citrus research international

CRI GROUP ANNUAL RESEARCH REPORT 2006

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

1 INTRODUCTION

CEO (CRI): Vaughan Hattingh

This report records the research results and technical support services of the CRI Group for the calendar year 2006. The CRI Group approach to industry research and technical support services was maintained and continued to be aimed at providing co-ordination of a balanced portfolio of research, focused on the industry's immediate and long-term needs. The strategy of utilizing specialist subject committees, to advise CRI on the composition and funding of the research programmes, was retained.

CRI continued to pursue a diversified income stream to augment the core funding provided by the levy on export citrus. The SA-EU Pesticide Initiative Programme (PIP) continued to provide a valuable source of additional funding. These funds were utilised to support research aimed both at defending the retention of valuable pesticide residue tolerances (MRLs) in the European Union and at reducing the industry's reliance on pesticides. The citrus industry's technology implementation company, River Bioscience, continued to show strong growth as a future source of additional research funding. The future implementation of FCM SIT is to be conducted by a subsidiary of River Bioscience. CRI continued to operate the Citrus Foundation Block with a financial self-sufficiency objective, but there was a sharp downturn in budwood sales in 2006 that precluded maintenance of a breakeven situation. As a consequence, some scheduled infrastructure developments at CFB were postponed, pending future recovery of the operation's income level.

There were a few changes to CRI's in-house capacity during the report period. The post of Assistant to the CEO was upgraded to a full time position, with technical market access support capabilities and Elma Carstens was appointed to this position. Dr Tony Ware, the research entomologist responsible for the fruit fly research project resigned. The research in this project has continued under the supervision of Dr Tim Grout, but a suitable entomologist is being sought to fill the vacancy. CRI's personnel structure was modified to cater for the re-introduction of the Research Programme Manager level of positions. Initially only the position of Research Programme Manager: Plant Pathology is to be filled and Dr Paul Fourie will commence in this post as of 2007. The CRI Board (with CGA ratification) also approved the future inclusion of a Citrus Nutritionist research post, although adequate funding for the position is still to be secured. The industry Diagnostic Centre benefited from an arrangement between CRI and DuRoi Banana laboratory, whereby Jacolene Meyer was seconded to CRI, providing increased molecular diagnostic capabilities to the DC. The Extension Division was strengthened by contracting the services of four citrus consultants (members of the professional citrus consultants association SASCCON) to assist with the coordination of some of the Technology Transfer Groups.

The gaining, retaining and optimising of access to markets, remained a central guiding priority throughout CRI's research and technical support activities. The CEO CRI continued to undertake technical coordination of phytosanitary market access issues as a portfolio of personal responsibility, in close collaboration with the CEO CGA. Access to most export markets continued to be increasingly challenged by sanitary and phytosanitary regulatory issues. A large proportion of the research portfolio continued to be directed at addressing such issues. The China export opportunity improved dramatically in 2006 with the acceptance by China of an amended export protocol and the registration of 393 farms to produce citrus for potential export to China. On a less positive note, USA continued to impose the extended (24 d) FCM cold treatment, despite the provision of data that supports SA's insistence on reversion to the 22 d treatment.

Progress was made with improving the scope of molecular identification techniques for plant pathogens of phytosanitary importance to the CIP. The Shoot Tip Grafting procedures at CRI's Nelspruit facilities were brought into full operation, with the renovation of a laboratory dedicated to this purpose. The process of developing the CIP into a statutorily legislated scheme under the Plant Improvement Act, was taken forward through engagement with the relevant responsible parties in the Department of Agriculture, the drafting of the scheme into a legal format and consultation with directly affected parties. The scheme will be formally submitted to the DoA in the near future.

The guiding principles for directing the industry's evaluation of cultivars and rootstocks was revised and the evaluation project was accordingly aligned with these principles. The local industry Cultivar Mutation Screening Project was launched, in pursuit of providing local growers with access to new cultivars on the most favourable terms and conditions. All southern African growers were invited to look for any such discoveries and draw these to the attention of the industry's cultivar development division of CRI (headed by Graham Barry), for potential development into new future cultivars. Furthermore, as previously called for by the citrus growers, the industry again became directly involved in the international pursuit of rights to new cultivars.

The diagnostic centre (DC) at CRI's Nelspruit facilities, has historically been operated as an industry technical support service. In addition to recovering a large proportion of its operating costs for the year, through fees for commercial diagnoses, the DC added considerably greater value than it cost, by providing diagnostic services to research projects. The DC entered a new era during the year, when it was equipped to begin conducting molecular diagnoses.

Both the export performance (of fruit from the preceding production cycle) and the reigning production conditions influence the research environment in any given calendar year. Whereas the 2005 year had been particularly difficult due to a combination of adverse market factors and record export volumes, the industry generally experienced a greatly improved 2006 export season. Likewise, the early production conditions during 2005 were subject to drought in the northern regions, but conditions were far more favourable for the 2006 early production period.

Lemon supply continued to be strong and market performance was generally good in the 2006 export season. The dry conditions in the early part of the production cycle, compounded by heavy rains shortly before harvest, hampered the start of the lemon export season. Consequently the 2006 lemon season had a somewhat delayed start with the northern region's crop being somewhat lower, with a tendency towards oversize and lower quality fruit. However, the season experienced considerable improvement as it progressed.

Soft citrus remained in short supply resulting in good price returns, although this opportunity was constrained by small fruit size. Considerable losses were experienced in USA towards the end of the 2006 soft citrus season due to a combination of factors, that included heavy supply volumes from Chile.

Grapefruit export fruit was in very short supply in 2006 due to drought experienced during the production season and a strong demand for processing fruit. The Japanese Grapefruit market experienced a dramatic recovery in comparison with the previous season's disaster. Unfortunately the good 2006 Japanese Grapefruit market was damaged for some suppliers due to difficulty experienced in complying with altered pesticide residue tolerances in this market.

The 2006 navel season started off poorly with oversize and low quality fruit (due to climatic conditions) from the North. Although this adversely impacted on the market conditions the quality of navels supplied improved after the early part of the season. Eastern and Western Cape yields were high but this was balanced by the prevalence of creasing and small fruit size. Valencias had a late start with reduced volumes, but the season strengthened considerably as it progressed, resulting in very good returns.

CRI strives to maintain a research portfolio with a balance between short term and long term industry needs, applied and basic research, and production and market focus. Whereas the production focus has always been strong, the need to ensure that this remains balanced with a market perspective, led to the Extension division strengthening the Research association with exporters. A Technical Committee was established to provide a forum for ongoing interaction between research and technical representatives from the exporter sector.

The annual revision of industry research priorities was undertaken by the Extension division. The priorities remained similar to the previous year, with continued top prioritisation of Citrus Black Spot, False Codling Moth, Fruit Flies, Post Harvest Pathology and Cultivar Development. CRI continued to apply the principle of guaranteeing that, in addition to addressing national priorities, it will also address every region's top priority, regardless of its national status.

INLEIDING

In hierdie verslag is die resultate vervat van die navorsing wat uitgevoer is en die tegniese dienste wat gelewer is deur die CRI Groep in die 2006 boekjaar. Die benadering van die CRI Groep tot die bedryf se navorsing en tegniese ondersteuningsdienste is daarop gefokus om die kort- en die langtermyn behoeftes te koördiner in 'n gebalanseerde navorsingsportefeulje. Die strategie van spesialis-komitees om CRI te adviseer in die samestelling en befondsing van die navorsingsprogramme is behou.

In die proses om die kernbron van inkomste - die heffings op uitvoervrugte - aan te vul het CRI voortgegaan om ander bronne van inkomste te vind. Die SA-EU Plaagdoder Inisiatiefprogram (PIP) was weereens 'n belangrike bron van addisionele befondsing. Hierdie fondse is aangewend om navorsing te ondersteun wat daarop gerig is om die residu toleransies (MRVs) van belangrike plaagdoders in die Europese Unie te verdedig en ook om die bedryf se staatmaking op plaagdoders te verminder. River Bioscience, die maatskappy van die sitrusbedryf wat verantwoordelik is vir die kommersialisering van tegnologie, het goeie

vooruitsigte getoon as 'n potensiële addisionele bron van befondsing vir navorsing. 'n Filiaalmaatskappy van River Bioscience sal ook verantwoordelik wees vir die verdere implementering van VKM SIT. CRI het voortgegaan om die Sitrus Grondvesblok op 'n self-onderhoudende basis te bestuur maar die skerp afname in die verkope van enthout in 2006 het tot 'n verlies gelei. Van die infrastrukturele ontwikkelings wat beplan is, is gevolglik uitgestel totdat die finansiële omstandighede verbeter.

Gedurende die verslagperiode het daar veranderinge binne die CRI se personeelkomponent plaasgevind. Die funksies van die pos van Assistent vir die HUB is uitgebrei en opgegradeer na 'n voldagpos om voorsiening te maak vir ondersteuning in die tegniese aspekte van marktoegang. Elma Carstens is aangestel in die pos. Dr Tony Ware, die entomoloog wat verantwoordelik was vir die vrugtevlieg navorsingsprojek, het bedank. Navorsing in hierdie projek het egter voortgegaan onder die leiding van Dr Tim Grout, maar 'n geskikte entomoloog word gesoek om die vakature te vul. Daar is ook verander aan die personeelstruktuur om voorsiening te maak vir die herinstel van die poste vir navorsingsprogrambestuurders. Die aanvanklike besluit is dat slegs die posisie van Navorsingsprogambestuurder: Plantpatologie gevul sal word en Dr Paul Fourie is aangestel om diens te aanvaar aan die begin van 2007. Die CRI Raad het ook die skepping van 'n navorsingspos vir 'n Sitrus Plantvoedingskundige goedgekeur. Die besluit is deur die CGA bekragtig maar die nodige befondsing vir die posisie moet nog gevind word. Die reëling tussen CRI en DuRoi Piesang Laboratorium waar Jacolene Meyer gesekondeer is aan CRI, was tot die voordeel van die bedryf se Diagnostiese Sentrum. Die sekondering het die molekulêre diagnostiese vermoëns van die Diagnostiese Sentrum aansienlik verbeter. Die Voorligtingsafdeling is ook versterk deur die kontraktering van die dienste van vier sitrus konsultante (lede van die sitrus konsultante vereniging - SASCCON) om hulp te verleen met die koordinering van sommige van die Tegnologieoordrag Groepe.

Die verkryging, behoud en verbetering van toegang tot markte bly 'n sentrale fokuspunt van CRI se navorsing en tegniese ondersteuningsaktiwiteite. Die HUB CRI is steeds persoonlik verantwoordelik, in noue samewerking met die HUB CGA, vir die tegniese koördinering van die fitosanitêre marktoegangsaksies. Toegang tot die meeste van die uitvoermarkte word op 'n toenemende basis bedreig deur sanitêre en fitosanitêre aksies. 'n Groot gedeelte van die navorsing was daarop gerig om die aksies aan te spreek. Die uitvoermoontlikhede na China het drasties verbeter in 2006 met die aanvaarding van die gewysigde protokol en die registrasie van 393 plase. Minder positief was die VSA se volharding met die verlengde (24 d) VKM-koue behandeling ten spyte daarvan dat inligting ter ondersteuning van SA se aandrang op verandering na 22 d voorgelê is.

Daar is vordering gemaak met die verbetering van die omvang van die tegnieke vir die molekulêre identifikasie van plant patogene wat van fitosanitêre belang is vir die SVP. Die groeipunt-entingsproses by CRI se fasiliteite in Nelspruit is nou in volle werking met die opknapping van die toegekende laboratorium. Die proses om die SVP te ontwikkel in 'n statutêre, wetlike skema onder die Plantverbeteringswet het ook verder gevorder. Skakeling het plaasgevind met die relevante verantwoordelike partye in die Departement van Landbou (DvL), die skema is opgestel in 'n wetlike formaat en daar is ook gekonsulteer met die partye wat direk geraak gaan word. Formele voorlegging van die skema aan die DvL sal in die nabye toekoms plaasvind.

Die riglyne van die bedryf se evalueringsproses vir kultivars en onderstokke is hersien en die evaluasieprojek is ook dienooreenkomstig aangepas. Die plaaslike bedryf se Kultivar-Mutasie-Siftingsprojek is van stapel gestuur om aan plaaslike produsente toegang te verleen tot nuwe kultivars volgens die beste bepalinge en voorwaardes. Al die suider Afrikaanse produsente is ook uitgenooi om te soek vir enige sulke ontdekkings en dit onder die aandag te bring van die bedryf se Kultivarontwikkelingsafdeling van CRI (onder leiding van Graham Barry) vir potensiële ontwikkeling van nuwe kultivars. Verder soos versoek deur die sitrus produsente het die bedryf ook direk betrokke geraak by die internasionale verkryging van regte tot nuwe kultivars.

Histories het die Diagnostiese Sentrum by CRI se fasiliteite in Nelspruit gefunksioneer as 'n tegniese ondersteuningsdiens aan die bedryf. Addisioneel tot die verhaling van kostes deur die heffing van fooie vir die uitvoering van kommersiële diagnostiese dienste, was die toevoeging in waarde deur die diagnostiese dienste wat gelewer is aan die navorsingsprojekte groter as die kostes betrokke. Die Sentrum het 'n nuwe era betree toe dit in die jaar toegerus is om ook molekulêre diagnostiese dienste te lewer.

Die omgewing waarin navorsing moet geskied in enige gegewe jaar word beïnvloed deur die prestasie van die uitvoere (van vrugte van die vorige seisoen) en die bestuur van die omstandighede tydens verbouing. Waar 2005 'n buitengewoon moeilike jaar was weens ongunstige faktore in die mark en rekord uitvoervolumes, het die bedryf in 2006 oor die algemeen 'n baie beter uitvoerseisoen beleef. So ook, was dit baie droog vroeg in die 2005 seisoen in die noordelike dele maar die omstandighede was baie meer gunstig vroeg in die seisoen gedurende 2006.

Die mark se vertoning asook die beskikbaarheid van suurlemoene was oor die algemeen goed tydens die 2006 uitvoerseisoen. Die aanvang van die seisoen is egter vertraag deur die droë toestande aan die begin tesame met die swaar reëns kort voor die oes. Die seisoen het dus laat begin met die noordelike streke se volumes wat minder was met 'n tendens van groot vrugte en 'nlaer vrugkwaliteit. Die seisoen het egter baie verbeter soos dit gevorder het.

Die tekort aan sagte sitrus het baie goeie pryse tot gevolg gehad het alhoewel die geleenthede beperk is deur die klein vruggroottes. Aansienlike verliese is gelei in die VSA aan die einde van die 2006 seisoen as gevolg van 'n kombinasie van faktore soos die groot volumes afkomstig van Chile.

Daar was 'n tekort aan pomelos in 2006 weens die droogte gedurende die seisoen en 'n sterk aanvraag na verwerkte vrugte. Die Japanese mark vir pomelos het dramaties herstel na die vorige seisoen se ramp. Ongelukkig was die goeie Japanese mark nie beskore vir sommige produsente nie weens die probleme wat ondervind is om te voldoen aan die gewysigde plaagdoder residue toleransies van die mark.

Die 2006 se nawelseisoen het nie 'n goeie begin gehad nie. Die vrugte van die Noorde was te groot en ook van 'n lae kwaliteit (weens klimaatstoestande). Alhoewel dit 'n nadelig invloed gehad het op die marktoestande het die kwaliteit van die nawels later in die seisoen verbeter. Die volumes in die Oos en Wes Kaap was hoog maar die voorkoms van kraakskil en klein vruggroottes het vir 'n balans gesorg. Valencias was ook laat met minder volumes. Die toestande het egter later verbeter wat goeie opbrengste tot gevolg gehad het.

CRI se doel is om 'n portefeulje van navorsing te onderhou, wat 'n balans sal verseker tussen kort en lang termyn bedryfsbehoeftes, toegepaste en basiese navorsing en verbouing en mark fokus. Die fokus op verbouing was altyd hoog en om te verseker dat dit in perspektief sal bly met die mark het die Voorligtingsafdeling navorsingsbande met die uitvoerders versterk. 'n Tegniese komitee is gestig om 'n forum daar te stel waar interaksie voortdurend kan plaasvind tussen navorsers en tegniese verteenwoordigers van die uitvoerders.

Die jaarlikse hersiening van die bedryf se navorsingsprioriteite is deur die Voorligtingsafdeling gedoen en die prioriteite was baie dieselfde as die vorige jaar met Sitrus Swartvlek, Valskodlingmot, Vrugtevlieë, Na-oes Patologie en Kultivarontwikkeling as die hoof prioriteite. CRI gaan voort om die versekering te gee dat alhoewel nasionale prioriteite aangespreek sal word, aandag ook aan elke streek se hoof prioriteit gegee sal word, ongeag die nasionale status wat daaraan toegeken is.

2 PROGRAMME: MARKET ACCESS TECHNICAL COORDINATION

Co-ordinator: Vaughan Hattingh (CEO)

2.1 PROGRAMME SUMMARY

A process of gaining **Chinese** acceptance for inclusion of cold treatment as an alternative FCM risk mitigation treatment was initiated in 2004 and the revised export protocol was signed by the relevant SA and Chinese government officials in 2006. A total of 393 farms were registered in 2006 for participation in the China export programme.

South Africa's submission to the **EU,** proposing a relaxation of the quarantine measures associated with CBS, had remained outstanding since SA submitted the last set of requisite data in 2004. The CEO CRI undertook two trips to Brussels in May and October 2006 to get the process moving. The EC working group was convened in June 2006. The long-waited response from the EC was received by SA DoA at the end of 2006 and will be dealt with as a priority in 2007. From the EU-meetings it was evident that SA must engage in a multi-facetted strategy to create a political and trade policy environment within the EU that will be conducive to a favourable resolution. This is to be done in parallel with continuation of the official technical interaction and the mobilisation of simultaneous pressure (political and trade) to bring about a favourable resolution. This was referred to CGA for implementation.

There were two major phytosanitary issues in **Japan** that received attention, namely (1) an application to open the Japanese market for the export of South African Clementines and (2) the adoption of a revised cold treatment condition for the export of all citrus types from South Africa to Japan.

In culmination of sustained efforts by SA over three years, USDA APHIS finally included additional Western Cape magisterial districts (within the winter rainfall region) in the **USA** export work plan, thus opening the way for export of citrus from these areas to USA. SA also requested reversion back to the 22 d FCM treatment and provided supporting data. Discussions were held with USDA APHIS scientists in Raleigh

during April 2006. Despite the validation data supplied by SA and the additional measures implemented by SA, USA thereafter demanded the implementation of various additional FCM risk mitigation measures. SA formally tabled its objection to the situation by holding a side-bar discussion with USA at the Geneva WTO-SPS talks. High level political engagement was sought and the Minister of Agriculture engaged with the USA Secretary of Agriculture on this matter in December 2006. The parties undertook to resolve the issue before the 2007 export season.

An intense official CBS survey was conducted in the Northern Cape and adjacent magisterial districts in the Free State, enabling SA to officially recognise the Northern Cape as a CBS pest-free area and request USDA to include this area in the USA citrus export programme. Attention was also given to potentially gaining access to the USA market through establishment of CBS pest-free places of production. DoA, assisted by CRI, proceeded in 2006 with intensive surveys of four production units in northern Limpopo to test the feasibility of the proposed system. The results confirmed that the proposed system is feasible.

The fast track implementation of FCM SIT was pursued as a priority in 2006. The industry-owned company Xsit (Pty) Ltd, was consequently formed to provide the implementation vehicle and CRI continued with essential further research and development on the FCM SIT technology. Attention was also given to development of irradiation as an alternative to cold for post-harvest disinfestation of FCM. Research conducted by CRI enabled the fast-track registration, in 2006, of a post-harvest treatment for grain chinch bug, an organism of phytosanitary concern in the USA programme.

Attention was given to pursuit of access to **South Korea** for SA lemons and grapefruit. Attention was also given to gaining access to the **Thailand** market, as well as lemons to **Israel**. **Australia's** failure to respond to SA's application to open the Australian market to SA citrus exports was raised by SA in an official side-bar discussion with Australia at the Geneva WTO-SPS meetings in 2006. **Indonesia** implemented the application of a mandatory, pre-shipping, fruit fly cold treatment requirement in 2006 and SA DoA was successful in obtaining approval to apply in-transit (during shipping) cold treatment.

Good progress was made in the generation of data to support the retention of **MRLs** (in the EU) for important insecticides and fungicides. The monitoring of changes in pesticide residue requirements in market countries continued in close cooperation with Paul Hardman as CGA Industry Affairs Manager. The SA Recommended Usage Restrictions, to assist in continued compliance with changing market residue tolerances, was periodically revised. Unfortunately, changes to triflumuron MRLs in Japan proved to be problematic and some producers experienced losses due to an inability to comply with the changed tolerances. The recommended usage practices for this active ingredient were consequently amended.

PROGRAMOPSOMMING

'n Proses is in 2004 begin om goedkeuring van **China** te verkry om koue behandeling as 'n alternatief te aanvaar as 'n beheermaatreël vir VKM. In 2006 is die gewysigde protokol geteken deur die Chinese en SA owerhede wat daartoe gelei het dat 'n totaal van 393 plase geregistreer is in 2006 vir deelname aan die mark.

Suid Afrika se voorlegging aan die **EU** waarin daar versoek is dat die kwarantyn maatreëls wat van toepassing is op Sitrus Swartvlek (SSV) verslap moet word, het uitstaande gebly sedert die laaste inligting, soos versoek, in 2004 aan hul voorgelê is. Die HUB CRI het twee reise na Brussels onderneem gedurende Mei en Oktober 2006 in 'npoging om die proses te bespoedig. Die EC se werksgroep het in 2006 vergader en die lang verwagte terugvoering is aan die einde van 2006 deur SA se Departement van Landbou (DvL) ontvang. Die aangeleentheid sal voorkeur aandag in 2007 geniet. Dit was duidelik vanuit die EU-vergaderings dat indien 'n gunstige uitslag verlang word, SA betrokke sal moet raak in die daarstelling van 'n veeldoelige strategie om 'n gunstige politieke- en handelsbeleids omgewing binne die EU te skep. Dit sal parallel moet plaasvind saam met voortsetting van amptelike tegniese interaksie en die mobilisering van gelyktydig druk (polities en handel). Hierdie aksie is verwys na die CGA vir implementering.

Daar was twee belangrike fitosanitêre sake met betrekking tot **Japan** wat aandag geniet het. Eerstens was dit die aansoek om marktoegang te verkry vir die uitvoer van Suid Afrikaanse Clementines en tweedens was dit die aanvaarding van gewysigde kondisies vir koue behandeling vir alle sitrussoorte wat na Japan uitgevoer word.

Toenemende volgehoue pogings deur SA gedurende die afgelope drie jaar het daartoe gelei dat USDA APHIS uiteindelik addisionele magistraatsdistrikte van die Wes Kaap (binne die winter reenvalstreek) ingesluit het in die **VSA** se werksplan vir sitrusuitvoere. Sitrus van hierdie areas kan nou na die VSA uitgevoer word. SA het ook versoek dat die 22 d VKM behandeling weer ingestel word en het data ter

ondersteuning daarvan voorgelê. Samesprekings is gehou met wetenskaplikes van USDA APHIS in Raleigh gedurende April 2006. Na hierdie samesprekings het die VSA steeds aangedring op die implementering van verskeie addisionele VKM beheermaatreëls ten spyte van die inligting wat voorsien is en die addisionele maatreëls wat reeds deur SA ingestel is. SA het formeel hul teenkanting teen die situasie aangeteken deur "side-bar" samesprekings aan te vra met die VSA tydens die WTO-SPS vergaderings in Geneva. Politieke betrokkenheid op hoë vlak is versoek en die Minister van Landbou het samesprekings gevoer met die VSA se sekretaris van Landbou in Desember 2006. Die partye het onderneem om die kwessie op te los voor die begin van die 2007 uitvoerseisoen.

'n Intensiewe, amptelike SSV opname is uitgevoer in die Noord Kaap en in die aangrensende magistraatsdistrikte van die Vrystaat. Dit het SA instaat gestel om die Noord Kaap amptelik te erken as 'n SSV-vrye area en die versoek is aan die USDA gerig om hierdie area in te sluit in die VSA sitrus uitvoerprogram. Om potensieël toegang tot die VSA mark te verkry is daar ook aandag geskenk aan 'n sisteem om SSV-vrye plekke van produksie te vestig. DvL in samewerking met CRI het in 2006 voortgegaan met intensiewe opnames op vier produksie-eenhede in die noordelike dele van Limpopo om te uitvoerbaarheid van die sisteem te toets. Die resultate het die uitvoerbaarheid van die sisteem bevestig.

Die vinnige implementering van VKM SIT is gesien as 'n prioriteit in 2006. Die maatskappy Xsit (Pty) Ltd wat aan die bedryf behoort, is gevolglik gestig om die aksie te dryf en CRI het voortgegaan met noodsaaklike navorsing en ontwikkeling van die VKM SIT tegnologie. Aandag is ook gegee aan die ontwikkeling van bestraling as 'n alternatief vir koue as na-oes disinfesteringsmetode van VKM. Navorsing wat deur CRI uitgevoer is het die vinnige registrasie van 'n na-oes behandeling vir die graanstinkbesie, 'n plaag van fitosanitêre belang in die VSA uitvoerprogram, in 2006 moontlik gemaak.

Aandag is gegee om moontlike marktoegang te verkry vir suurlemoene en pomelos na **Suid Korea,** alle sitrussoorte na **Thailand** en suurlemoene na **Israel**. Tydens die WTO-SPS vergaderings in Geneva in 2006 het SA in amptelike "side-bar"-besprekings met **Australië** die uitstaande terugvoering van Australië op SA se aansoek om marktoegang te verkry, bespreek. 'n Verpligte voor-verskepings koue behandeling teen vrugtevlieë is deur **Indonesië** in 2006 geïmplementeer. SA-DvL was suksesvol in 'n aansoek om goedkeuring te verkry dat die behandeling in-transit toegepas kan word.

Goeie vordering is gemaak met die verkryging van data om die voortbestaan van **MRVs** in die EU vir belangrike plaagdoders te ondersteun. Die monitering van veranderinge in die plaagdoder residu vereistes in die lande waarheen ons uitvoer is voorgesit in noue samewerking met CGA se Bestuurder: Bedryfsaangeleenthede (Paul Hardman). SA se Aanbevole Gebruiksbeperkinge is periodiek hersien om op datum te bly met die veranderinge in die markte se residu toleransies en om produsente te help om te kan voldoen aan die vereistes. Ongelukkig het veranderinge aan die MRVs van triflumuron in Japan probleme veroorsaak en sommige produsente het verliese gelei as gevolg van die onvermoë om te kan voldoen aan die gewysigde toleransies. Die aanbevole gebruikspraktyke vir hierdie aktiewe bestanddeel is dus aangepas.

2.2 **CHINA**

The 2005 export season had been the first full year that SA citrus had access to the Chinese market, but the quantities of fruit successfully exported were disappointingly small. The principal constraint had been that the original export protocol required that fruit for export to China had to be produced in orchards that were free of FCM. A process aimed at gaining Chinese acceptance for inclusion of cold treatment as an alternative FCM risk mitigation treatment was initiated in 2004. A large scale trial to validate the efficacy of the cold treatment was successfully executed in 2005. A protracted delay ensued before the revised export protocol was signed by the relevant SA and Chinese government officials. Signature opened the way for Chinese inspectors to conduct audit inspection in June 2006 on a sample of the farms that were potentially eligible to register for export of citrus to China. Due to these developments having taken place with very short notice, it was necessary to limit the farms that would initially be potentially eligible to register for participation in the China programme, to those that were already involved in special export programmes with similar requirements. Nonetheless, 393 farms were registered in 2006 for potential participation in the China export programme, whereas there had only been four farms registered the previous year. The citrus industry nonetheless requested that China consider registration of additional farms in 2007. At the end of the report period, China reverted indicating that they would consider registration of additional farms in 2007, as well the inclusion of some southern Chinese ports for SA citrus exports.

Despite having validated (in the presence of Chinese officials) the efficacy of a 22 day cold treatment (at temperatures below 0°C) for the disinfestation of FCM, China imposed a 24 day cold treatment requirement. China had no technical justification for insisting on a 24 day treatment, since a 22 day treatment had been

validated as providing the requisite level of phytosanitary security, in accordance with internationally accepted standards of Probit 9 efficacy. China defended their position on the basis that USA had imposed a 24 day cold treatment requirement, even though USA could also not provide suitable technical justification for its extended treatment requirement. Although this position taken by China could legitimately be challenged by SA, attention has been focussed on resolving the issue of the cold treatment duration with USA, being the source of the problem.

In discussions leading up to conclusion of the revised (2006) China export protocol, the SA citrus industry had requested SA Department of Agriculture to pursue retention of the option to export fruit from FCM-free orchards, in addition to the option of applying a post-harvest cold treatment for the disinfestation of FCM. Unfortunately negotiations between the SA DoA and China were not successful in this regard. This effectively resulted in a situation where lemons (and to a large extent also Grapefruit) were excluded from this programme because of their sensitivity to cold. Guidelines on how to manage chilling injury risk on Grapefruit were distributed in the form of a Cutting Edge article.

A potential means of circumventing the restraint imposed by cold treatment on the opportunity to export lemons to China, is to demonstrate that lemons are not a suitable host for FCM, making the application of cold treatment superfluous. Conclusive evidence of potential non-host status has not as yet been attained, but the investigation has also not yet been completed. The potential of irradiation as an alternative post-harvest disinfestation treatment also received attention.

2.3 **EUROPE**

South Africa's submission to the EU, proposing a relaxation of the quarantine measures associated with CBS on citrus fruit imported from South Africa, had remained outstanding since SA submitted the last set of requisite data in 2004. Despite numerous requests from SA DoA to the relevant EC officials, to expedite a response from the EU, by the end of the previous report period no response had been forthcoming. VH undertook a trip to Brussels in May 2006 in an attempt to get the process moving. Discussions were held with a wide range of parties, including: the EC officials responsible for reverting to SA with a response; officials from the EC Directorate Trade and EC Directorate Agriculture; the SA Embassy (including the SA Ambassador, the SA Agricultural Attaché and SA's Department of Trade and Industry representatives in Brussels); and Freshfel (an EU fruit trade organisation). The EC provided assurance that they would proceed with convening an EU CBS working group to review the SA data and revert with a response. The Ambassador and Agricultural Attaché had follow up meetings with the DG of the EC DG SANCO, the Directorate responsible for handling the matter.

The EC working group was convened in June 2006. Although no official response had been received by October 2006, Freshfel had obtained unofficial indications that the EU response was not going to be favourable for SA. VH undertook another trip to Brussels in October 2006. Discussions were held with: EC DG SANCO officials (the responsible EC Directorate); the SA Embassy; Freshfel; and a potential lobbyist. Follow up discussions were also held with Spanish citrus industry leaders. It was evident that SA must engage in a multi-facetted strategy to create a political and trade policy environment within the EU, that will be conducive to a favourable resolution. This is to be done in parallel with continuation of the official technical interaction and the mobilisation of simultaneous pressure (political and trade) to bring about a favourable resolution. In the meeting with EC DG SANCO, the EC officials gave an undertaking to revert to SA with their official response by the end of the year or early 2007 at the latest. The EC response was received by SA DoA at the end of 2006 and will be dealt with as a priority in 2007.

Whereas there had been reports of numerous FCM interceptions in SA citrus arriving in Spain in 2005, the awareness campaign in SA appeared to be successful in that SA received no official notifications of such interceptions in 2006. It is essential that SA continues to improve the efficacy of pre-harvest FCM control measures and to diligently implement these and other FCM risk management practices.

The fruit fly risk mitigation procedures required by the EU, include certification that the fruit is free of non-Mediterranean fruit flies. The EU plant health inspections seemed to have focussed increased attention on the fruit-fly-free status of fruit imports in 2006. This highlights the importance of SA continuously improving the efficacy of its fruit fly control strategies. The presence of *Bactrocera invadens* in central African countries (a newly introduced invasive fruit fly of Indonesian origin) continued to be monitored, but there was no evidence of an expansion in its distribution.

2.4 **JAPAN**

There were two major phytosanitary issues in Japan that received attention, namely (1) an application to open the Japanese market for the export of South African Clementines and (2) the adoption of a revised cold treatment condition for the export of all citrus types from South Africa to Japan.

With regard to Clementines, a large scale cold treatment validation trial (14 d at -0.4°C) was successfully conducted by SA in the presence of a Japanese scientist in 2005 and subsequently a report on the trial was submitted to Japan MAFF. Japan indicated that the next step would be to hold a public hearing in Japan to invite comment on MAFF's intention to grant access to SA Clementines. Throughout 2006, enquiries were repeatedly made about progress with the process (both official enquiries through SA DoA and unofficial enquiries through trade contacts). Although assurances were repeatedly given that the matter was in process, no public hearing had been held in Japan by the end of the report period. It will be necessary for SA to engage diplomatic and trade channels to facilitate progress in 2007.

With regard to the amended cold treatment standard for Japan, although there was no resolution as to whether it would be necessary to repeat phase 4 of the validation trial on each citrus type, SA continued in 2006 with phase 4 on oranges. The target shipping temperature for which validation was being sought was 1°C ± 0.5°C. Given that Japan tends to adopt the upper end of this range of experimental conditions as the mid-point temperature for the shipping protocol (although the technical justification for this is contested by SA), it was necessary to conduct the trial under conditions where the experimental conditions would be 1.5°C ± 0.5°C. Under these conditions some larvae survived and the trial will have to be repeated in 2007 at a lower temperature or with a longer exposure period.

2.5 **USA**

In culmination of sustained efforts by SA over three years, USDA APHIS finally included additional Western Cape magisterial districts (within the winter rainfall region) in the USA export work plan, thus opening the way for export of citrus from these areas to USA.

In response to reports that a live FCM had been found in SA citrus fruit exported from SA to USA in 2005, the USA extended the post-harvest cold treatment for FCM disinfestation from 22 d to 24 d. This took place in the early part of the 2005 export season. On successful completion of the 2005 season, SA requested reversion back to the 22 d treatment and provided supporting data. USA rejected the proposal to revert back to the validated 22 d treatment, but invited SA to send a delegation to have a technical meeting with USA scientists. Consequently VH, Hendrik Hofmeyr and Isabel Bezuidenhout (DoA) held discussions with USDA APHIS scientists in Raleigh during April 2006. Whereas SA could provide data validating the Probit 9 efficacy of the 22 d treatment, and USDA APHIS had no validation for the extended treatment besides the reported find of a survivor in 2005, USDA continued to reject SA's call to revert to the 22 d treatment.

SA subsequently also provided analysis of the 2005 shipping temperature logs to demonstrate that the implementation of the minimum 22 d cold treatment necessitated exposing the fruit to far longer periods at these insecticidal temperatures and that imposing the extra 2 d treatment had little chance of improving efficacy but did have serious adverse effects on fruit quality. SA also advised the USA that it had made the implementation a Good Agricultural Practice for the pre-harvest control of FCM a pre-requisite for participation (as of 2006) in the USA export programme. SA indicated that this represented the implementation of measures that materially increased the level of phytosanitary security and thereby provided technical justification of re-instatement of the shorter-duration (22 d) cold treatment.

Despite the validation data supplied by SA and the additional measures implemented by SA, USA thereafter demanded the implementation of various additional FCM risk mitigation measures. SA responded by indicating that it was of the opinion that it had fulfilled its obligations with regard to the International Plant Protection Convention guidelines, thus implying that the USA's restrictions had become technically unjustified and contrary to the World Trade Organisation's requirements for phytosanitary regulation of trade.

At the end of the 2006 export season, SA analysed the pre-clearance (before export) inspection records, indicating that the FCM infestation levels in fruit presented for export inspection in 2006 were markedly lower than in 2005. This supported SA's position that the implementation of the FCM GAP had provided increased levels of phytosanitary security. This information was provided to USA and DoA had follow up meetings with USDA APHIS in USA, but without resolution. SA formally tabled its objection to the situation by holding a side-bar discussion with USA at the Geneva WTO-SPS talks. High level political engagement was sought and the Minister of Agriculture engaged with the USA Secretary of Agriculture on this matter in December 2006. The parties undertook to resolve the issue before the 2007 export season.

Citrus producers in several production areas had previously requested CRI support in potentially gaining access to the USA market through establishment of CBS pest-free areas or the development of a risk management system that would enable export from CBS pest-free places of production (farms) within circumscribing areas of low CBS pest prevalence. CRI had conducted preliminary surveys in several such areas over the preceding years. At the end of 2005 CRI had supported DoA to conduct an intense official survey of the Northern Cape and adjacent magisterial districts in the Free State. The 450 samples collected were analysed in both the DoA quarantine laboratory in Stellenbosch and QMS Agrisciences laboratory (under the auspices of the DoA). No evidence of CBS was found, enabling SA to officially recognise the Northern Cape as a CBS pest-free area and request USDA to include this area in the USA citrus export programme. Finalisation of the official survey report and submission of the application to USA is to receive attention in early 2007.

Intensive surveys for CBS were conducted by DoA (assisted by CRI) in December 2005 in the northern Limpopo region. This was conducted towards developing a system for potential recognition of CBS pest-free places of production within circumscribing areas of low pest prevalence. CRI had the samples analysed by QMS Agrisciences and University of Pretoria Plant Pathology Laboratories, under the auspices of the DoA. One sample tested positive for CBS, demonstrating that the procedure was able to detect the presence of very low infection levels. The results of the surveys conducted over the preceding four years (September 2001 – December 2005) were consolidated into a report and submitted to DoA. SA had first submitted a draft of this proposed system to USDA APHIS in 2004 for comment. Despite repeated re-submissions of the information and requests for a response, USDA failed to officially respond to SA's proposal to implement such a system. This failure of APHIS to give attention to SA's requests to consider improved market access was raised at the political level in an attempt to expedite a response.

The fast track implementation of FCM SIT was pursued as a priority in 2006. An industry decision was taken to proceed with implementation in a subsidiary of River Bioscience. The company Xsit (Pty) Ltd was consequently formed to provide the implementation vehicle and CRI continued with essential further research and development on the FCM SIT technology.

SA had previously (2004) requested USDA APHIS to endorse an experimental protocol for validating the efficacy of post-harvest irradiation of citrus fruit as a potential FCM disinfestation treatment. Towards the end of 2006, USDA APHIS reverted with a response, and CRI will give further attention to the matter in 2007.

CRI generated data that supported the efficacy of a novel post-harvest treatment for the control of Grain Chinch Bug as a phytosanitary "hitch hiker" on citrus exports to USA. The data, together with industry support for fast-track authorisation, was supplied to the Registrar Act 36 of 1947, enabling the registration of the product in time for use during the 2006 export season.

2.6 SOUTH KOREA

South Africa has been pursuing the inclusion of lemons and Grapefruit into the South Korean export programme for some time. In 2005 the South Korean authorities notified South Africa that they would accept South African lemons, on condition that they are subjected to the 22 d FCM cold treatment, as for oranges. However, lemons and Grapefruit display extensive chilling injury when exposed to these conditions. An amended FCM risk mitigation procedure was formulated for lemons and Grapefruit and provided to DoA in December 2005, for presentation to South Korea. South Korea responded in April 2006 and counter-proposals for alternative risk mitigation measures for lemons and Grapefruit were provided to DoA in July 2006, for communication with S Korea. No further response had been received from S Korea by year end. The opportunity to include soft citrus in the S Korean export programme was identified for attention in the next year.

S Korea followed the USA by insisting that the FCM cold treatment period be extended to 24 d. SA held side-bar discussions with S Korea at the Geneva WTO-SPS talks to indicate that SA objects to this since there is no technical justification for insisting on a 24 d cold treatment period. S Korea indicated that they would be prepared to revert to 22 d once USA did so.

2.7 THAILAND

Whereas South Africa and Thailand were close to concluding an export protocol for citrus from South Africa in 2003, subsequent delays in exchange of information and changes in the Thailand administration, frustrated attempts to make progress. Thailand indicated in 2005, that they would revisit the Pest Risk Analysis (PRA) that had previously been conducted in 2003. SA and Thailand exchanged information on the

validity of reverting to this earlier stage of the process. In July 2006 SA DoA officials visited Thailand and it was resolved that SA must respond to a list that Thailand supplied in March 2005 concerning organisms of phytosanitary concern to Thailand. CRI compiled a data package in response and submitted it to DoA. However, prior to DoA proceeding with such communication, SA DoA received a revised draft export protocol from Thailand (dated September 2006). By year end the DoA had not as yet provided it to the industry for input.

2.8 ISRAEL

SA and Israel had previously exchanged information in connection with an application to consider accepting the export of lemons from SA to Israel. In December 2005, Israel requested additional information from SA. The situation was assessed by the Market Access Working Group (convened under the auspices of the DoA) and it was concluded that SA should advise Israel that, at this point, SA does not wish to continue pursuing the PRA for lemons to Israel.

2.9 AUSTRALIA

Australia had requested additional data to support SA's application to potentially authorise commencement of SA citrus exports. The requisite data package was compiled and forwarded to DoA in February 2005, for submission to Australia. Despite repeated official queries by SA, no response was received from Australia. SA raised the issue in an official side-bar discussion with Australia at the Geneva WTO-SPS meetings in late 2006. A response to the information submitted by SA in 2005 is expected in early 2007.

2.10 OTHER MARKET ACCESS AND FOOD SAFETY ISSUES

Indonesia implemented the application of a mandatory, pre-shipping, fruit fly cold treatment requirement in 2006. SA DoA was successful in obtaining approval to apply in-transit (during shipping) cold treatment. SA became an associate member of CLAM (the Mediterranean citrus industry's co-ordination forum) in 2005. CLAM sent a delegation to SA in May 2006, and the exchange of information on market conditions was thereby initiated. Good progress was made in the generation of data to support the retention of MRLs in the EU for important insecticides and fungicides. The monitoring of changes in pesticide residue requirements in market countries was continued in close cooperation with Paul Hardman as CGA Industry Affairs Manager. The SA Recommended Usage Restrictions, to assist in continued compliance with changing market residue tolerances, was periodically revised. Unfortunately, changes to triflumuron MRLs in Japan proved to be problematic and some producers experienced losses due to an inability to comply with the changed tolerances. The recommended usage practices for this active ingredient were consequently amended.

3 PROGRAMME: INTEGRATED PEST MANAGEMENT

3.1 PROGRAMME SUMMARY

By Tim G Grout (Manager: Research & Technical)

Phytosanitary requirements continue to be the driving force behind most IPM research and false codling moth research once again received the most funding and attention. The research breakthrough of the year was the successful control of FCM on 35 ha of citrus using the Sterile Insect Technique. This led to further development of apparatus and techniques for the mass rearing of FCM and the formation of the spin-off company XSIT. Various other approaches to orchard control of FCM were under investigation including the use of larval parasitoids and entomopathogenic nematodes. Means of improving the residual control of Cryptogran, the continued search for female attractants and the host status of lemons, also received attention. Fruit fly research is the next most funded after FCM, again due to the phytosanitary significance of this pest. Unfortunately, a 16-day cold treatment at 1.5°C was not sufficient to provide probit-9-level control of Medfly and this research will have to be repeated at 1°C. The developmental thresholds for South African Natal fruit fly were found to differ from those found by other researchers for Natal fruit fly on the island of Reunion, suggesting that these represent different strains. Research on control techniques showed that the application of baits to the ground-cover rather than the tree to avoid residues is ineffective. The control of cosmetic pests often has a detrimental effect on natural enemies of other pests and the search for more IPM-compatible treatments continued. Further promising results with the virus Helicovir for bollworm control were obtained and a comparison of all available abamectin formulations for citrus thrips control showed that there were no significant differences in efficacy between them. Further setbacks were experienced in the rearing of a parasitoid of the citrus thrips and populations of citrus grey mite suitable for research were not found. Ants also disrupt natural enemies and further research was conducted on control methods. Unfortunately, the best repellents were only effective against the pugnacious ant for five days. The non-target effects of commonly-used pesticides on green lacewing larvae were determined to assist in conserving this predator of mealybug and other soft-bodied insects. More parasitoids of the oleander mealybug were discovered but the dominance of this species of mealybug has declined and citrus mealybug once again appears to be the most commonly found species in citrus orchards in South Africa. A means of controlling the hitchhiker pest grain chinch bug with post-harvest dips of natural pyrethrum, was verified but no suitable IPM-compatible treatments could be found as alternatives to endosulfan for the control of citrus psylla. An attempt to use mass trapping as a means of control for psylla actually increased levels of infestation on new leaves. A considerable amount of time was spent on the development of residue breakdown data for the determination of pre-harvest intervals for old insecticides that are no longer being supported by the original manufacturers.

PROGRAMOPSOMMING

Deur Tim G Grout (Bestuurder: Navorsing & Tegnies)

Fitosanitêre vereistes bly steeds die dryfveer agter die grootste gedeelte van IPB navorsing en die meeste tyd en befondsing is weereens spandeer aan valskodlingmot navorsing. Die navorsings- deurbraak van die jaar was die suksesvolle beheer van VKM op 35 ha sitrus deur gebruik te maak van die Steriele Insek Tegniek. Dit het daartoe gelei dat apparate en tegnieke ontwikkel is vir grootskaalse teling van VKM asook die stigting van die maatskappy XSIT. Verskeie ander benaderings vir die beheer van VKM in boorde is ook ondersoek soos die gebruik van larwale parasitoïede en entomopatogeniese nematodes. Verdere aandag is ook geskenk aan metodes om die beheer van residue van Cryptogran te verbeter, die soektog na lokmiddels vir wyfies sowel as die gasheerstatus van suurlemoene. Naas VKM-navorsing is die meeste befondsing toegeken aan vrugtevlieg-navorsing ook weens die fitosanitêre belangrikheid van die plaag. 'n Koue behandeling van 16 dae by 1.5°C was ongelukkig nie suksesvol genoeg vir 'n probit 9-vlak beheer van Medvlieg nie en die eksperiment sal herhaal moet word by 'n temperatuur van 1°C. Ander navorsers het gevind dat die ontwikkelingsdrempelwaardes van die Natalse vrugtevlieg wat in Suid Afrika voorkom verskillend is van die vlieë wat in Reunion voorkom wat moontlik 'n aanduiding mag wees dat dit verskillende rasse is. Navorsing op tegnieke om vrugtevlieg te beheer en om residue te voorkom deur die aanwending van lokmiddels op grondbedekkings eerder as die boom was nie effektief nie. Die beheer van kosmetiese plae het dikwels 'n nadelinge uitwerking op die natuurlike vyande van ander plae en dus gaan die soektog na meer IPB-verenigbare behandelings voort. Verdere belowende resultate is verkry in die beheer van bolwurm met die Helicovir-virus en 'n vergelyking tussen al die beskikbare formulasies van abamectin vir die beheer van sitrus blaaspootjie het getoon dat daar nie noemenswaardige verskille is tussen die effektiwiteit van die formulasies nie. Verdere terugslae is ondervind in die teling van 'n parasitoïed vir sitrus blaaspootjie en geskikte populasies van die sitrus grysmyt vir navorsing kon nie gevind word nie. Miere kan ook natuurlike vyande ontwrig en verdere navorsing is uitgevoer op metodes om miere te beheer. Ongelukkig was die beste afweermiddels net effektief teen die malmier vir 5 dae. Die nie-teiken effekte van chemikalieë wat algemeen gebruik word, is bepaal op larwes van die gaasvlerkie ter ondersteuning van die bewaring van

hierdie predator van witluise en ander sagte dop insekte. Meer parasitoïede van die oleander witluis is ontdek maar die voorkoms van die spesie het egter afgeneem en die sitrus witluis skyn om nou weer die mees algemene spesie te wees wat in Suid Afrikaanse sitrus boorde gevind word. 'n Metode om die graanstinkbesie, 'n ryloper plaag, te beheer met na-oes dompelings in natuurlike pyrethrum is geverifiëer. Geen geskikte IPB-verenigbare behandelings kon egter gevind word as alternatiewe vir endosulfan in die beheer van die sitrus bladvlooi nie. 'n Poging om massa uitvangste te gebruik as 'n metode van beheer vir die sitrus bladvlooi het egter die vlakke van besmetting op nuwe blare verhoog. 'n Aansienlike hoeveelheid tyd is spandeer aan die ontwikkeling van residuele afbrekingsdata van ou insekdoders wat nie meer deur die oorspronklike vervaardigers gedoen word nie, vir die bepaling van die weerhoudingsperiodes.

3.2 PROJECT: FALSE CODLING MOTH

Project Coordinator: Hendrik Hofmeyr (CRI)

3.2.1 Project summary

A wide and divergent range of subjects relating to false codling moth, was investigated during 2005-2206 by researchers from the CRI, University of Stellenbosch and the private sector. The purpose of the investigations was to find out more about FCM's habits, and to develop new methods to suppress the pest pre- and postharvest. FCM's preferences, life style and habitat received attention. An incisive look was given at better methods of artificial rearing and the insect's sexual urges were exploited as a means of suppression. Last, but not least, it has become more dangerous for the pest to infest fruit, as progress was made with treatments that could target and kill it in its food source.

The extensive survey of chemical attractant and repellent compounds were continued (3.2.2). The investigation has entered a new phase and certain promising products were evaluated in traps under more natural conditions than in the laboratory.

Research on the role of inherited sterility in FCM in a Sterile Insect Release programme has been conducted since 2002. This work was continued and culminated in a successful application when the pest was commercially suppressed during the whole season in 35 ha of citrus (3.2.3). Commercial development of the Sterile Insect Technique for FCM on an area wide scale is being fast-tracked and a mass rearing facility will be built in Citrusdal during 2007. Design of the facility has almost been completed and good progress was made with the design of new equipment to be used for mass rearing purposes. Attempts are continually made to improve the mass rearing of FCM. Research has shown that formaldehyde is still an excellent egg disinfectant and that the current treatment application can be modified for better results (3.2.4).

The contribution of larval parasitoids to suppress FCM biologically, was investigated (3.2.5). Surprisingly high parasitism of larvae by *Apophua* sp. was found in the Eastern Cape, which may be exploited for FCM control in future. Despite the progress, the parasitoids remain difficult to rear artificially and attempts to improve this aspect are continued.

A range of alternative host plants which can sustain FCM development, was identified in the Eastern Cape (3.2.6). These observations may help growers to improve their management of FCM control programmes.

Studies continued to improve the efficacy and persistence of the granulovirus, Cryptogran (3.2.7). Good progress was made and two products, lignin and Wetcit, were identified which respectively improved the product's resistance to UV breakdown and knockdown effect. A second viral product, Cryptex, gave noticeably poorer results against FCM in a field trial. Research on FCM control was not limited to the above-ground environment and the pest may even find it less safe underground in future. The role that nematodes can fulfil in FCM suppression was actively investigated and several isolates were discovered from which the potential for FCM suppression will be determined in biotests (3.2.8).

Many importers of southern African fruits are extremely concerned about the possibility that FCM can settle in their countries when infested fruit are imported. It is therefore important to study the distribution of FCM in the divergent climatical areas of southern Africa to enable predictions of the possibility that it may adapt and settle in suitable habitats in foreign countries. Surveys were therefore initiated to determine the geographical distribution of FCM in our country (3.2.9).

Lemons are very cold sensitive and it is currently impossible to export this cultivar to China as long as the obligatory cold treatment protocol for South African citrus is enforced. An investigation was launched to study the potential of FCM to infest lemons in orchards containing fruit which comply with export regulations. If this fruit is unacceptable to FCM it may be possible to waive the cold treatment protocol (3.2.10). Apart from lemons, most citrus cultivars exported are variably sensitive to cold treatment damage. It is therefore

necessary to either replace the current cold treatment protocol completely with an alternative safe treatment, or to modify the current treatment to reduce its hazards. It is potentially possible to shock treat larvae inside fruit with carbon dioxide and decrease their cold tolerance (3.2.11). This possibility was investigated and certain promising treatments were found which merit further research.

The Olifants river valley in the Western Cape was criss-crossed the past season in an intensive investigation into possible factors influencing the spatial and temporal distribution of FCM (3.2.12). The investigation also included a search for alternative host plants. The surveys show that moth activity, measured by trap catches of male moths, increased as the pest was monitored progressively nearer orchards from the fynbos veld. The same phenomenon was noticed in orchards, *viz.* greater activity nearer the edge of orchards adjoining fynbos veld. No alternative host plants were found in the fynbos veld to account for the increased activity.

With this report certain of the abovementioned research subjects are concluded. Each of those contributions added to a better understanding of the FCM problem and better management of its suppression. The research field remains large and the possibility that more progress will be made with the management of the FCM problem, increases satisfactorily. In this respect the continued support from the citrus industry for this project is much appreciated.

Research to develop a larval disinfestation treatment for export fruit based on gamma irradiation (exp. 719), was temporarily delayed. Previous research had demonstrated that the technique could produce good results. However, research to establish a dose range for the treatment of citrus fruit without detrimentally influencing fruit quality, had to be concluded before a decision could be made as to the continued viability of a gamma irradiation treatment (see Section on Fruit Production and Quality). A start was made to develop a research proposal for this technique that would be used to establish an export protocol.

Projekopsomming

'n Uiteenlopende reeks onderwerpe wat op valskodlingmot betrekking het, is gedurende 2005-2006 deur navorsers van CRI, die Universiteit van Stellenbosch, asook die privaatsektor, ondersoek. Dié ondersoeke het ten doel gehad om meer van die mot se lewensgewoontes uit te vind en nuwe maniere te ontwikkel waarmee dit voor- en na-oes onderdruk kan word. Die valskodlingmot se voorkeure, leefstyl en blyplekke het aandag geniet. Daar is indringend gekyk hoe dit makliker kunsmatig geteel kan word en die plaaginsek se seksdrange is gebruik om hom hok te slaan. Les bes, het dit vir die insek gevaarliker geword om vrugte te besmet, aangesien daar vordering gemaak is met behandelings wat hom na-oes in sy voedsel kan opspoor en doodmaak.

Die uitgebreide ondersoek na lok- en afdryfmiddels vir VKM is voortgesit (3.2.2). Die ondersoek het 'n volgende fase betree met die evaluasie van sekere belowende produkte in lokvalle onder natuurliker toestande as in die laboratorium.

Navorsing op die rol van oorgeërfde steriliteit in VKM in 'n Steriele-insek Loslaatprogram word sedert 2002 uitgevoer. Dié werk is voortgesit met 'n suksesvolle evaluasie waartydens die plaag seisoenlank kommersieel in 35 ha sitrus onderdruk is (3.2.3). Die Steriele-insek Tegniek vir VKM word vinnig ontplooi en daar sal in 2007 met die bou van 'n nuwe massateelfasiliteit in Citrusdal begin word. Dié gebou is ontwerp en daar word baie goeie vordering gemaak met die ontwerp van toerusting wat in die gebou vir die massateel van VKM gebruik moet word.

Daar word voortdurend gepoog om die kunsmatige VKM-teelproses te verbeter. Navorsing het gewys dat formalien nog steeds 'n uitstekende eierdisinfeksieproduk is en dat die huidige gebruikspatroon van die produk aangepas kan word om beter resultate te lewer (3.2.4).

Die biologiese beheer van VKM met behulp van larweparasitoïede is ondersoek (3.2.5). Daar is verrassend-hoë parasitisme van larwes deur *Apophua* in die Oos-Kaap opgemerk, wat moontlik in die toekoms vir VKM-bestryding uitgebuit kan word. Alhoewel daar vordering gemaak word, blyk die parasitoïede moeilik om kunsmatig te teel en pogings om dit te vermag word voortgesit.

'n Reeks alternatiewe gasheerplante vir VKM is in die Oos-Kaap geïdentifiseer waarin VKM-larwes kan ontwikkel (3.2.6). Dié waarnemings kan produsente help om hul VKM-bestryding in sommige gevalle beter te bestuur.

Studies is voortgesit om die doeltreffendheid en nablywende werking van Cryptogran te verbeter (3.2.7). Daar is goeie vordering gemaak en twee produkte, lignien en Wetcit, is gevind wat onderskeidelik die

produk se UV-weerstand en uitklopvermoë verbeter het. 'n Tweede virusproduk, Cryptex, het sigbaar swakker teen VKM in 'n boordproef gevaar.

Daar word nie slegs na bogrondse metodes gekyk om VKM hok te slaan nie. Die plaag kan binnekort ook selfs ondergronds nie meer veilig voel nie. Die rol wat nematodes in VKM-bestryding kan speel word aktief ondersoek en verskeie isolate is gevind waarvan die potensiaal vir VKM-onderdrukking verder in biotoetse ondersoek gaan word (3.2.8).

Baie invoerders van suider-Afrikaanse sitrus is bekommerd oor die moontlikheid dat VKM in hul lande kan vestig wanneer besmette vrugte ingevoer word. Dit is derhalwe belangrik om die verspreiding van VKM in die uiteenlopende klimaatstreke van suidelike Afrika vas te stel sodat dit kans op vestiging in soortgelyke streke in die buiteland bereken kan word. Opnames is van stapel gestuur om die geografiese verspreiding van VKM in ons land vas te stel (3.2.9).

Suurlemoene is baie kouegevoelig en dit is tans onmoontlik om dié kultivar na China uit te voer, as gevolg van die verpligte kouebehandeling wat op alle sitrusvrugte toegepas moet. 'n Ondersoek word daarom uitgevoer om te bepaal of onge-oeste suurlemoene wat vir uitvoer geskik is, moontlik so swak, of glad nie, deur VKM besmet word, dat die kultivar nie kouebehandel te word nie (3.2.10).

Nie slegs suurlemoene nie, maar die meeste sitruskultivars wat uitgevoer word en 'n kouebehandeling moet ondergaan, is wisselend vatbaar vir kouebeskadiging. Dit is dus nodig om om die voorgeskrewe kouebehandeling óf heeltemal met 'n veilige behandeling te vervang, óf die huidige behandeling só aan te pas dat dit veiliger is. Dit is potensieel moontlik om vrugte met koolsuurgas te behandel en enige lewendige larwes daarin 'n skok te gee wat dit vatbaarder vir 'n gematigder kouebehandeling maak (3.2.11). Dié moontlikheid word nagevors en sekere behandelings is gevind wat genoeg belofte toon om verdere ondersoek te regverdig.

Die Olifantsriviervallei in die Wes-Kaap is die afgelope deurkruis in 'n intensiewe ondersoek na moontlike faktore wat die verspreiding, beide gebieds- en seisoensgewys, van die plaag kan beïnvloed (3.2.12). Daar is terselfdertyd na potensiële gasheerplante gesoek. Die opnames toon dat motaktiwiteit, gemeet aan mannetjievangste in lokvalle, in die fynbosveld toeneem hoe nader daar na die boorde gemonitor word. Dieselfde verskynsel geld vir mannetjies in die boorde – daar is groter vangste nader aan die rande van boorde aangrensend aan die fynbosveld. Dié groter aktiwiteit van mannetjies in die veld vind plaas in die afwesigheid van enige noemenswaardige gasheerplante.

Sommige van bogenoemde navorsingsondersoeke word met dié verslag afgesluit. Elkeen van hulle het 'n bydra gemaak om VKM beter te leer verstaan en beter bestuur van die probleem bevorder. Die ondersoekveld is egter nog groot en die moontlikheid dat bevredigende vordering met die bekamping van die VKM-probleem gemaak gaan word, raak al hoe beter. In dié opsig word die volgehoue ondersteuning vir die projek wat van bedryfskant ondervind word, hoog op prys gestel.

Geen navorsing op die ontwikkeling van 'n larwedisinfestasiebehandeling met gammabestraling vir uitvoervrugte (proef 719), is uitgevoer nie. Vorige navorsing het gewys dat so 'n metode goeie resultate kan lewer. Navorsing om die dosisreeks wat vir die behandeling van sitrusvrugte geskik is sonder om kwaliteit in te boet, moes egter eers afgehandel word voordat 'n besluit oor die lewensvatbaarheid van die prosedure geneem kon word (raadpleeg Afdeling oor Vrugproduksie en Kwaliteit). Daar is begin om 'n navorsingsvoorstel te ontwikkel wat tot 'n behandelingsprotokol vir die VSA-uitvoermark kan lei.

3.2.2 **Development of semiochemical odorants for the attraction and repellence of false codling moth in citrus**

Experiment 648 by Christo Smit (Desense Pest Control, Citrusdal)

Opsomming

Gedurende die eerste deel van die seisoen is 'n verdere reeks reukstowwe in olfaktometers geëvalueer vir VKM- aantrekking. Die merendeel van die proefwerk is egter daarna in opelugproewe uitgevoer deur van geel delta-lokvalle in sitrusboorde te gebruik met kunsmatig-aangevulde VKM-bevolkings. Die soektog na reukstowwe in boordproewe wat die aantrekkingskrag van die VKM-geslagsferomoon beïnvloed, het drie antranilate gelewer wat die aantrekkingskrag van die geslagsferomoon sterker bevorder as etielantranilaat wat vroeër geïdentifiseer is, nl. kantoksalmetielantranilaat, sitronellielantranilaat en fenieletielantranilaat. Ander antranilate soos die dekiel-, butiel- en sikloheksielantranilaatesters het die aantrekkingskrag van die seksferomoon amper of heeltemal uitgeskakel.

Wat die nie-geslagsferomoonreukstowwe betref, is molasse tentatief as potensieel waardevolle basiese gemeenskaplike bestanddeel geïdentifiseer, tot welke produk VKM-sinergistiese aantrekkende reukstowwe bygevoeg kan word.

Kardamomolie en sinnamielisobutiraat kombineer goed met molasse om die vangste te verbeter. Die hemiterpene, prenielasetaat, prenol en tigliensuur kombineer weer goed met eersgenoemde twee reukstowwe. Die maksimum aantrekkingskrag wat al bereik is met nie-geslagsferomoonreukstowwe, is nog baie swakker as geslagsferomoon opsigself, maar teen die tempo waarmee nuwe proefdata bykom, is die verwagting van sterker nie-geslagsferomoon aantrekmiddels heeltemal realisties.

Introduction

The major objective of this season's experiments was to identify odorant attractants for FCM, initially in olfactometers and subsequently in delta traps in orchard conditions.

Material and methods

- **Olfactometer experiments**: Olfactometers (consisting of calibrated glass tubes) and the experimental method used to determine relative migration indexes were similar to those reported in the CRI Annual Report for 2005. Approximately 100 FCM were loaded per exposure per olfactometer glass tube.

- **Open air delta traps**: Yellow plastic delta traps together with their sticky pads as supplied by Chempack, Paarl, were used. The odorant dispensers consisted of 20 mm square six-ply pieces of toilet paper stapled together onto each of which 0,5 ml of a particular odorant was absorbed. A more volatile odorant would be rolled into a 30 mm wide strip of heavy duty aluminium foil, the open ends being pressed together to allow a small slit for the vapour to escape through.

- **Layout of field experiments**: A block of 30 year old Olinda Valencias (tree spacing 7 m x 5 m) were used for the trapping experiments. Delta traps were suspended inside the canopy of every fourth tree of every second row. Odorant dispensers were placed on the sticky pads inside the traps. A closed paper bag (230 mm x 120 mm x 70 mm) containing approximately 200 FCM, was attached to the underside of each delta trap with staples. When all the prepared traps were suspended in the trees, the paper bags were slit 90 mm from the bottom to enable the moths to leave the bags voluntarily.

- **Filling the paper bags with test moths**: From the time the test FCM are collected at Ceder Biocontrol insectary, they are kept at less than 16°C in a refrigerator, cool bag or in the temperature controlled test laboratory. In the cooled laboratory, they are measured out at one tablespoon per paper bag (about 200) which is then stapled shut; later to be fixed to the bottom of the traps. All orchard tests are initiated and run in the early evening after sunset.

- **Time span of artificially created FCM population densities**: It became clear that with this test procedure, nearly all FCM trap catches always happened during the first night of a test run. If the trap catches are to be increased, a second batch/application of FCM in a paper bag to the same traps 2 days later on, can achieve this, as can be seen in the table on FCM sex pheromone (FCM-SP)/odorant interactions.

- **Sources of odorants**: Test odorants are obtained from the companies Aldrich (USA), Fluka (Switzerland), Bedoukian (USA) and R C Treatt (Britain). There was a long delay until October in the execution of the order for the Sigma-Aldrich chemicals.

- **Exploratory investigations**: In the case of the non-sex pheromone candidate attractants, initially only one trap per odorant treatment was used because of the wide range of odorants and their combinations which are to be covered. Those treatments which caught FCM will be replicated later on.

- **Looking for the best combinations of odorants**: The female FCM sex pheromone is a fairly complex mixture – as is the male sex pheromone or aphrodisiac of the closely related Oriental fruit moth. Similarly, the odours emanating from citrus or cotton as FCM host plants are also a complex mixture. Judging from the above examples on sex pheromones as natural FCM attractants, it is expected that the best results on FCM attraction will be obtained with the assembling and usage of odorant mixtures with the right components of positively interactive odorants rather than single odorants. Potential useful partners for these mixtures will have to be identified beforehand experimentally.

- **Selecting test odorants**: The procedure for selecting candidate test odorants was to build upon previous experience. For example, in the 2002/3 project annual report ethyl anthranilate was identified as probably the strongest of a series of candidate FCM-SP synergists tested. To follow up this cue, as first choice, all available anthranilates and other amino-benzenes have already been or are planned to be included in experiments on FCM-SP attractive interactions. Subsequently these experiments will be extended to all other chemical groups. It will also include all of those non-sex pheromone substances which in combination with FCM-SP increased trap catches in field experiments as was reported in the 2002-2003 annual report.

The non-sex pheromone substance caryophyllene, which in the same annual report was reported to catch some FCM in field tests, was chosen as a common component to be combined with representatives of the various other chemical groupings. Future open air tests with artificial FCM test populations will be extended to cover all non-sex pheromone odorants which was reported to catch FCM in orchards using natural populations as was indicated in the 2002-2003 report.

The ingredients of the aphrodisiac mixture of the FCM (which causes male induced female attraction) is still unknown, but is expected to be chemically related to that of the OFM because of their biological relatedness as Tortricids. The ingredients of the OFM aphrodisiac is ethyl cinnamate (from the benzyl propenyl group), mellein or 2-hydroxy chromone (from the O-heterocyclic group), methyl jasmonate and methyl epi-jasmonate (jasmone group). The possible role of cinnamyl isobutyrate (CI) as component of such an aphrodisiac mix, equivalent to ethyl cinnamate in OFM, was implied by the strong stimulation of sexual activity of both male and female FCM by CI in the olfactometer glass tubes in last season's experiments. Various alternatives /chemical variants of the components of the OFM aphrodisiac mixture will be investigated for FCM attraction. Also, it will be investigated to what degree male FCM in small mesh cages, loaded inside delta traps, are able to attract female FCM by means of their aphrodisiac emissions.

Results

1 **Olfactometer experiments**

The main purpose of the olfactometer screening of odorants is for selecting candidate test attractants for testing in open air experiments in the orchard. The results presented in Table 3.2.2.1 are mean relative dispersion indexes of two replicates, expressed as percentages of the dispersion indexes of the comparable blank olfactometer tubes of the same battery.

Table 3.2.2.1. Olfactometer attractiveness of various odorants towards FCM

Test Date 2006	Odorant	Mean Relative dispersion index (Blank control =100%)
	Plant oils and –extracts	
19/12	Lemon ginger sesquiterpenes	120,0
	Cardammon oil	112,5
	Parsley seed oil	108,9
	Ginger grass oil	99,4
	Clove oil	98,2
	Celery seed oil	95,0
	Peppermint oil	80,6
04/12	**Sesquiterpenes**	
	Alpha-Bisabolol	110,0
07/12		
	Alpha-Bisabolol	113,1
	Farnesyl acetate	117,8
04/12	**Diterpenes**	Means
	Geranyl-linalool	111,3
07/12	Geranyl-linalool	115,8
04/12	**Triterpenes**	Means
	Squalene	112,5
04/12	**Unsaturated olefins**	Means
	Phytol (3,7,11,15-Tetramethyl 2-hexadecenol)	116,6
07/12		
	Phytol	131,2

	Oxygen containing ring structures	
04/12	Maltol (3-Hydroxy 2-methyl 4-pyrone)	91,0
	6-Amyl pyrone	90,6
	Benzyl alkyl ketones	
09/11	4-Methoxyphenyl 2-butanone	120,5
	4-Hydroxyphenyl 2-butanone	105,6
	4-Acetoxyphenyl 2-butanone	105,9
	3,4-Dimethoxy phenyl acetone	105,5
	4-Hydroxy 3-methoxy phenyl acetone	96,7
	1-Methoxyphenyl 2-propanone	98,8
07/12	4-Methoxy acetophenone	128,6
	4-Methoxyphenyl 2-butanone	120,0
28/11	4-Methoxy acetophenone	117,2
	Aceto-vanillone	103,0
	Vanillyl acetone	102,2
	Vanillylidene acetone	89,9
	4-Hydroxy 3-methoxy phenyl acetone	98,9
	2-Hydroxy 4-methoxy phenyl acetone	95,5
	3,4-Dimethoxy acetophenone	89,9
	Cinnamyl compounds	
13/12	Cinnamyl isobutyrate	123,4
	4-Methoxy 3-hydroxy cinnamic aldehyde	115,6
	3,5-Dimethoxy 4-hydroxy cinnamic aldehyde	102,1
	2-Methoxy cinnamic aldehyde	101,1
	alpha-Amyl cinnamic aldehyde	95,9
	Hydro-cinnamic aldehyde	80,2

The more successful compounds from the above table will be used as candidate test odorants in open air delta trap experiments and are the following:

- Plant oils and extracts

Lemon ginger sesquiterpenes and cardamom oil

- Sesquiterpenes

Farnesyl acetate and alpha-bisabolol
(Farnesyl acetone and bisabolene from the previous season's evaluations seem to be better choices)

- Diterpenes

Geranyl-linalool

- Triterpenes

Squalene

- Unsaturated olefins

Phytol

- Benzyl alkyl ketones

4-Methoxyphenyl acetophenone 4-methoxy phenyl 2-butanone

- Propenyl benzenes

Cinnamyl isobutyrate and 4-hydroxy 3-methoxy cinnamic aldehyde

Switch to orchard experiments

After discussions with the project coordinator, it was decided to temporarily stop with olfactometer experiments and start with open air attraction experiments with delta traps in citrus orchards.

2 Open air experiments with FCM odorant attraction in delta traps.

2.1 Working with FCM sex pheromone

The usefulness of the experimental set-up with delta traps as described in "Materials and Methods" was evaluated in these experiments.

2.1.1 Experiment 1: Effect of FCM-SP concentrations on trap catches

The results below (Table 3.2.2.2) were regarded as evidence that the experimental setup is useful to compare degrees of attraction of FCM-SP to other odorants with similar attractive capabilities over distance as FCM-SP (no non-sex pheromone odorants tested thus far have been successful).

Table 3.2.2.2. FCM attraction as affected by FCM-SP concentration (in Citrex oil)

FCM-SP concentration	FCM trap catches (Mean of 2 replicates)
2,0%	28,5
1,0%	18,5
0,5%	11,5
0,2%	8,5

Biggest trap catches for all odorants, including FCM-SP, were realised during the first night after test application and to a much lesser extent the second night; thereafter mostly none, presumably as the result of FCM dispersion from the release sites. Therefore, results of all non-sex pheromone experiments below are presented as trap catches collected during the first two nights after moth release, except for the section on FCM-SP interactions described where the experiment ran for 5 days.

FCM sex pheromone interactions

The following are combined results of three experiments on FCM-SP interactions; FCM trap catches over 5 days.

2.1.2 Experiments 2, 3 and 4: FCM sex pheromone interactions with other odorants

Both synergistic and antagonistic interactions of FCM-SP with various odorants exposed in the traps were observed previously. This experiment (Table 3.2.2.3) was directed to a large extent at anthranilate compounds as this compound was found to be one of the strongest synergists for FCM-SP in previous orchard experiments with natural FCM populations (2002-2003 annual report).

Table 3.2.2.3. Interaction of FCM sex pheromone with several odorants

Odorant	Exp. 2 (1 rep)	Exp. 3* (2 reps., 2 FCM releases)	Exp. 4 (2 reps)	Mean catch /applic.
FCM-SP 0.2% alone	9	18,0	8,0	8,8
FCM-SP (0.2%) + anthranilates				
SP + canthoxal methyl anthranilate	15	30,5	11,5	14,3
SP + citronellyl anthranilate	13	28,5	10,5	13,0
SP + phenylethyl anthranilate	13	32,0	9,5	13,6
SP + ethyl anthranilate	15	23,0	10,0	12,0
SP + anisaldehyde methyl anthranilate	15	15,0	14	11,0
SP + dimethyl anthranilate	15	18,0	9,3	10,6
SP + geranyl anthranilate	13	15,7	9,0	9,4
SP + triplal methyl anthranilate	8	-	-	8,0

Odorant	Exp. 2 (1 rep)	Exp. 3* (2 reps., 2 FCM releases)	Exp. 4 (2 reps)	Mean catch /applic.
SP + decyl anthranilate	1	-	-	1,0
SP + butyl anthranilate	0	-	-	0,0
SP + cyclohexyl anthranilate	0	-	-	0,0
SP + other				
SP + cinnamyl isobutyrate	10	21,0	11,5	10,5
SP + isobutyl cinnamate	-	-	2,0	2,0
SP + neryl acetate	15	21,0	11,0	11,8
SP + prenol	-	-	5,0	5,0
SP + isoprene	-	-	7,5	7,0
SP + dodecyl methacrylate	-	-	7,0	7,0
SP + bisabolene	-	-	4,5	4,5
SP + orange oil	-	-	2,5	2,5

***Note**: In experiment 3, FCM was released a second time two days after the first. This explains the much higher trap catches (about double) in this experiment relative to the two other experiments. FCM was released once only in experiments 2 and 4.

In these experiments with artificially created FCM populations, the FCM-SP + ethyl anthranilate combination attracted 12,0 FCM to 8,8 with FCM-SP only.

Anthranilates which further enhanced FCM attraction relative to ethyl anthranilate were canthoxal methyl anthranilate (14,3), citronellyl anthranilate (13,0) and phenylethyl anthranilate (13,6).

On the other side of the spectrum, decyl anthranilate (1,0), butyl anthranilate (0,0) and cyclohexyl anthranilate (0,0) suppressed FCM-SP attraction totally (or almost so).

Other groups of odorants

In experiment 3 comparison between FCM-SP combinations with cinnamyl isobutyrate (CI) and isobutyl cinnamate (IC) showed a slight increase in the case of FCM-SP + CI (10,5) to the FCM-SP only (8,8), in contrast to the strong suppression of FCM attraction with the FCM-SP + IC combination (2,0). This indicates a preference of FCM for CI rather than IC in combinations with FCM-SP.

Neryl acetate, like CI, combines well with FCM-SP with trap catches of 11,8. Neryl acetate and cinnamyl isobutyrate on their own, also show good FCM attraction in the olfactometer. However in the orchard experiments both were only very weakly attractive to FCM on their own relative to FCM-SP.

FCM-SP combinations with prenol, isoprene, dodecyl methacrylate bisabolene and orange oil all suppressed FCM trap catches when compared to FCM-SP only.

2.2 Screening for non-sex pheromone FCM attractants

In the screening of non-sex pheromone odorants for FCM attraction, only one replicate was used. The intention is to replicate the evaluation of those odorants with positive attraction in follow-up experiments.

2.2.1 Experiment 5: FCM trap catches with molasses (treacle) and several combinations

As standards for comparison FCM-SP (2% solution in Citrex oil) attracted 15 FCM and FCM-SP (0,2%) attracted 8 FCM in two replicates.

- **Some attraction**: Molasses + cardamom oil (5), molasses + cinnamyl isobutyrate (3), molasses + orange oil + Yield-Plus (2), molasses alone (2), molasses + neryl acetate (1) and molasses + Yield-Plus (1).

- **No attraction**: Molasses combinations with orange oil, prenol or citronellyl anthranilate. Also molasses + orange oil combinations with squalene, phytol, dimethyl anthranilate, bisabolene, N-4-methoxyphenyl acetamide or acetyl choline chloride.

Comment

Molasses seems to have potential as a basic component for FCM-SP attractant mixtures as it creates the possibility of poisoned baits simultaneously aimed at FCM and fruit flies (molasses independently attracts Medflies).

2.2.2 **Hemiterpenes(=methyl butenyl substances) and their combinations**

Further experiments concentrated mostly on hemiterpenes because of their consistent, but weak, attraction in initial field attraction experiments.

As standards for comparison FCM-SP (0,2%) caught respectively 5,5 and 8,5 FCM in two replicates for the duration of the non-sex pheromone experiments described below.

2.2.2.1 **Experiment 6: Hemiterpenes** (Screening - one replicate only)

- **Prenol (=3-methyl 2-buten 1-ol) and combinations**

Prenol + cinnamyl isobutyrate (3), prenol + cardamom oil (3), prenol + caryophyllene (2), prenol + lemon ginger sesquiterpenes (2), prenol + Litsea cubeba oil (2), prenol + ginger grass oil (2), prenol + valencene (1), prenol only (0).

- **Other hemiterpene combinations**

3-methyl 2-buten 1-ol + caryophyllene (2), 3- Methyl 3-buten1-ol + caryophyllene (1), 2-methyl 3-buten 1-ol + caryophyllene (1), 2-methyl 2-buten 1-al + caryophyllene (1).

The following hemiterpene combinations attracted no FCM

- **Caryophyllene combinations with:**

3-Methyl 3-buten 1-ol, 2-methyl 3-buten 2-ol, 3-methyl 1-butanol ethyl 3-aminobenzoate and butyl 4-aminobenzoate.

- **Prenol only and in combination with any of the following:-**

Verbenone, beta-pinene, allo-ocimene, sinensal, bisabolene, elemol, nerolidol, bisabolol, cedrene, farnesyl acetate, aurantiol, dimyrcetol, geranyl-linalool, neroli oil, orange oil, chamomile oil, cedar wood oil and cananga oil.

2.2.2.2 **Experiment 7: Hemiterpene combinations** (continued)

Prenyl acetate (=3-methyl 2 butenyl acetate) + cardamom oil (3), prenyl acetate + cinnamyl isobutyrate (2), prenol + cinnamyl isobutyrate (2), 2-Methyl 2-butenoic acid (= tiglic acid) + cinnamyl isobutyrate (2), tiglic acid + cardamom oil (2) and 3-methyl 1-butanol + cardamom oil (1).

The following combinations attracted no FCM

- **Cardamom oil in combination with any of the following**:

3-Methyl 2-butenal, 2-Methyl 3-buten 2-ol, 2-methyl 2-butenal (=tiglic aldehyde), 3-methyl 3-buten 1-ol, 2-methyl butyric acid, cinnamyl isobutyrate, ginger grass oil, lemon ginger sesquiterpenes, retinyl acetate, Yield Plus, E2,E4-octadienal and iso-leucine.

2.2.2.3 **Experiment 8: Hemiterpene combinations** (continued)

- **Tiglic acid (TA) = 2-Methyl 2-butenoic acid combinations.**

TA + neryl acetate (3), TA+ 3-formyl 6-methyl chromone (3), TA + isobutyl cinnamate (2), TA + 2-methoxy naphtalene (2), TA + cardamom oil (2), TA + ethyl anthranilate (1), TA + methyl jasmonate (1), TA + indalone (1) and TA + L-cysteine (1).

The following combinations attracted no FCM

- Tiglic acid alone or in combination with any of the following: Cinnamyl isobutyrate, lemon ginger sesquiterpenes, prenyl acetate, allo-ocimene, cedrene, squalene, dimethyl anthranilate, Vitamin E, dodecyl methacrylate, indole, E2,E6-nonadienal, E2-hexenol, retinyl palmitate, Yield Plus and N-4-methoxyphenyl acetamide.

2.2.2.4 **Experiment 9: Hemiterpene combinations** (continued)

- Prenyl acetate (PA) only (2), PA+ prenol (2), PA + TA (2), PA + isobutyl cinnamate (1), PA + cardamom oil (2), PA + isobutyl cinnamate + methyl jasmonate + 3-formyl 6-methyl chromone (2), PA + dimethyl anthranilate (1), PA + 3-formyl 6-methyl chromone – 1, PA + dodecyl acrylate (1) and PA + dodecyl methacrylate (1).

- TA + cardamom oil (2), TA + isobutyl cinnamate (1), TA + isobutyl cinnamate + methyl jasmonate + 3-formyl 6-methyl chromone (1).

The following combinations attracted no FCM

- Tiglic acid (TA) alone and in combination with neryl acetate or 3-formyl 6-methyl chromone.

- Prenyl acetate (PA) in combination with lemon ginger sesquiterpenes or methyl jasmonate.

2.2.3 **Other odorants and their combinations**

2.2.3.1 **Experiment 10: Neryl acetate and combinations**
For reference, FCM-SP (0.2%) caught 5 FCM (2 reps). Neryl acetate (NA) only (2), NA + triplal methyl anthranilate (2), NA + E2,E6-nona-dienal (2), Neryl acetate + isobutyl cinnamate (2), NA + molasses (1), NA+ phytol (1).

The following NA combinations caught no FCM
- Neryl acetate (NA) in combination with any of the following: Cis-Jasmone, lactone of cis-jasmone, jasmin lactone, methyl jasmonate, dimethyl anthranilate, menthyl anthranilate, 4,5-dimethoxy anthranilate, 4-methoxy benzamide, N4-methoxy phenyl acetamide, 3-methyl 2-butenal, 2-methyl 2-butenal, dodecyl acrylate, dodecyl acetate, indalone, squalene and vitamin E. Also singly isobutyl cinnamate.

2.2.3.2 **Experiment 11: Cinnamyl isobutyrate combinations**

Some trap catches

- Cinnamyl isobutyrate alone (1), cinnamyl isobutyrate + ethyl anthranilate (2), cinnamyl isobutyrate + prenyl acetate (2), cinnamyl isobutyrate + prenol (2), cinnamyl isobutyrate + 2-Methyl 2-butenoic acid (= tiglic acid) (2), Cinnamyl isobutyrate + Yield-Plus (1).

- **Yield-Plus** (= acetyl thiazolidine carboxylic acid, a plant growth stimulant) **plus combinations**
Yield Plus + lemon ginger sesquiterpenes (2), Yield-Plus + cinnamyl isobutyrate (1), Yield-Plus + neryl acetate (1), Yield-Plus + molasses (1), Yield-Plus + caryophyllene (1).

- **Ethyl anthranilate combinations**
Ethyl anthranilate only (2), ethyl anthranilate + cinnamyl isobutyrate (2), ethyl anthranilate + caryophyllene (1), ethyl anthranilate + tiglic acid (1).

- **Water**
Water bottles with wet wick and sticky pads on outside – 1 to 2.

- **Male FCM in small mesh cages, loaded inside delta traps**

The aim of this experiment was to test for male aphrodisiac emissions attracting females. Six FCM were caught, but of mixed sexes and not females only, as expected. The experiment will be repeated.

The following combinations caught no FCM

- **Cinnamyl isobutyrate in combination with any of the following:-**
Caryophyllene, dodecyl acetate, decanol, molasses + Yield-Plus, nerolidol + 3-formyl 6-methyl chromone, methyl jasmonate + 3-formyl 6-methyl chromone, Yield-Plus + dimethyl anthranilate, tiglic aldehyde, 2-methyl butyric acid, 3-methyl 1-butanol, 3-methyl 3-buten 1-ol, 2-methyl 3-buten 2-ol, 3-methyl 2-butenal, lemon ginger sesquiterpenes, ginger grass oil, iso-leucine, E2,E4-octadienal, Yield Plus, neryl acetate and retinyl acetate.

Discussion

In the initial olfactometer experiments some additional candidate test odorants for use in open air orchard experiments with delta traps had been identified in the previous season, including lemon ginger sesquiterpenes, farnesyl acetate, geranyl-linalool, phytol, 4-methoxyphenyl acetophenone, 4-methoxy phenyl 2-butanone and 4-hydroxy 3-methoxy cinnamic aldehyde.

In the subsequent orchard experiments with delta traps, the usefulness of the experimental setup of paper bags with FCM attached to the bottom of the traps, had been established in experiments with FCM-SP as attractant; firstly in a dilution series of FCM-SP from 2% to 0,2%, and secondly in a series of FCM-SP interaction experiments with other odorants. From previous season's results, the 2% FCM-SP oily solution absorbed on the odorant dispenser initially attracted approximately the same number of FCM as the Lorelei pheromone dispenser. For experimental purposes, i.e. to measure enhancing or suppressing effects of odorant additives on FCM-SP attraction on trap catches, the 0,2% dilution was selected. This weak dilution of FCM-SP was selected in order not to mask the weaker attraction of the non-sex pheromone additives.

Regarding the experiment on FCM-SP/odorant interactions which focussed on anthranilates, it seems as if canthoxall methyl anthranilate, citronellyl anthranilate and phenyl-ethyl anthranilate have a still better synergistic effect on FCM attraction with FCM-SP than ethyl anthranilate; which was reported on in the CRI annual report for 2002-2003. Cinnamyl isobutyrate and neryl acetate both combined well with FCM-SP. However, other anthranilates, such as the decyl-, butyl- and cyclohexyl esters almost or totally eliminate FCM-SP attraction.

The best non-sex pheromone attractants independently compare very weakly to FCM-SP. The present aim is therefore to put together the right choice of synergistic odorant partners in an attractant mixture. Molasses had been tentatively identified as a potentially useful basic ingredient for the assembly of FCM attractant mixtures. Cardamom oil and cinnamyl isobutyrate combines well with molasses and hemiterpenes such as prenyl acetate, prenol and tiglic acid. Several other potentially useful combinations, e.g. with neryl acetate and ethyl anthranilate, were also identified. The process of trying to assemble strongly attractant FCM attractant mixtures for both FCM sexes, is ongoing.

The test procedure to artificially create reliable and uniform FCM populations at the site of the field experiments seems to overcome the previous seasons' problems with very unreliable and variable natural FCM populations.

Although it was possible to attract some FCM by using male FCM confined in small mesh cages inside delta traps, the attempt to assemble an artificial equivalent of an OFM aphrodisiac related odorant combination to attract female FCM was unsuccessful until now. However, the investigation will continue.

Conclusion

The search for sex pheromone interacting odorants yielded several anthranilates which improved the attractiveness of FCM-SP in orchard experiments beyond that of the previously identified ethyl anthranilate, i.e. canthoxall methyl anthranilate, citronellyl anthranilate and phenyl-ethyl anthranilate. Other anthranilates such as the decyl-, butyl- and cyclohexyl anthranilate esters almost or totally eliminated FCM-SP attraction.

Concerning non-sex pheromone odorants, molasses had been tentatively identified as potentially useful basic ingredient for the assembly of FCM attractant mixtures. Cardamom oil and cinnamyl isobutyrate combined well with molasses and also with hemiterpenes such as prenyl acetate, prenol and tiglic acid.

Future research

The search for odorants interacting with FCM-SP will continue, as will the screening of non-sex pheromone odorants with the purpose of identifying the best components to be included in synergistically interacting FCM attractive mixtures.

3.2.3 **Bestryding van valskodlingmot deur middel van Steriele Insekloslatings**

Proef 662 deur Hendrik en Marsheille Hofmeyr (CRI)

Summary

A pilot experiment to investigate the potential for the Sterile Insect Technique (SIT) to suppress false codling moth (FCM) in citrus orchards, was conducted on a semi-commercial scale in Citrusdal, West Cape. One

thousand FCM irradiated with 150 Gy gamma irradiation at ARC Infruitec, Stellenbosch, were released twice a week for 56 weeks in 35 ha of orange trees. The releases were made by hand from a quad bike at 40 m intervals and were monitored with delta traps and Lorelei FCM pheromone. It was established that the released moths distributed very well and fully overlapped between release rows. The desired overflooding ratio of 10 release males to one wild male was exceeded by margins of up to 180:1 during the major part of the release period. Fruit drop surveys were conducted once a week in release and control orchards. Fruit drop due to FCM was reduced by 94,4% in the release area compared to the control orchard.

A new insectary aimed at the commercial control of FCM by mass rearing and Sterile Insect Releases (SIR) is planned. Techniques utilized in existing insectaries are inadequate for the mass rearing of FCM and this endeavor therefore necessitates the complete redesign of established procedures and equipment. To this end research was conducted to test new rearing jars and closure systems, egg laying containers, pupation substrate, diet sterilization technique and moth release equipment. Progress was made and the new equipment will be fine-tuned and tested in a small pilot plant during 2007.

Inleiding

In die CRI-verslag vir 2004-2005 is verslag gelewer van navorsing wat uitgevoer is om die Steriele-insek Tegniek (SIT) vir VKM verder te ondersoek. In hokproewe is vasgestel dat die doeltreffendheid van Steriele-insek Loslatings (SIL) vergroot kan word deur eierparasitoïede, *Trichogrammatoidea cryptophlebiae*, saam met gesteriliseerde motte los te laat. Om die toepassing van SIT op semi-kommersiële skaal in ’n loodsprojek moontlik te maak, was gespesialiseerde toerusting nodig om VKM te teel, versamel, verkoel, vervoer, gammabestraal en los te laat. Heelwat tyd is in dié ontwikkelingsfase spandeer om toerusting te vervaardig en te toets. Die loodsprojek is aan die einde van bogenoemde verslagtydperk ingelei.

Die loodsprojek word volledig In die huidige verslag bespreek. Navorsing om toerusting te ontwerp wat in ’n toekomstige insektarium vir die teel van genoeg VKM vir die kommersiële bestryding van VKM in 6 000 h sitrus gebruik kan word, is ook ingelei. Daar is terselfdertyd met die alomvattende beplanning van ’n nuwe insektarium begin – beide in terme van toerusting, gebou-ontwerp en arbeidsbeplanning om werkers só aan te wend dat massateling glad kan verloop.

Materiale en metodes

A **Ontwikkeling van toerusting vir semi-kommersiële loodsproef en nuwe SIT-insektarium**

Die huidige teelprosedure is vir meer as 4 dekades met min kritiese veranderinge in bestaande VKM-insektaria toegepas. Die toerusting wat gebruik was, was ook geskik vir die omvang van die teling wat uitgevoer moes word. Kritiese dele van die prosedure, veral dié aspekte wat met insekhantering te doen gehad het, was egter nie vir die loodsproef geskik nie en moes herontwerp word (CRI-jaarverslae vir 2004 en 2005). Die loodsproef was egter slegs ’n oorgangsfase tussen die bestaande insektaria en ’n nuwe insektarium wat geskik moet wees vir massateling op ’n skaal wat vir SIL vereis word. Die vereistes vir VKM-teling op loodsproef- en massateelvlak verskil egter ook heeltemal en toerusting wat vir eersgenoemde ontwerp en gebruik was, is nie vir laasgenoemde geskik nie. Daar is derhalwe gedurende die verslagtydperk na afloop van die loodsproef begin om toerusting vir die nuwe SIT-insektarium te ontwerp en te toets.

A1 **Motversamelapparaat**

Die ontwerp van twee perspekstrôe wat in die semi-kommersiële loodsproef (Afd. A2) gebruik moes word om motte op relatiewe groot skaal vir die loodsproef te versamel, is in die CRI Jaarverslag vir 2005 bespreek. Die trôe het uitstekend gewerk en ’n groot aantal motte kon in ’n kort rukkie bymekaar gemaak word. Daar is egter binne enkele weke na aanvangs van die loodsproef waargeneem dat baie motte tussen die kartonproprolle (wat die kokonne bevat het) onderaan die deurskynende perspeksdeksels bly sit het nadat hulle ontpop het. Dit was ongewéns aangesien dié motte nie vinnig genoeg in die versamelbakkies beland het nie. In die bestaande Ceder Biocontrol insektarium word motte wat in ontpoppingkiste in die donker ontpop, deur ’n ronde gat van 100 mm deursnee na kamerlig aangelok en word dan in 2 ℓ plastiekbottels versamel. Die perspeksdeksels van die trôe is na aanleiding van dié verskynsel swart geverf om die motte se gedragspatroon te benut. Die kartonproprolle is sodoende redelik goed teen skerp invallende omgewingslig in die laboratorium beskut terwyl die res van die trôe deur omgewingslig verlig is. Die motte het toe vinniger teen die liggradiënt na die beter beligte dele beweeg, teen die gladde, skuins (55°) trogwande probeer sit, maar het onmiddelik afgegly om in die versamelbakkies te beland. Die aanlokkingskrag van gewone lig vir pas-ontpopte motte sal ook vir nuwe motversamelapparaat in die nuwe SIT-insektarium benut word.

A2 **Semi-kommersiële loodsproef**

Laboratorium- en hokproewe wat sedert 2002 uitgevoer was, het die weg vir die uitvoer van 'n loodsproef van semi-kommersiële omvang gebaan. Dié proef is in November 2005 op die plaas Hexrivier, Citrusdal, ingelei.

- **Die proefperseel**: Die proefperseel waarin VKM losgelaat was, was 35 ha groot en het uit 14 boorde volwasse sitrusbome bestaan wat van 1,0 tot 3,93 ha in grootte gewissel het. Die blokke bome was in 'n 7 x 2 patroon gerangskik en het 9 aangrensende Washingtonnawelboorde, 4 Valencia- en een Minneola Tangeloboord behels. 'n Enkele boord met nawelbome op 'n aangrensende plaas, nagenoeg 800 m vanaf die proefboord, was as kontrole gebruik. Die proefperseel was relatief geïsoleer – die naaste sitrusboorde was ongeveer 500 m weg en was van die proefperseel deur die Olifantsrivier geskei. VKM was in laasgenoemde aanplantings met behulp van paringsontwrigting en Cryptogran-bespuiting onderdruk.

- **Algemene plaagbestryding**: Met die uitsondering van die VKM SIL is geen ander bestrydingsmaatreëls teen VKM getref nie. Insekdoderbespuitings vir die kommersiële bestryding van rooidopluis en witluis in die proefboorde is slegs soos benodig toegedien. Applaud, Nemesis, Ultracide, Lannate en Phosdrin is op verskillende tye in verskillende boorde vanaf die middel tot die einde van Desember toegedien.

- **Motproduksie**: Motproduksie is deur Ceder Biocontrol onderneem. Die konvensionele teeltegniek vir VKM-larwes is toegepas. 'n CRI-ontwikkelde mieliemeel-gebaseerde dieët (Moore & Richards, 2001) is in heuningflessies met watteproppe geplaas, hitte-gesteriliseer en die volgende oggend met 24-uur oue VKM-eiers ingeënt. Die flesse is nagenoeg 15 dae by 26°C gehou totdat die larwes volwassenheid bereik het. Die watteproppe is toe verwyder en met riffelkartonproppe vervang sodat die larwes daarin kon pupeer (Fig. 3.2.3.1).

Fig. 3.2.3.1. Teelflesse met riffelkartonproppe – 'n blou PVC-dop met vleklosestaal-gaasdraad beskerm die kartonprop.

Die kartonproppe is 3 dae lank op die flesse gelaat, daarna verwyder en aan weerskante toegemaak sodat larwes wat nog nie pupeer het nie, nie kon ontsnap nie. Die proppe is 'n verdere 3 dae lank onversteur gelaat sodat pupering voltooi kon word. Die proppe met papies is vervolgens in perspekstrôe geplaas in afwagting op motontpopping (Fig. 3.2.3.2).

Fig. 3.2.3.2. Perspekstrog met plastiekbakkies waarin motte na ontpopping versamel is.

Waarneming het getoon dat die deursigtige perspeksdekses wat die trôe bedek het, baie lig deurgelaat het. Die motte het derhalwe geneig om in relatiewe groot getalle tussen die kartonproppe, waar hulle ontpop het, te versamel. Dit het vertragings met die versamelingsproses veroorsaak. Die deksels is gevolglik swart geverf (nadat die foto in Fig. 3.2.3.2 geneem is) en dit het tot gevolg gehad dat die motte na skerper lig tussen die trogwande aangelok is. Hulle het sodoende vinniger in die versamelbakke beland.

Besendings papies is 2 keer per week deur die insektarium gelewer en die motte het gewoonlik binne 24 uur begin uitkom. Ontpopping het normaalweg 24-36 uur later 'n piek bereik. Die motte, wat daagliks versamel is, is onmiddelik na versameling in 'n koelkamer by 4°C geplaas sodat hulle geïmmobiliseer kon word om vertrapping te voorkom en hantering te vergemaklik. Motouderdom is aangeteken om te verseker dat die jongste motte deurentyd vir loslating gebruik is. Die tussenposes tussen die gelewerde papiebesendings is só gereël dat die aantal benodigde motte (35 000 per loslating) binne 72 uur versamel kon word om optimum lewenskragtigheid te verseker. Genoeg motte vir elke loslating is egter gedurende die hele proefverloop met enkele uitsonderings binne 48 uur versamel. Op die middag voor 'n loslating is elke besending motte met fluoriserende poeier gemerk. Vier verskillende kleure poeier is agtereenvolgens gebruik sodat motte van opeenvolgende loslatings van mekaar onderskei kon word. Nagenoeg 467 motte is daarna volumetries in elk van 75 plastiek Petribakkies (110 mm x 15 mm) afgemeet. Die motte is deurgaans in 'n lugsuiweringskabinet hanteer om lugbesoedeling met motskubbe te verhoed (Fig. 3.2.3.3).

Fig. 3.2.3.3. Lugsuiweringskas waarin koue-geïmmobiliseerde valskodlingmot hanteer is. 'n Deel van 'n aksiale suigwaaier, wat lug van voor na agter deur 'n filtreerder in die kabinet gesuig het, is aan die agterkant sigbaar.

Twintig Petribakkies (4 stapels van 5 bakkies elk) is vervolgens in elk van 4 polistireen-koelkissies (310 mm x 310 mm x 180 mm; 30 mm dik wande) verpak. Die koelkissies is die volgende oggend in 'n groter vervoerkoelkis van polistireen (1320 mm x 600 mm x 580 mm; 100 mm dik wande) verpak waarin 10 vriesblokke (170 mm x 120 mm x 40 mm) geplaas is om die klein koelkissies koel te hou.

- **Gammabestraling**: Gammabestraling van die motte is met die $Kobalt_{60}$-bron van LNR Infruitec in Stellenbosch uitgevoer. Die koelkissies met motte is in die bestralingskompleks uit die vervoerkoelkis verwyder en is as sodanig met die Petribakkies binne-in bestraal om te verseker dat die motte nie deur stygende temperature geheraktiveer word nie (Fig. 3.2.3.4).

Fig. 3.2.3.4. Polistireen-koelkissies met valskodlingmot op gammabestralingsplatform te LNR Infruitec, Stellenbosch.

Die digtheid, vorm en grootte van enige voorwerp wat gammabehandel moet word, bepaal hoe lank dit neem om 'n sekere bestralingsdosis akkuraat toe te dien. 'n Uitgebreide dosisbepalingstudie is derhalwe aan die

begin van die loodsproef deur Dr. Kobus Slabbert (Bestraling Biofisika) van iThemba LABS (**L**aboratory for **A**ccelerator **B**ased **S**ciences), Faure, uitgevoer om 'n geskikte mot/mothouer-opstelling en korrekte bestralingstyd te bereken. Gammabestraling veroorsaak dat die Fe^{3+}-konsentrasie in 'n ystersulfaatoplossing met stygende bestralingsdosisse toeneem. Dié toename word met behulp van 'n spektrofotometer gemeet. Lesings van 'n bestralingsreeks wat van 50 tot 250 Gy (50 Gy inkremente) gewissel het, is in die studie gebruik. Ystersulfaat-bevattende dosimeters is in verskillende plekke in die Petribak/koelkis-opstelling geplaas om die bestralingverstrooiing te bepaal. Met behulp van dié lesings is 'n gepaste opstelling bepaal waarin die bestralingverstrooiing met minder as 10% in verskillende posisies in die opstelling gewissel het – 'n afwyking wat heeltemal aanvaarbaar is. Die vasgestelde bestralingstyd is elke twee maande aangepas om vir die natuurlike afname in bestralingsenergie van die Kobaltbron voorsiening te maak. Die bestralingstyd het sodoende van 28 minute 12 sekondes tot 31 minute 30 sekondes gedurende die proefverloop van 6 maande toegeneem. 'n Bestralingsdosis van 150 Gy, wat in vorige navorsing as die geskikste dosis vasgestel is (Bloem *et al*, 2003), is deurgaans in die proef gebruik.

Die koelkissies is onmiddelik na bestraling weer in die vervoerkoelkis teruggeplaas en na Citrusdal teruggneem.

- **Steriele-insek Loslating**: Vyf en sewentig werksrye, eweredig in die perseel versprei, is vooraf gemerk en alle loslatings is in dié rye uitgevoer. 'n Geskikte aantal Petribakkies met motte is in die proefperseel uit die polistireen-koelkissies verwyder en in 'n Coleman-tipe koelkis oorgeplaas. Dié koelkis was 'n minder-doeltreffende koelhouer en die motte was ten tye van hul loslating alreeds grotendeels uit hul verkoelde toestand ontdooi. Dit het gehelp dat hulle aktief kon vlieg om skuiling te vind sodra hulle losgelaat is.
Die motte is vanaf middel-November 2005 met die hand vanaf 'n vierwielaangedrewe motorfiets losgelaat. Die motte van 'n Petribakkie is so eweredig as moontlik op elke derde boom in in elke ry toegedien, ongeag die rylengte. Eenduisend bestraalde VKM (mannetjies en wyfies in 'n nagenoeg 1:1 verhouding) is twee keer per week per hektaar op Maandae en Donderdae in die proefperseel losgelaat. Die roetine van twee loslatings per week is sonder uitsondering gedurende die proefverloop gehandhaaf. Loslatings moes egter by enkele geleenthede weens onbeheerbare omstandighede met 'n dag, of uitsonderlik twee dae, vervroeg of vertraag word. Motte is tot einde-Mei 2006 losgelaat op welke tydstip die boorde ge-oes is.

- **Proefevaluasie**

a **Lokvalopname**: Dertien deltalokvalle, toegerus met Lorelei-feromoonvrystellers, is in die proefperseel versprei. 'n Kontrolelokval is in twee boorde, onderskeidelik in die kontroleboord (verwysing hierbo) en in 'n Valenciaboord 600 m van die proefboord af, geplaas. Alle lokvalle is twee keer per week ondersoek. Gedurende elke inspeksie is die lokvalbodems met nuwes vervang. Die gebruiktes is na die laboratorium vervoer waar die gevangde mannetjies met behulp van 'n ultraviolet(UV)-lamp (verstelbaar van 254 nM tot 365 nM) getel is. Aantekening is gemaak van (i) die aantal mannetjies wat in die UV-lig gefluoriseer het (losgelate mannetjies) en (ii) ongemerkte mannetjies (óf wilde óf F1-mannetjies).

b **Vrugvalopname**: Vyf aangrensende databome in 'n ry by elke lokval wat in 'n nawelboord geplaas is, is vir vrugvalopnames gebruik. Elke lokval met vyf databome is as 'n datapunt beskou. Daar is sodoende 'n totaal van 12 datapunte gebruik – 11 in die proefperseel en een in die kontrole-nawelboord. Alle afvalvrugte onder die databome is een keer per week opgetel, oopgesny en vir tekens van VKM-besmetting ondersoek. Enige vrug met 'n larwe of enige simptome van VKM-besmetting, is as besmet beskou.

c **Mededingendheid van bestraalde, losgelate motte**: Die kwaliteit van bestraalde motte is nagegaan om te verseker dat hulle mededingend met wilde motte was:

(i) **Oorvloedingsverhouding, vliegvermoë en oorlewing**: Die aantal losgelate motte wat in die lokvalle gevang is (hierbo bespreek) is gebruik om die oorvloedingsverhouding na te gaan. Dié verhouding moes verkieslik groter as 10 losgelate mannetjies tot een wilde mannetjie wees. Die loslaatrye in die proefboorde is aan die begin van die proef gemerk en motte is deurentyd in die gemerkte rye losgelaat. Aan die hand van merk&loslaatproewe wat gedurende 2005 uitgevoer was, is besluit om die motte in boomrye nagenoeg 40 m van mekaar af los te laat. Motte is met een geleentheid in Desember met twee kleure fluoriserende poeier (verskillend van die normale loslatingskleur op daardie tydstip) gemerk en in een loslaatry elk, weerskante van 'n lokvalbevattende ry losgelaat. Twee herhalings van dié uitleg, 300 m van mekaar af in die proefboorde versprei, is gebruik. Dit is gedoen om vas te stel of die mannetjies in staat was om só van loslaatry tot loslaatry te versprei dat daar nie onbehandelde dele in die boorde tussen die loslaatrye geskep sou word nie. Daar kon ook van die data afgelei word hoe lank hul in die boord aanwesig was voordat hul gevrek of buite bereik van die lokvalle versprei het.

(ii) **Paringplatforms**: Die vlieg- en opsporingsvermoë van bestraalde mannetjies en die roep(=aanlok)-vermoë van bestraalde wyfies is met behulp van paringplatforms getoets. Elke platform was 'n ronde pasteipanvormige houer van "Correx"-plastiek vervaardig en 500 mm in deursnee. Die 50 mm hoë wande het uit stywe, 1 mm dik verdigte polistireenmateriaal bestaan, wat aan die binnekant met Teflon-kleefband bedek was om motontvlugting te verhoed. Die Teflonband was om dieselfde rede bykomend met talkumpoeier bestuif. Elke platform is op so 'n wyse aan 'n 2,5 m hoë hoekysterraamwerk gehang dat dit op en af gehys kon word om mothantering en tellings te vergemaklik (Fig. 3.2.3.5).

Fig. 3.2.3.5. Paringplatform om die vlieg- en paringsvermoë van gammabestraalde mannetjies en wyfies te ondersoek.

Drie proewe is met een- tot tweedae-oue wyfies uitgevoer. In die eerste proef is 10 bestraalde en 10 onbestraalde wyfies op elke paringplatform in die proefperseel geplaas. Twintig onbestraalde wyfies is in elk van die orige twee proewe gebruik. Die linkerkantse voor- en agtervlerke van die wyfies is vooraf afgeknip om te verhoed dat hulle kon wegvlieg. Die bestraalde wyfies is met fluoriserende poeier gemerk om hulle van die onbestraaldes te onderskei. Die wyfies is om 1800 op die paringplatforms geplaas. Bykomende mannetjies is nie vir die paringsproewe losgelaat nie, maar is staatgemaak op mannetjies wat deel van die roetine-loslatings was. Dié loslatings het, soos voorheen vermeld, elke 3 tot 4 dae plaasgevind. Twee platforms is per proef gebruik. Die wyfies op die platforms is elke 30 minute onder rooi lig ondersoek om nie die motte te versteur nie. Alle motte wat tot nagenoeg 01:00 *in copulo* waargeneem is, is versigtig versamel om nie die paringsproses te versteur nie en in 30 ml plastiekbakkies geplaas. Die gepaarde motte is die volgende oggend onder UV-lig ondersoek om vas te stel of hulle bestraal was en die wyfies is daarna gedissekteer om te bepaal of paring suksesvol was. Die aanwesigheid van 'n spermatofoor in die *bursa copulatrix* van 'n wyfie is as bewys van 'n suksesvolle paring beskou. Die herkoms van die mannetjies is ook sover moontlik ondersoek, aangesien dit een van drie tipes kon wees, t.w. 'n losgelate, bestraalde P1-mannetjie (gemerk), 'n wilde mannetjie (ongemerk), of 'n F1-mannetjie (ongemerk). Laasgenoemde twee tipes mannetjies sou van mekaar onderskei kon word indien van hulle suksesvol sou paar, maar geen vrugbare eiers ná paring gelê is nie. In so 'n geval sou die betrokke mannetjie moontlik die F1-nageslag van 'n bestraalde mannetjie gewees het (indien aanvaar word dat die wyfie wel vrugbaar was).

d **Opspoor van F1-steriele VKM**: Die sukses van die SI-tegniek is op die steriele F1-beginsel geskoei. Alle bestraalde wyfies is volkome steriel na bestraling met 150 Gy en kan geen nageslag hê nie al paar hulle met wilde, vrugbare mannetjies. Daar is egter 'n klein persentasie van die losgelate mannetjies wat slegs gedeeltelik deur die gamma-behandeling gesteriliseer word. Parings van sulke mannetjies met wilde, vrugbare wyfies kan tot gevolg hê dat vrugte besmet word. Enige nageslag (F1) wat uit dié besmette vrugte ontwikkel, is egter steriel. Sulke F1-motte kan geensins in die boord met die blote oog van wilde motte onderskei word nie. Dit beteken dat ongemerkte mannetjies wat in lokvalle in SIL-boorde gevang word, nie

noodwendig wild is nie, maar ook F1-indiwidue kan wees wat uit vrugte ontwikkel het. Daar is op twee maniere tussen dié mannetjies onderskei:

(i) **Mikroskopiese tegniek**: Twintig ongemerkte mannetjies wat uit lokvalle of vanaf paringsplatforms verwyder was, 10 een- tot 2-dae oue insektariumgeteelde F1-mannetjies, asook 10 insektariumgeteelde kontrolemannetjies, is in die ondersoek gebruik. Die mannetjies se interne geslagsorgane is onder 'n mikroskoop verwyder en deur 'n spesiale proses op mikroskoop-voorwerpglasies gefikseer en met Giemsa-kleurstof gekleur (Carpenter, pers. kom.) Dié kleurstof reageer met chromatienmateriaal in spermbundels en kleur duidelik homogenies in vrugbare mannetjies. Die hoeveelheid chromatienmateriaal in die spermbundels van F1-steriele mannetjies wissel egter baie en kleur daarom nie homogenies nie.

(ii) **Herwinning van F1-motte uit natuurlik-besmette vrugte**: Vyftien afvalvrugte is met twee geleenthede in elk van die kontrole- en SIL-boorde versamel en indiwidueel in 500 ml plastiekbakkies met gaasdeksels in die laboratorium geplaas. Elke mot wat uit 'n vrug ontwikkel het, is met 'n insektarium-geteelde mot van die teenoorgestelde geslag in 30 ml plastiekbakkies ingehok. Die pare is 7 dae lank *in situ* gehou. Die mannetjies is daarna verwyder en vernietig, terwyl die wyfies gedissekteer is en vir die aanwesigheid van spermatofore (as teken dat paring plaasgevind het) ondersoek is. Die bakkies is nog 4 dae lank gehou sodat alle vrugbare eiers kon uitbroei. Die eiers is daarna onder 'n mikroskoop ondersoek om die eiermortaliteit te bepaal. Wyfies wat uit besmette vrugte herwin was en in sulke parings onvrugbare eiers gelê het, moes uiteraard die steriele F1-nageslag van 'n bestraalde mannetjie gewees het. In die omgekeerde paringskombinasie waar onvrugbare eiers die gevolg van 'n kruising tussen 'n Insektarium-geteelde wyfie en 'n herwonne mannetjie was, moes die mannetjie derhalwe ook die F1-nageslag van 'n bestraalde mannetjie gewees het.

Resultate en bespreking

Die insekdoders wat in die proefboorde gebruik was, het verbasend min uitwerking op VKM gehad en daar was geen opsigtelike afname in die totale vangste van wilde en losgelate motte nie. In die enkele gevalle waar 'n motloslating 'n nagtoediening van Lannate, Ultracide of Phosdrin voorafgegaan het, is motvangste tydelik onderdruk, maar het gewoonlik na die volgende loslating tot normaal teruggekeer. Dit is slegte nuus in terme van enige onderdrukking van die natuurlike bevolking wat 'n produsent moontlik van sulke bespuitings sou verlang het. Daar was egter ook 'n relatiewe klein en kortstondige invloed op die losgelate motte, wat beteken dat daar nie spesiale maatreëls getref hoef te word om die motte in 'n SIL-program onder kommersiële omstandighede te beskerm nie. Wat die werklike invloed ook al is, moet onthou word dat enige sodanige invloed op beide die wilde en die losgelate motte van toepassing is. Die relatiewe loslaatverhouding tussen die twee groepe motte sal dus deurentyd gehandhaaf word – indien die losgelate motte se getalle met 90% deur 'n insekdoder verminder word, sal 90% van die wilde motte ook uitgeklop word - en die *status quo* sal gehandhaaf word.

a **Lokvalopname**: Motvangste in die kontroleboord was van die begin af redelik hoog en die lokvaldrempelwaarde van 15 mannetjies per deltalokval per week is dikwels oorskry (Fig. 3.2.3.6). In teenstelling is relatief min mannetjies aanvanklik in die SIL-proefboorde gevang - selfs voordat die loslaat van bestraalde motte die natuurlike bevolking kon begin onderdruk het. Navorsing is in die verlede verskeie jare lank in dié boorde uitgevoer op verskillende aspekte van VKM-bestryding, insluitend paringsontwrigting, die lok&vrekbenadering en lokval- en feromoonontwikkeling. Lokvalopnames in daardie proewe het daarop gedui dat VKM-getalle sonder uitsondering in die begin van die seisoen klein was en dikwels eers in Februarie-Maart begin toeneem het. Dit lyk dus asof dié neiging herhaal is, met die verskil dat die motbevolking nie in dié proef later tot skadelike vlakke opgebou het nie, waarskynlik danksy die SI-loslatings.

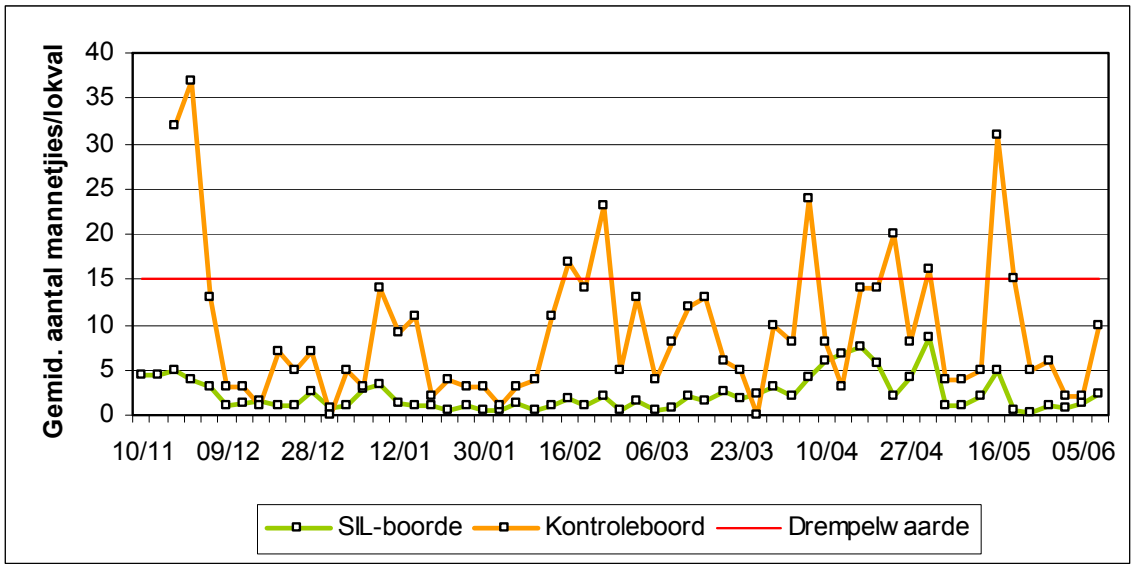

Fig. 3.2.3.6. Vangste van valskodlingmotmannetjies in kontrole-(wild) en SIL-(wild plus F1) proefboorde.

b **Vrugvalopname**: Min vrugte is gedurende die eerste ses weke in die proefperseel en kontroleboord deur VKM besmet (Fig. 3.2.3.7). Die oesskade in die kontroleboord het daarna geleidelik gestyg en vanaf die middel van Maart het die gemiddelde vrugval deurentyd bo die ekonomiese drempelwaarde (een besmette vrug per boom per week) gebly en later tot 'n maksimum van amper ses besmette vrugte per boom gestyg. Gedurende die laaste telling wat met oesdag in die boord uitgevoer was, is 'n gemiddeld van 17 besmette vrugte per boom aangeteken. Dit verteenwoordig egter alle besmette vrugte wat op daardie dag van die bome afgepluk is en dié wat alreeds afgeval het. Dié data is daarom vir berekeningsdoeleindes weggelaat. Die algehele oesskade is egter ernstig, veral as in gedagte gehou word dat die kontroleboord se bome relatief klein was - slegs nagenoeg 1,8 m hoog. 'n Totaal van gemiddeld 51 vrugte op elke kontroleboom is vanaf middel-Desember tot oestyd deur VKM besmet. Vrugval weens VKM-besmetting in die SIL-boorde was deurentyd laag en slegs 2,9 vrugte per boom is in dieselfde tydperk deur VKM beskadig is. Die oesskade is dus met 94,3% verminder. Dit is 'n goeie resultaat en vergelyk uitstekend met die beste onderdrukking wat in die verlede met enige chemiese insekdoder verkry was.

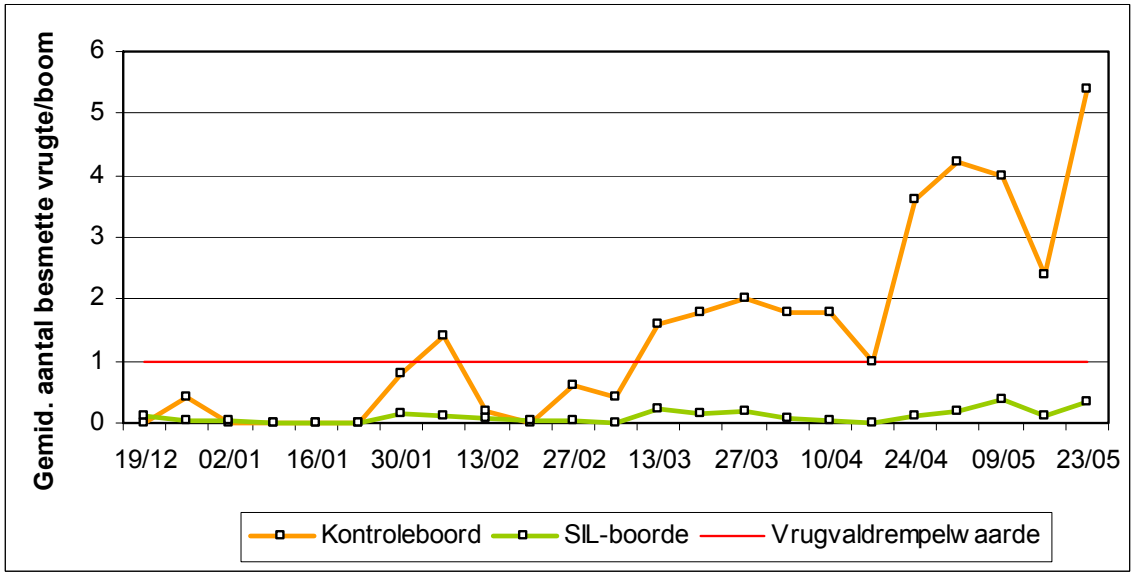

Fig. 3.2.3.7. 'n Vergelyking van oesskade weens valskodlingmotbesmetting in kontrole- en SIL-proefboorde.

c **Mededingendheid van bestraalde, losgelate motte**:

(i) **Oorvloedingsverhouding, vliegvermoë en oorlewing**:

- **Oorvloedingsverhouding**: Die oorvloedingsverhouding van losgelate tot ongemerkte (wilde plus F1-mannetjies) het heelwat met verloop van die proef gewissel (Fig. 3.2.3.8). Die verhouding het tot aan die

einde van Maart van 40:1 tot 180:1 gewissel. Daarna, tot aan die begin van Mei, het die aantal wilde mannetjies effens toegeneem en die verhouding het tot tussen 10:1 en 20:1 gedaal. Dit is die vlak wat as mikpunt vir doeltreffende VKM-onderdrukking deur SIL gestel is. Die gemiddelde nagtemperature het daarna gedaal en selde bo 10°C gestyg, wat die aktiwiteit van beide losgelate en ongemerkte mannetjies aan bande gelê het. Die oorvloedingsverhouding het onder dié toestande tot minder as 10:1 afgeneem.

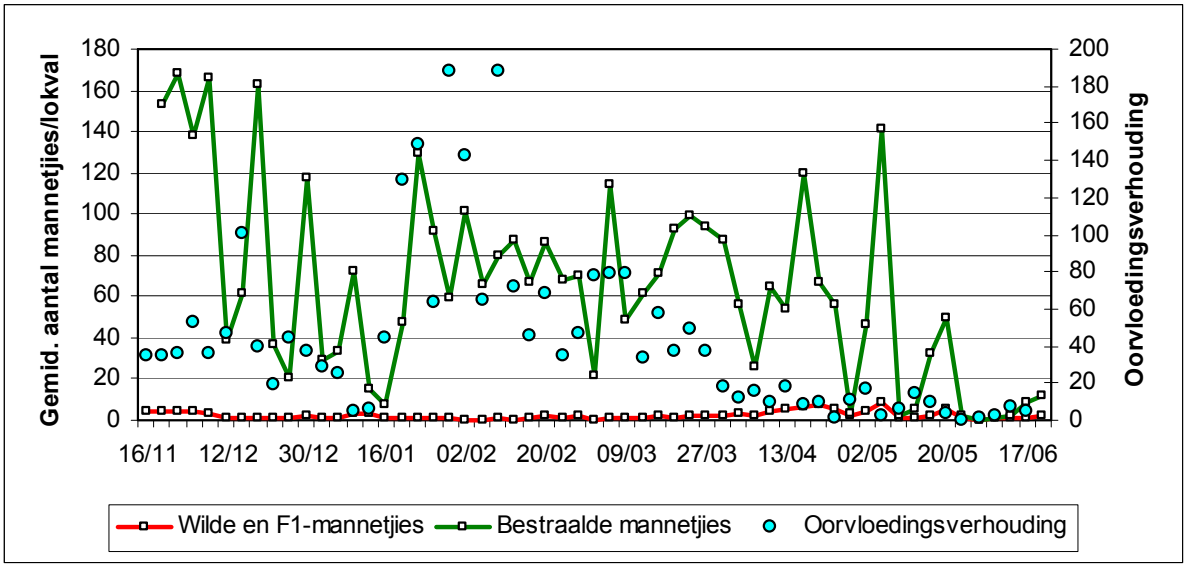

Fig. 3.2.3.8. Gemiddelde lokvalvangste van ongemerkte (wild en F1-nageslag) en bestraalde, losgelate mannetjies in SIL-proefboorde.

- **Vliegvermoë**: Mannetjies wat met die twee kleure fluoriserende poeier gemerk was, is binne 4 dae na loslating in onderskeidelik 8 en 11 van die 13 lokvalle gevang. In 'n tweede telling 12 dae later is van dié mannetjies in onderskeidelik 6 en 7 van die 13 lokvalle aangetref. Dié vangste dui daarop dat die mannetjies goed versprei en beslis nie tot die loslaatrye of in die onmiddelike omgewing daarvan, verbind bly nie. Die meeste mannetjies is in 'n lokval 40 m van die naaste loslaatry af gevang. 'n Redelike aantal mannetjies is 185 m ver in lokvalle gevang, terwyl een mot binne die eerste 4 dae na loslating in 'n lokval 285 m van die naaste loslaatpunt af gevang is. Dié data bevestig dat die 40 m afstand tussen loslaatrye goed genoeg is om 'n groot mate van oorvleueling tussen motte wat in aangrensende rye losgelaat is, te bewerkstellig, wat verseker dat hulle wilde motte maklik sal kan bereik.

- **Oorlewing**: Die meeste mannetjies is binne 3 tot 4 dae na loslating in die lokvalle gevang. Mannetjies van elke loslating is ook deurgaans in die proef 'n week na loslating in veel kleiner, maar steeds beduidende getalle, in die lokvalle aangetref. Vangste het daarna afgeneem, alhoewel mannetjies van 'n betrokke loslating dikwels tot twee weke later nog in lokvalle opgemerk is. Dit bevestig dat dubbele loslatings elke week goed genoeg is om te verseker dat daar deurentyd bestraalde, lewenskragtige mannetjies in die boorde sal wees om op wyfies se roepseine te reageer. Dié eienskap dra uiteraard daartoe by dat wilde wyfies 'n goeie kans het om deur bestraalde mannetjies gedek te word en sodoende tot 'n kleiner nageslag by te dra. Alhoewel dit onmoontlik is om vas te stel wat die toedrag van sake in die geval van die losgelate wyfies is, kan daar redelikerwys aangeneem word dat hulle ook minstens só lank bly lewe het en derhalwe ook tot die onderdrukking van die wilde bevolking bygedra het.

(ii) **Paringplatforms**: In die eerste proef het geen paring tot 10:00 die aand plaasgevind nie en is waarnemings gestaak. Lesings het getoon dat die temperatuur kort na sonsondergang alreeds om en by 16°C was. Dié koue het motaktiwiteit waarskynlik onderdruk. Dié verskynsel stem ooreen met waarnemings in vorige proewe (CRI-jaarverslag vir 2004, Fig. 3.2.5.39) ten opsigte van die inaktivering van VKM-geslagsaktiwiteit deur temperatuur laer
as nagenoeg 16°C.

In die tweede proef is 5 paar motte op die twee platforms vanaf 22:30 tot 24:00 *in copulo* versamel. Waarnemings vir die aand is om 01:00 afgesluit toe geen verdere paringsaktiwiteit waargeneem is nie. Die motte is *in situ* gelos en die volgende oggend om 07:30 is 'n sesde paartjie *in copulo* versamel. Al 6 mannetjies het onder UV-lig gefluoriseer en was dus bestraalde, losgelate mannetjies. Die mannetjies is 3 dae vroeër losgelaat en staaf bogenoemde data dat bestraalde mannetjies van dié ouderdom nog steeds seksueel aktief is. Een van die 6 betrokke wyfies het slegs onvrugbare eiers gelê, terwyl die orige wyfies eiers gelê het waarvan 50-80% nie uitgebroei het nie – dus 'n tipiese P1-bestraalde mannetjiereaksie. Daar is by nie een van die gepaarde wyfies 'n enkele spermatofoor in hul bursae copulatrix gevind nie. Dit dui

daarop dat spermatofore moontlik nie met die eerste paring gevorm word nie, maar slegs in daaropvolgende parings.

In die derde proef is 3 paar motte op die twee platforms vanaf 21:30 tot 22:00 *in copulo* versamel. Die temperatuur het daarna tot onder 16°C gedaal en geen verdere motaktiwiteit is waargeneem nie. Al 3 mannetjies het onder UV-lig gefluoriseer en was dus bestraalde, losgelate mannetjies. Die mannetjies in dié proef was 4 dae vroeër losgelaat. Al 3 wyfies het vrugbare eiers gelê, maar eiermortaliteit was weer eens hoog en het van 57-95% gewissel. Dit is weer kenmerkend van bestraalde P1-mannetjies wat grotendeels steriel is. Die wyfies en mannetjies van al 3 pare het voor 24:00 geskei. Een van die pare was egter alweer teen die volgende oggend aan die paar en 'n spermatofoor is in dié wyfie gevind, in teenstelling met die ander twee, waar geen spermatofore aanwesig was nie. Dit dien as verdere bevestiging dat spermatofore nie gedurende die eerste paring gevorm word nie. Vrugbare eiers is egter wel gelê, waar daarop dui dat vrugbare sperme desnieteenstaande met sukses oorgedra kan word.

d **Opspoor van F1-steriele VKM**:

(i) **Mikroskopiese tegniek**: Mikroskooppreparate is van 40 mannetjie se geslagsorgane gemaak en mikroskopies ondersoek. Tekens van verskille in die hoeveelheid chromatienmateriaal tussen die verskillende motgroepe is gevind. Die verskille was egter te wisselvallig om tot 'n gevolgtrekking te kom oor die bruikbaarheid van dié metode om tussen normale en steriele F1-motte te onderskei. Navorsing sal voortgesit word om die tegniek verder te ondersoek.

(ii) **Herwinning van F1-motte uit natuurlik-besmette vrugte**: In twee kruisings tussen insektariummotte en herwonne motte uit die kontroleboord, is geen vrugbare eiers gelê nie. Spermatofore is ook nie in die gedissekteerde wyfies gevind wat op onsuksesvolle paring gedui het. 'n Verdere 8 kontrolekruisings het almal vrugbare eiers tot gevolg gehad; die natuurlike eiermortaliteit was nagenoeg 5% – uit vorige ervaring 'n normale verskynsel. Al 24 kruisings tussen insektariummotte en herwonne motte uit die SIL-boorde was suksesvol – spermatofore is in alle wyfies gevind. Die natuurlike eiermortaliteit in kruisings waar vrugbare eiers gelê was, was normaal. Vyf van die 24 kruisings het egter slegs onvrugbare eiers tot gevolg gehad en die gevolgtrekking kan gemaak word dat die herwonne mot in elkeen van dié parings die steriele F1-nageslag van bestraalde VKM was. Dié nagenoeg 20% onvrugbare parings lyk relatief laag en en moet 'n direkte weerspieëling van die persentasie F1-steriele motte in die proefperseel wees. Dié verskynsel skyn moeilik vereenselwig te word met die groot sukses waarmee vrugte in die SIL-boorde teen VKM-besmetting beskerm was. Die uitwerking van die loslating van groot getalle steriele wyfies en deels-steriele mannetjies moet dus 'n baie groot direk-onderdrukkende invloed op die natuurlike bevolking hê. Daar moet onthou word dat die bykomende beskikbaarheid van groot getalle wyfies in die SIL-boorde ongetwyfeld die aandag van wilde, vrugbare mannetjies geniet het. Die kans dat sulke mannetjies by wilde, vrugbare wyfies kan kom, word verminder en vrugbesmetting word sodoende voorkom. Dié verskynsel kan die loslating van beide geslagte regverdig (wat somtyds bevraagteken word). Die bydraende invloed van F1-steriliteit is derhalwe moontlik nie so belangrik as wat gereken is nie.

Ondanks die verskeie tegnieke wat toegepas was om die doeltreffendheid van die loodsproef te evalueer, kan daar nog steeds 'n mate van twyfel oor die oënskynlik goeie resultaat in vergelyking met die kontroleboord bestaan. Bestraalde VKM is van die begin van die seisoen af in die proefboorde losgelaat toe daar geen tekens van 'n werklike of selfs potensieel beduidende VKM-besmetting was nie. Die omvang van die oesskade wat later in die seisoen deur 'n natuurlike VKM-bevolking van onbekende grootte in die afwesigheid van SIL aangerig sou gewees het, kan dus nie bereken word nie. Die enigste wyse waarop 'n beter idee (maar nog steeds nie bo alle twyfel nie) verkry sou kon word, sou gewees het om 'n onbehandelde kontroleboord aangrensend aan die SIL-terrein te gebruik. Selfs dan sou die werklike besmettingsvlakke nog nie bo alle twyfel vasgestel kon word nie, aangesien daar 'n goeie kans sou wees dat losgelate motte na die kontroleboord sou versprei (sien latere bespreking hieronder). Ander faktore moet derhalwe in aanmerking geneem word om die verkreë resultate in perspektief te plaas, naamlik:

- Die 35 ha sitrusboorde wat vir die SIT-loodsprojek gebruik was, is alreeds dikwels in die verlede vir proefdoeleindes gebruik. Historiese inligting wat ingewin was, toon dat die betrokke proefboorde elke seisoen sonder uitsondering aan matige tot hewige VKM-besmetting blootgestel was.

- Baie boorde in die Olifantsriviervallei wat nie gedurende 2005-2006 met behulp van 'n intensiewe bestrydingsprogram (boordsanitasie, paringsontwrigting met Isomate en/of 'n Cryptogran-bespuitingsprogram) behandel was nie, hewig deur VKM besmet geraak het.

Beide bogenoemde faktore versterk die aanname dat daar inderdaad 'n uitstekende kans is dat die SIL-proefboorde hewig deur VKM besmet sou gewees het indien die SIT nie doeltreffend was nie.

A3 **Ontwikkeling van toerusting vir die nuwe SIT-insektarium**:

(a) *Eierlê-houers ("eierpanne")*: In VKM-insektaria word eiers deur wyfiemotte onder huishoudelike meelsiwwe (200 mm deursnee) op waspapier gelê. Dié tegniek is egter om verskeie redes ondoeltreffend:

- Die waspapier word swak benut omdat 'n groot gedeelte daarvan op die hoeke ('n ronde sif op 'n vierkantige stuk papier) weggegooi moet word.
- Die siwwe word langs mekaar op rakke gepak wat baie spasie in beslag neem.
- Motaktiwiteit onder die siwwe veroorsaak dat die motte hulle skubbe verloor. Dié skubbe versamel nie net onder die siwwe op die waspapier nie, maar dring deur die siwwe en versprei op die rakke en in die vertrek waarin eierlegging plaasvind. Dit hou 'n groot gesondheidsgevaar vir alle mense in die omgewing in.

Dit was derhalwe nodig om 'n alternatiewe houer te ontwikkel wat vir die versameling van die eiers gebruik kan word. 'n Vleklose staal "eierpan" is ontwerp wat 'n eierlê-oppervlakte van 1200 cm^2 het, in vergelyking met 314 cm^2 van 'n enkele sif. Dit het dus die oppervlakte van nagenoeg vier siwwe, maar beslaan slegs die bruikbare spasie wat deur twee siwwe in beslag geneem word. Elke eierpan het 'n soliede bodem, dak en wande met splete op strategiese plekke om die waspapier te manipuleer en water aan die motte te verskaf. Elke eierpan word aan 'n outomatiese lugsuigstelsel gekoppel wat los skubbe na 'n sentrale punt in die insektarium uitsuig waar dit versamel en vernietig word. Dié stelsel skakel skubbesoedeling amper volledig uit. Nagenoeg 270 van dié eierpanne word in die nuwe insekatrium benodig. Een probleem moet nog opgelos word, naamlik 'n gewoonte van die motte om hul eiers nie slegs op die bodem van die eierpanne op die waspapier te lê nie, maar ook op die dak en wande. Laasgenoemde moet dus met 'n materiaal bedek word wat nie 'n gunstige oppervlakte vir eierlegging bied nie. Verskillende grade skuurpapier, tapytmateriaal, sandgeblaasde metaal, vleklose staal gaasdraad en 'n verskeidenheid Velcro-tipes (die hakiekant) is getoets. Alhoewel daar 'n groot reeks materiaal, beide van natuurlike of sintetiese oorsprong te kry is, is hulle deurgaans nie geskik om in die eierpanne vasgeheg te word nie. Slegs die vleklose staal gaasdraad en een tipe Velcro het enige belofte getoon en word tans verder ondersoek. Eiers is op al die ander proefmateriale gelê.

(b) *Alternatiewe teelflesse vir VKM-larwes*: Larwes kan op twee maniere geteel word, naamlik in dieët wat in oop of toe houers geplaas word. Eersgenoemde houers hanteer makliker in 'n massateel-prosedure, maar het die groot nadeel dat dit in 'n vertrek gebruik moet word wat so steriel is dat infeksies van die dieët en die larwes wat daarin voed, deur skadelike organismes soos bakterieë, swamme en virusse verhoed kan word. Alhoewel nie onmoontlik nie, is sulke tegnologie duur en veeleisend om toe te pas. Indien iets verkeerd sou loop, word die produksie van 'n hele vertrek in gevaar gestel. In teenstelling hiermee, word 'n toe houer (glasflesse in VKM-insektaria) as 'n geslote, steriele stelsel hanteer. Dit skakel die gebruik van gespesialiseerde steriele vertrekke uit. Enige infeksie is ook gewoonlik tot slegs 'n gedeelte van die flesse beperk en die potensieel katastrofiese invloed van enige infeksie kan sodoende makliker beperk word.

Die probleem met heuningflesse (375 ml inhoudsmaat), wat tradisioneel in alle VKM-insektaria gebruik om die larwes in te teel, het onder andere twee probleme. Eerstens is die flesse te klein om in 'n massateelstelsel te gebruik. Elke heuningfles word met nagenoeg 140 g aangemaakte VKM-dieët gevul, wat meebring dat 20 000 van dié flesse per dag in die nuwe insektarium gebruik sal moet word om die vereiste aantal motte te teel. Tweedens word die flesse met watteproppe toegemaak. Dit is omslagtig en duur om die watte te gebruik, terwyl blootstelling aan die mikroskopiese wattevesels wat vrylik tydens hantering afgegee word, binne 'n kort rukkie uiters irriterend vir werkers raak en dus 'n gesondheidsgevaar inhou. Daar is dus gepoog om die bestaande blikdeksels, wat saam met die flesse verskaf word, só te verander dat dit die watte kan vervang. 'n Gat van 25 mm tot 50 mm deursnee is in verskillende deksels gemaak. Elke gat is met 'n membraan toegemaak wat saam met die deksel op die fles kon vasskroef. Verskeie alternatiewe glasflesse, gate van verskillende groottes en 'n verskeidenheid papiermembrane, is op die proef gestel. Slegs twee glasflesse is gevind wat hoegenaamd in terme van grootte en vorm vir die doel geskik sou wees, te wete 'n 500 ml blatjangfles (90 mm hoog x 100 mm breed) en 'n afgespitste 500 ml inlêfles (140 mm hoog x 85 mm breed bo en 65 mm breed onder), beide van Consul Glass. Meer dieët kon in beide dié flesse gebruik word, wat potensieel tot 'n groter larweproduksie per fles sou bydra. Afhangende van die hoeveelheid dieët wat per fles gebruik word, sou die aantal flesse wat gebruik moes word tot minder as 10 000 per dag verminder kan word. Dit is 'n beduidende arbeidsbesparende vermindering. Vyf proewe is uitgevoer om die produksievermoë van die flesse met die heuningflesse te vergelyk. Die volgende is vasgestel:

- Wanneer die larwes volwassenheid nader, word die dieët deeglik deurgewerk, wat tot gevolg het dat die dieëtvlak in die flesse "rys". Daar is gerieflik plek vir tot drie keer meer dieët (3x-dieët) in

die blatjang- en inlêflesse as in die heuningflesse, naamlik 420 g dieët per fles. Die deurgewerkte dieët in die blatjangflesse het egter tot teen die deksels “gerys”, wat ongewens is. Daarnenewens het ’n gedeelte van die dieët onder in die blatjangflesse nat gebly en die larwes het dié dele vermy. Dit het meegebring dat die produksie per fles afgeneem het en daar nie soveel meer larwes per fles geproduseer is dat dit die 50% bykomende hoeveelheid dieët geregverdig het nie (Fig. 3.2.3.9). Dié verskynsel is ook by die 375 ml heuningflesse en die 500 ml inlêflesse, beide met 2x-dieët, waargeneem, wat hulle as potensieel-bruikbare flestipes uitgeskakel het.

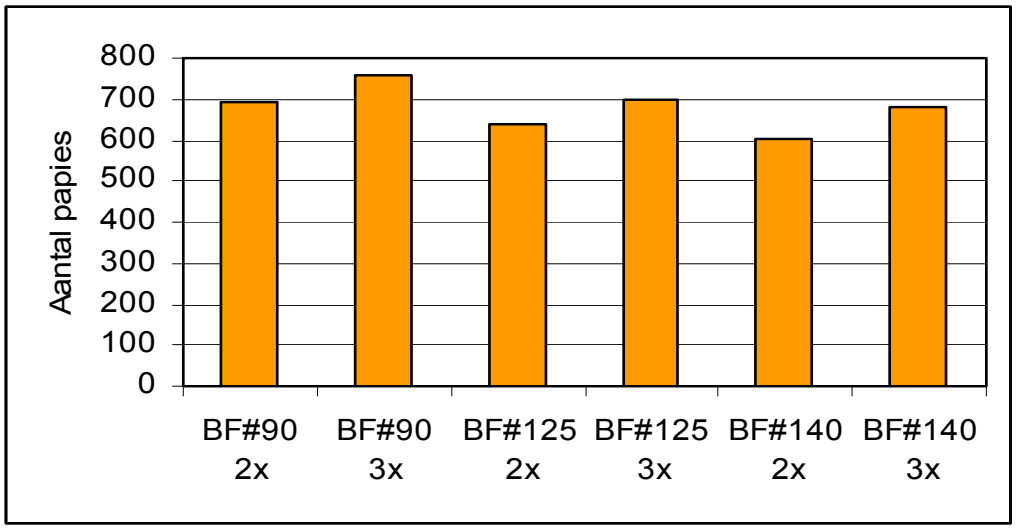

Fig. 3.2.3.9. Aantal valskodlingmotte wat in blatjangflesse met 2x- en 3x-dieët geproduseer was (#90, #125 en #140 is verskillende grade papiermembrane wat in die flesdeksels getoets was).

Die blatjangflesse met 2x-dieët het veel beter as die heuningflesse pesteer en daar kon nagenoeg dubbel soveel larwes per fles as met ’n heuningfles geproduseer word (Fig. 3.2.3.10).

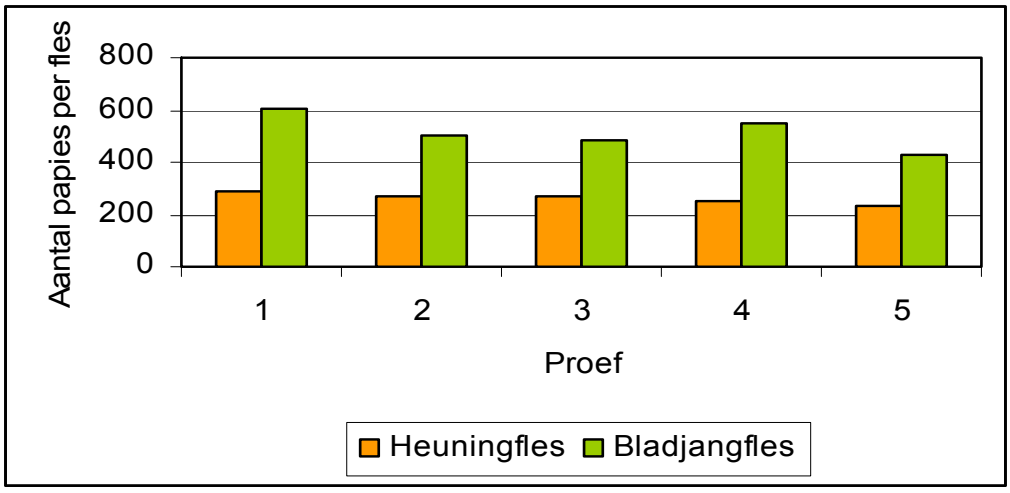

Fig. 3.2.3.10. Aantal valskodlingmotte wat in twee verskillende flestipes geproduseer kan word.

Van verskeie membraantipes wat getoets was, het Sappi Kraft Stratoseal die beste in kombinasie met ’n 40 mm deursnee dekselgat presteer. Die Stratoseal is ’n 80 g/m^2 graad papier wat natword verdra en toelaat dat die dieët teen die verlangde tempo uitdroog sonder om uitermatig nat te bly of te vinnig uit te droog. Dié membraan het die beste resultaat saam met ’n 40 mm deursnee dekselgat gegee.

- Larwes wat in flesse met drie keer die normale dieët geteel word, ontwikkel baie stadiger as larwes in flesse met twee keer die hoeveelheid dieët (Fig. 3.2.3.11). Dit is ongewéns aangesien die ontwikkelingstempo so vinnig as prakties moontlik moet wees om die gebruik van meer toerusting, wat deur ’n stadiger ontwikkelingstempo teweeggebring sal word, te verhoed.

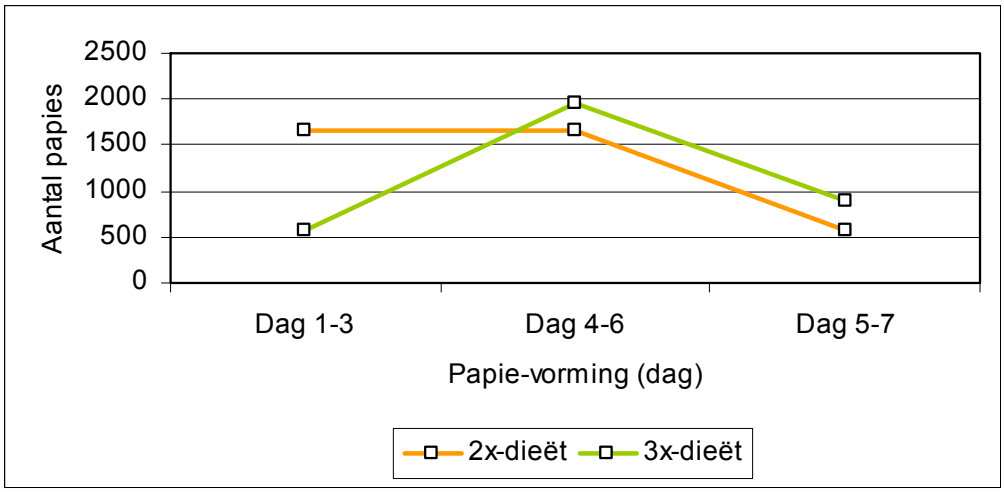

Fig. 3.2.3.11. Ontwikkelingstyd van valskodlingmot in 2x- en 3x-dieët in 500 ml blatjangflesse.

- 'n Beperkte aantal insekte kan per fles geteel word met beide die heuning- en blatjangflesse. Indien dié aantal oorskry word, word die insekte kleiner. Dié grens is nagenoeg 300 motte per heuningfles (Fig. 3.2.3.12) en 600 motte per blatjangfles (Fig. 3.2.3.13).

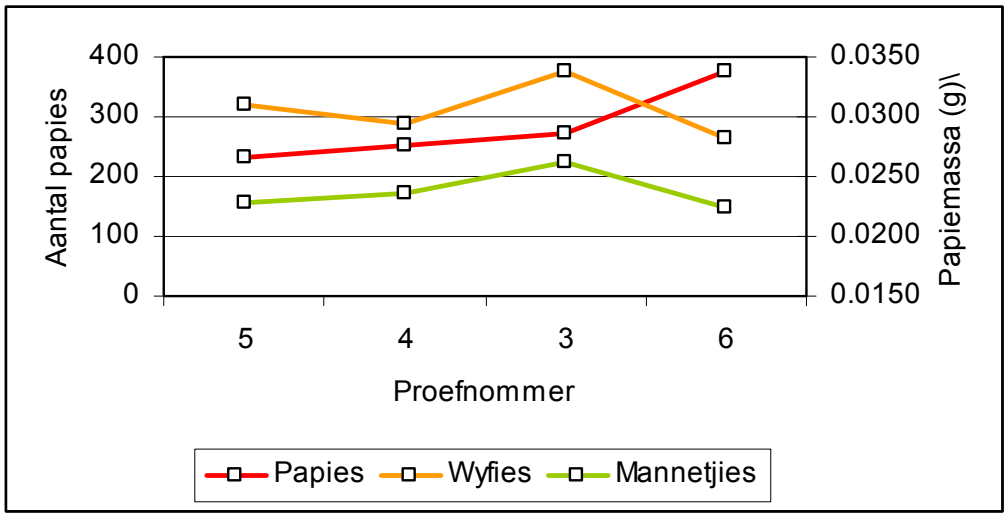

Fig. 3.2.3.12. Produksie van valskodlingmot in 375 ml inhoudsmaat heuningflesse met 140 g dieët per fles.

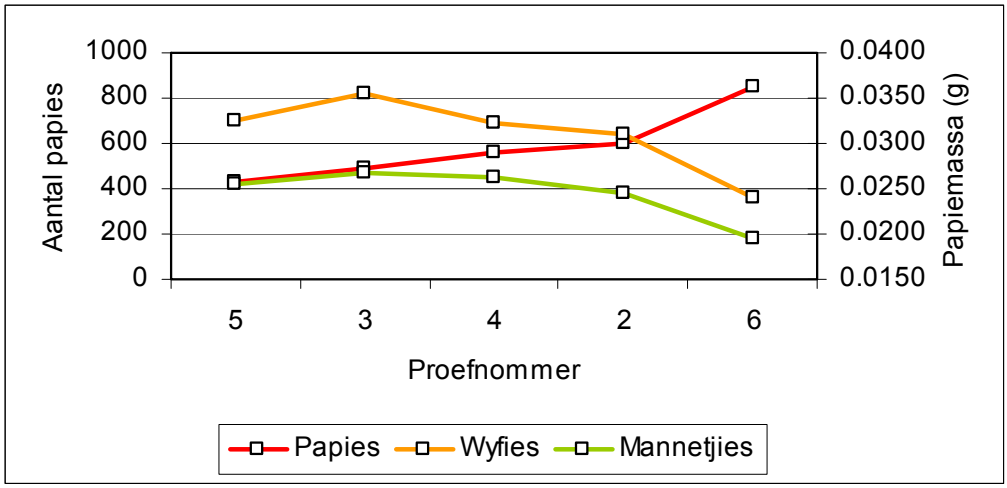

Fig. 3.2.3.13. Produksie van valskodlingmot in 500 ml inhoudsmaat blatjangflesse met 280 g dieët per fles.

Die invloed van grootte op die mededingendheid en lewensverwagting van verskillende groottes VKM is nie ondersoek nie. Gegewens wat in die VSA vir Pienkbolwurm, *Pectinophora gossypiella*, ingesamel is, dui daarop dat kleiner mannetjies gedurende die eerste paar dae na ontpopping goed met groter eweknieë meeding (Miller, pers. kom.). Hul lewensverwagting is egter nog nie ondersoek nie en dit is moontlik dat kleiner motjies nie so lank soos grotes sal lewe nie.

- **Alternatiewe pupeersubstraat**: Larwes in die bestaande insektaria pupeer in die watteproppe op die heuningflesse. Daar moet dus 'n alternatief ontwikkel word sodat larwes wat in die blatjangflesse volwassenheid bereik, plek vir pupering het. Daar is relatief min substrate wat vir dié doel geskik is. Slegs twee is die moeite werd geag om verder te ondersoek, naamlik vermikuliet en 'n heuningkoekmateriaal wat algemeen in die lugvaartbedryf gebruik word. Die vermikuliet was 'n goeie pupeersubstraat en die larwes het goed daarin pupeer. Dit is egter relatief duur om op groot skaal te gebruik aangesien dit nie hergebruik kan word nie. Dit moet ook in plat panne aan larwes blootgestel word, wat bykomende koste meebring. Die heuningkoek kan in verskillende materiale verkry word wat van vleklose staal tot aluminium, verskeie plastiekstowwe en aramiedpapied met fenoliese hars geïmpregneer, te kry is. Dit is gewoonlik in verskillende diktes en in 'n verskeidenheid selgroottes beskikbaar (Fig. 3.2.3.14).

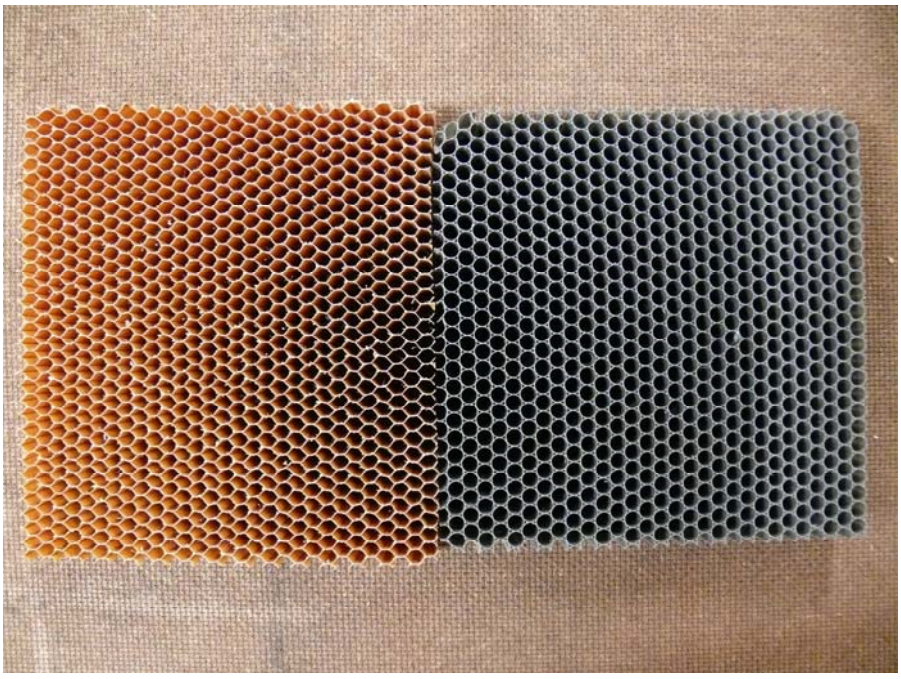

Fig. 3.2.3.14. Heuningkoekmateriaal vervaardig van aramiedpapier (links) en polipropileenplastiek (regs).

Die heuningkoekmateriaal het ook goed gewerk en die larwes het vrylik daarin pupeer (Fig. 3.2.3.15). Daar is dus besluit om belangrike aspekte van die teelstelsel om dié materiaal te ontwikkel. 'n Draadraammandjie is ontwerp waarin 25 dieëtgevulde teelflesse regop geplaas kan word. Wanneer die larwes gereed maak om te pupeer, word die flesdeksels verwyder en die flesse word op hul sye geplaas, sodat die larwes maklik daaruit kan klim. Die draadmandjies is só ontwerp dat 'n heuningkoekvel van geskikte grootte onder die flesse ingeskuif kan word. Waanneer die larwes die flesse verlaat, sak hulle direk met sydraadjies af op die heuningkoekvel en pupeer in die selle. Wanneer pupering afgehandel is, word die heuningkoekvel verwyder en in 'n motontpoppingskabinet geplaas sodat die papies 'n paar dae later kan ontpop. Dié stelsel is nog nie ten volle ontwikkel nie en aspekte soos die heuningkoekmateriaal se eienskappe en die draadmandjies word nog verfyn.

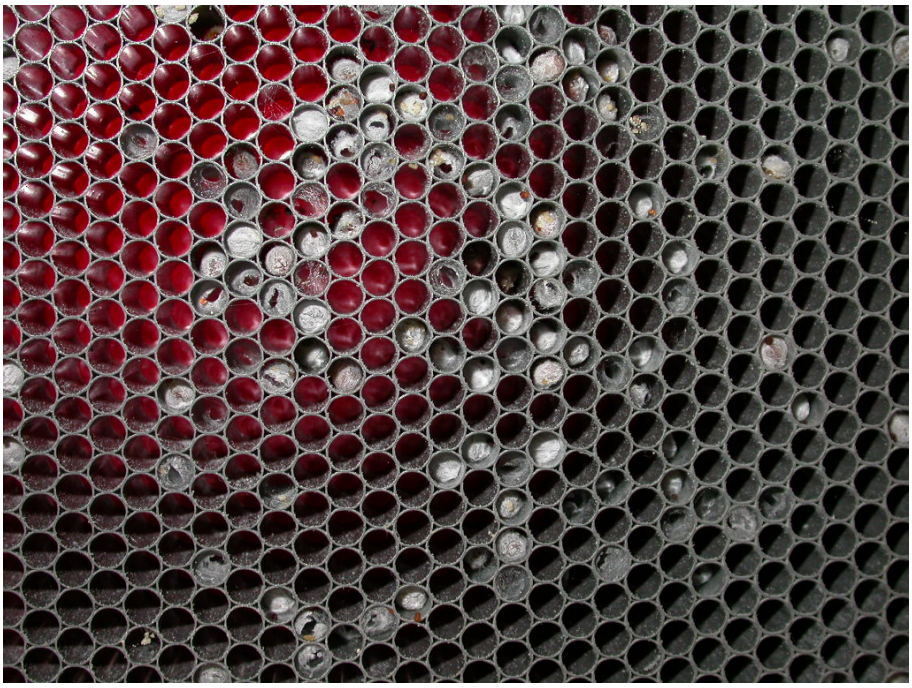

Fig. 3.2.3.15. Plastiekheuningkoek met valskodlingmotkokonne in die selle.

Daar is gedurende bogenoemde navorsing waargeneem dat die larwes, nadat hulle die teelflesse verlaat het, 'n instinktiewe drang het om te versprei en nie onmiddelik, selfs onder omstandighede wat as ideaal beskou word, wil begin om kokonne te spin nie. Dié gedrag van die larwes is nie tot die heuningkoekmateriaal beperk nie, maar is opgemerk terwyl ander pupeersubstrate ook getoets is. In proewe het tot 40% van die larwes die pupeersubstraat verlaat en weggeloop, wat uiteraard nie in die praktyk geduld kan word nie. 'n Reeks proewe is uitgevoer om toerusting te ontwikkel waarmee die larwes op die pupeersubstraat ingeperk kan word. Daar is gevind dat geen gladde oppervlakte of meganiese versperring van enige aard (ingesluit 'n versperring wat alreeds internasionaal vir die inperking van bolwurmlarwes gebruik was), VKM-larwes kan inperk nie. 'n Geslote houer sou uiteraard doeltreffend wees, maar dit is nie as prakties beskou om sulke toerusting te ontwikkel nie. Die enigste doeltreffende versperring wat getoets was, was 'n elektriese versperring. 'n Wisselstroom van nagenoeg 12V het die larwes doeltreffend sonder ooglopende nadelige gevolge afgeweer en gesorg dat hulle tot die pupeersubstraat beperk word. Ontwikkeling van dié versperring vir grootskaalse gebruik word voortgesit.

- **Motloslaattoerusting**: Die motte wat vir SIL geteel word, moet op een of ander wyse in die boorde losgelaat word. Sulke loslatings kan met die hand deur arbeiders, of meganies met 'n masjien, gedoen word. Eersgenoemde tegniek is gebruik om motte in die loodsprojek (Afd. A2) los te laat. Dit is egter arbeidsintensief en die tegniek sal nie koste-doeltreffend op 'n bevredigende manier op 6 000 ha sitrus toegepas kan word nie. Daar is dus begin om toerusting te ontwerp wat gebruik kan word om motte van 'n vierwielmotorfiets af los te laat. Soortgelyke toerusting word kommersieel in Kanada gebruik om Kodlingmot in 6 000 sagtevrugte deur middel van SIT te bestry. Een van dié masjiene (Fig. 3.2.3.16) is deur USDA-ARS vir navorsing aan CRI geskenk. Die toerusting is getoets, maar was ontoereikend en sou selfs met veranderinge nie vir die loslaat van VKM geskik wees nie. Die grootste enkele probleem was dat koue-geïmmobiliseerde Kodlingmot met die apparaat versprei word. Die motte kan nie vlieg wanneer hulle losgelaat word nie en beland op die grond. Hier ontdooi hulle mettertyd en soek dan skuiling. Wanneer die

motte in 'n aktiewe toestand in die apparaat geplaas word, klou hulle in bondels saam en verstop sodoende die toerusting.

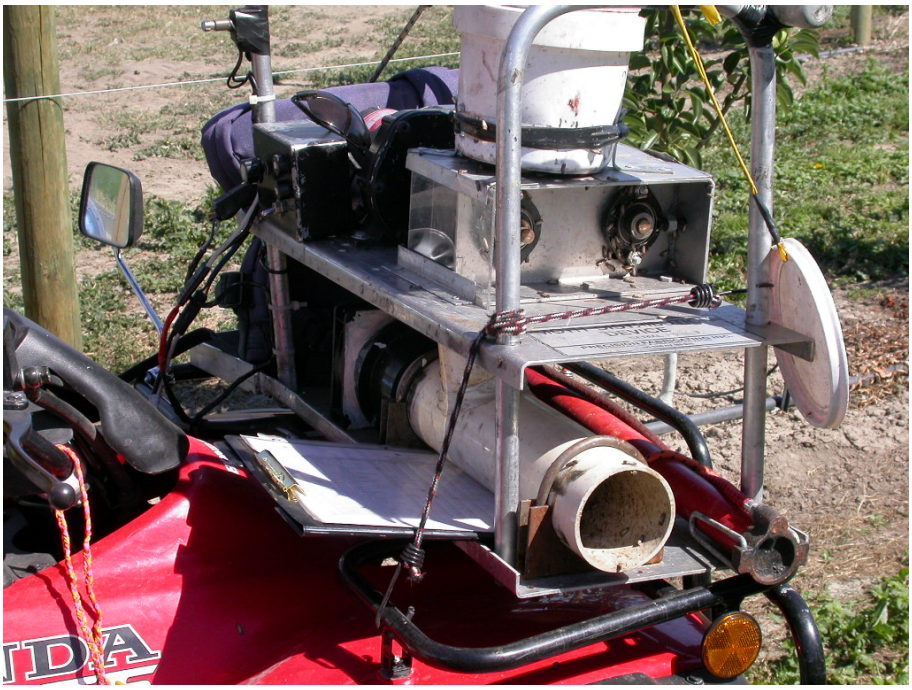

Fig. 3.2.3.16. Motloslaattoerusting op 'n vierwielmotorfiets gemonteer wat vir die loslaat van Kodlingmot in Kanadese appelboorde gebruik word.

VKM kan nie onder suider-Afrikaanse klimaatsomstandighede in 'n koue-geïmmobiliseerde toestand losgelaat word nie, aangesien hulle op die warm grond tussen die bome sal doodbrand voordat hulle hul beweegbaarheid kan herwin. Alternatiewe toerusting is daarom ontwerp waarmee die motte in hul aktiewe toestand losgelaat kan word (Fig. 3.2.3.17). Vollediger inligting oor dié apparaat sal bekend gemaak word wanneer sekere noodsaaklike veranderings aangebring is en dit behoorlik getoets is.

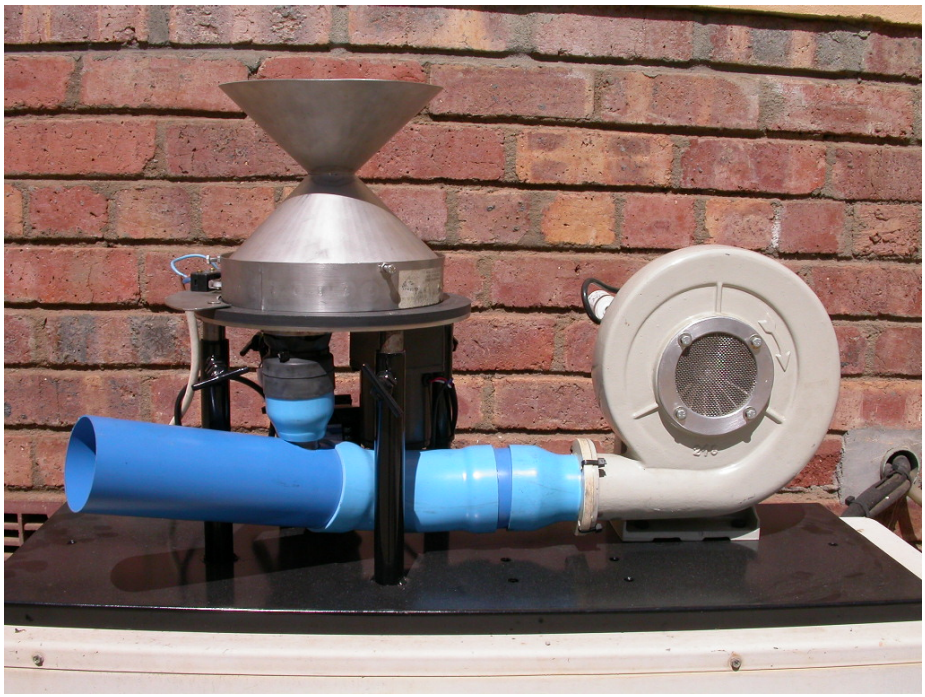

Fig. 3.2.3.17. 'n Apparaat vir die loslaat van gesteriliseerde valskodlingmot in sitrusboorde. Die toerusting word op 'n vierwielmotorfiets vasgebout.

- **Dieëtsterilisasie**: Dieëtgevulde teelflesse moet gesteriliseer word voordat VKM-eiers daarop ingeënt word. Dié proses word gewoonlik met behulp van outoklawe gedoen. Dié apparaat is egter te klein en te duur om in 'n massateelsituasie te gebruik. Die outoklawe in Ceder Biocontrol se insektarium te Citrusdal was gestel om 'n lugtemperatuur van 120°C te verskaf wat vir 20 minute teen nagenoeg 1,2 kg/cm² druk gehandhaaf moes word. Dit was nie moontlk om die dieëttemperatuur in die outoklawe te bepaal nie. 'n Konveksie-tipe oond is terselfdertyd vir 'n kort rukkie in die insektarium vir dieselfde doel gebruik. Gebruiksaanwysings vir die oond is op 'n subjektiewe wyse deur die insektariumbestuur benader en was grotendeels gebaseer op die voorkoms van die dieët na hittebehandeling, asook die sukses waarmee swam- en virusbesmettings daarna in die VKM-kultuur verhoed is. Enkele metings van lugtemperatuur gedurende die aanvangstydperk het daarop gedui dat die lugtemperatuur om en by 150°C was. Geen metings van dieëttemperature was moontlik nie. 'n Ondersoek is deur die skrywer geloods om die hittevereistes vir deeglike dieëtsterilisasie vas te stel. Sekere metings is uitgevoer, maar voordat die ondersoek afgehandel kon word, is die konveksie-oond deur die insektarium verkoop:

'n Digitale termometer met 'n teflon-bedekte termokoppel is gebruik om lug- en dieëttemperature in die oond gedurende die normale gebruik daarvan te meet. Die verhittingstyd het deurgaans 30 minute lank geduur, waarna die oond outomaties afgeskakel het. Die lug- en dieëttemperatuur in die oond het wesenlik verskil na gelang van die posisie van die termokoppel in die oond en hoeveel teelflesse daar in die oond was. Die metings het aangetoon dat die dieëttemperatuur gedurende die 30 minute verhittingstyd vinniger gestyg het indien die oond halfvol teelflesse gepak was, as wanneer dit volgepak was (Fig. 3.2.3.18). Die lugtemperatuur het binne 20 minute 'n maksimum van 160°C bereik en daarna gestabiliseer. Die teelflesse was egter veel kouer en die temperatuur daarin het aanhou styg gedurende die 30 minute voordat die oond afgeskakel het en geen verdere verhitting kon plaasvind nie. Die afkoelkurwe van die dieët in die flesse is nie ondersoek nie. Die lug- en dieëttemperature (volgepakte oond) het wesenlik van mekaar verskil – onderskeidelik ca. 165°C en 75°C. In 'n halfvol-gepakte oond het die dieëttemperatuur tot ongeveer 93°C gestyg. Goeie dieëtsterilisasie is deurgaans gehandhaaf, wat daarop dui dat 'n dieëttemperatuur van om en by 75°C waarskynlik voldoende is.

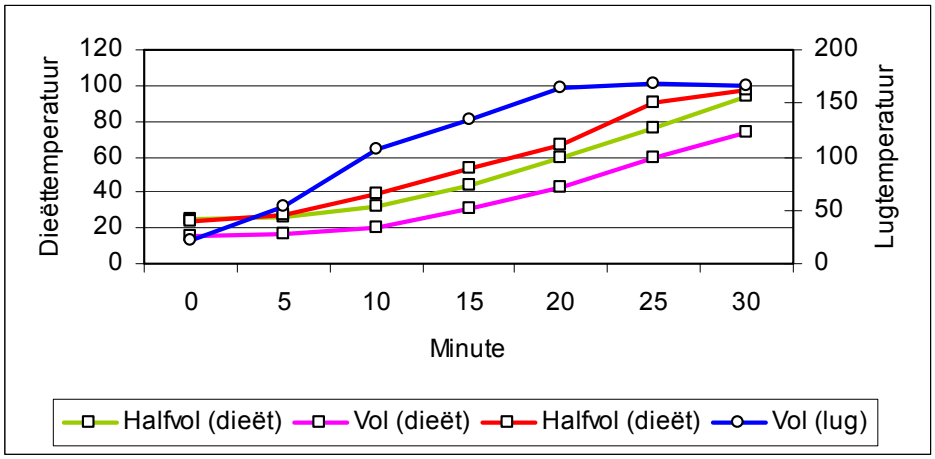

Fig. 3.2.3.18. Dieët- en lugtemperature in 'n konveksie-oond gedurende dieëtsterilisasie (groen, pers en blou = heuningflesse; Rooi = blatjangflesse). "Halfvol" en "vol" verwys na die aantal teelflesse wat in die oond gepak was.

Dit is interessant om daarop te let dat pasteurisasie plaasvind wanneer 'n produk tot 77°C vir 30 sekondes verhit word (temperatuur en tyd verskil effens afhangende van welke produk gepasteuriseer word). Die dieëttemperatuur van amper 75°C wat gemeet is, grens dus redelik naby aan wat as 'n gemiddelde pasteurisasieproses beskou kan word en is moontlik 'n aanduiding dat die uiteindelike behandeling wat vir dieëtsterilisasie ontwikkel word, nie veel sal verskil nie.

Daar word beoog om 'n dieselaangedrewe oond in die nuwe Xsit-insektarium in te bou. Bogenoemde inligting sal as basis dien vir 'n voortgesette ondersoek waarin die benodigde verhittingskedule noukeuriger vasgestel sal kan word. Dit sal tot gevolg hê dat spesifikasies vir die bedryf van die Xsit-oond opgestel sal kan word.

- **Teelfles-dekselmembrane**: Daar is vantevore gemeld dat ondersoeke geloods is wat ten doel gehad het om die watteproppe met 'n praktieser stelsel te vervang. Verskeie proewe is uitgevoer om die produksievermoë van die groter blatjangflesse met die bestaande heuningflesse te vergelyk (Fig. 3.2.3.19).

Die invloed van deksels met verskillende groottes gate daarin, op vogverlies uit die flesse, is in 'n volgende proef ondersoek.

Fig. 3.2.3.19. Toetsing van verskillende teelflesse, hoeveelheid dieët en deksel/membraankombinasies.

Gedurende die verloop van verskeie proewe is opgemerk dat die dekselmembrane van enkele flesse gedeeltelik gedurende die hittesterilisasieproses losgeskeur het. Dié membraanskeure het die vorm en grootte van die gat in die deksel gehad en die halflos stuk membraan is in die fles ingetrek. Daar word vermoed dat druk in die flesse opbou gedurende verhitting, die membrane nat word van die stoom in die flesse en dan skeur. Dit is onbekend waarom die geskeurde membraan in die fles ingetrek word – tensy dit slegs inkrul wanneer dit droog word. Dié probleem is ook ondersoek. Die volgende behandelings is getoets (Tabel 3.2.3.1):

Tabel 3.2.3.1. Verskillende behandelings om die invloed van los en vasgedraaide deksels op membraanbeskadiging en vogverlies uit teelflesse te ondersoek

Tipe fles	Flessluitstelsel	Grootte van gat in deksel	Deksel los of vasgedraai op fles
Heuningfles	Watteprop		
Blatjangfles	Sappi Stratoseal 80-membraan	-	-
Blatjangfles	Sappi Stratoseal 80-membraan	40 mm	Los
Blatjangfles		40 mm	Vas
	Sappi Stratoseal 80-membraan	40 mm	Los inmaakflesdeksel*
		50 mm	Los
	Sappi Stratoseal 80-membraan		

*Die los inmaakflesdeksel is ná hittebehandeling verwyder en met 'n Stratoseal 80-membraan deksel vervang.

Die flesse met dieët is op die gewone manier na hittebehandeling met VKM-eiers ingeënt en geïnkubeer. Die flesse is een keer per week op 'n analitiese weegskaal geweeg om die omvang van vogverliese te ondersoek.

Produksie in die verskillende behandelings was deurgaans goed en gemiddeld 850 VKM is per fles geproduseer. Die insekte was egter oor die algemeen klein (raadpleeg Proef A3b hierbo). Die dieët in die blatjangflesse wat met deksels met 40 mm deursnee gate toegemaak was, was effens vogtiger in die middel op die bodem van die flesse. Dié oortollige vog is nie by die flesse met 50 mm gate opgemerk nie. Die invloed van die groter gate is duidelik in die gemiddelde vogverlies uit die flesse te sien (Fig. 3.2.3.20).

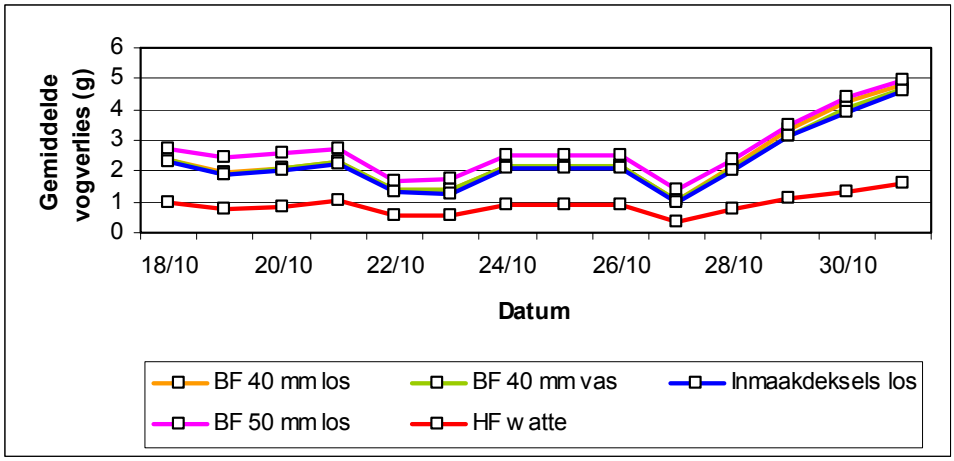

Fig. 3.2.3.20. Invloed van flesdekselgatgrootte op vogverlies uit teelflesse (BF = Blatjangfles; HF = Heuningfles).

Die stadiger vogverlies uit die blatjangflesse met die 40 mm dekselgate bevestig die waarneming dat die dieët onderin dié flesse natter was. Vogverlies deur die watteproppe op die heuningflesse kan as ideaal beskou word aangesien die larwes baie goed daarin aanteel en daar geen ooglopende nablywende vog aan die einde van larwe-ontwikkeling in die flesse gesien kan word nie. Vogverlies sou uiteraard baie stadiger geskied aangesien slegs nagenoeg 70 ml water in die heuningflesse gebruik word om in die dieët te meng (2x-dieët in die blatjangflesse, dus 140 ml water). Daar is alreeds daarop gewys dat te veel vog in die flesse larwe-produksie kan strem. Dit kom op dié tydstip voor asof die 50 mm gate in die flesdeksels beter as kleiner gate van oortollige vog ontslae kan raak.

Toekomstige navorsing

As gevolg van die dringendheid waarmee 'n geskikte, doeltreffende bestrydingmetode vir VKM gevind moet word, het die sitrusbedryf besluit dat SIT-navorsing so gou moontlik gekommersialiseer moet word. Daar is derhalwe in die tweede helfte van 2006 besluit dat 'n nuwe insektarium, wat vir die massateel van VKM geskik is, opgerig moet word. Dié insektarium moet teen die einde van 2007 in bedryf gestel word om VKM in nagenoeg 6 000 ha in die Olifantsriviergebied, Wes-Kaap, deur middel van SIL te onderdruk. Navoring gedurende 2007 sal derhalwe op die verdere ontwikkeling van toerusting wat vir VKM-teling in die insektarium gebruik moet word, toegespits wees.

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3.2.4 Development of a technique for mass rearing of FCM for SIT purposes

Experiment 689 by Sean Moore & Wayne Kirkman (CRI)

Opsomming

Die Steriele-insek Tegniek (SIT) vir valskodlingmot-bestryding word tans in die Citrusdalse sitrusproduserende gebied van die Wes-Kaap gekommersialiseer. 'n Belangrike vereiste om sukses met SIT te behaal is die ontwikkeling van 'n stelsel vir die massateel van baie groot getalle insekte. Die aantal VKM wat met huidige stelsels vir parasitoïed- en virusproduksie geteel kan word, is ontoereikend. In hierdie proef is die oppervlaksterilisasie van VKM-eiers met verskillende konsentrasies formalien vir verskillende tye ondersoek. Daar is bevestig dat oppervlaksterilisasie van VKM-eiers 'n baie belangrike stap in die massatelingsproses van VKM is en dat formalien 'n baie doeltreffende middel daarvoor is. 'n 10% oplossing van 37% aktiewe bestandeel formalien, toegedien vir 10 minute, was die doeltreffendste behandeling vir beide swam- en virusbeheer sonder om die oorlewing en ontwikkeling van die eiers aan te tas. Geen verdere werk word op hierdie onderwerp beplan nie.

Introduction

The sterile insect technique (SIT) for false codling moth (FCM) control is in the process of being commercialised for use in the Citrusdal citrus growing region of the Western Cape. An important prerequisite to achieving success with SIT is to develop a system for mass rearing extraordinarily large numbers of insects. The numbers of FCM that can be produced with currently known systems, used for parasitoid production, are inadequate. Improvements in FCM mass rearing techniques have already been made (see exp. 402; Moore & Richards, 2000 & 2001; Moore, 2002). However, these changes were not made specifically with SIT in mind. Improvements geared specifically towards SIT have been investigated and developed since 2002 (Moore *et al.*, 2002; 2003; 2004 & 2005). Changes are aimed at increasing production while keeping labour inputs and costs as low as possible.

Materials and methods

Formalin is currently used commercially at 25% (of 37% active ingredient) in water for surface sterilising FCM eggs, before inoculation into diet jars. A trial was conducted to test whether formalin can be used at lower concentrations, albeit for longer periods of time (Table 3.2.4.1).

Table 3.2.4.1. Concentrations and duration of formalin dip treatments for surface-sterilisation of FCM eggs

Treatment no	Formalin concentration (as % of 37% active ingredient)	Duration of treatment (minutes)
1	Untreated control	-
2	2	2
3	2	5
4	2	10
5	5	2
6	5	5
7	5	10
8	10	2
9	10	5
10	10	10

Four egg squares, ca. 400 mm^2, each containing approximately 100 eggs, were subjected to each treatment. Two of the egg squares were placed into Petri dishes to allow eggs to hatch. Percentage egg hatch was determined after several days, allowing sufficient time for all possible egg hatch to occur. The other two egg squares were placed directly onto potato dextrose agar (PDA) plates underneath a laminar flow hood. Lids were placed onto these plates, which were kept at 27°C. After a week, fungal growth on the PDA was recorded.

A second trial was conducted in which egg squares were dipped into 5%, 10% and 15% solutions of 37% formalin, for 10 minutes each. In an effort to simulate the process intended to be used for mass production of moths for SIT, whole egg sheets (i.e. before cutting the sheets into squares for inoculation into diet jars) were dipped into the formalin solutions. These were then cut into squares, using sterile equipment, after drying in the laminar flow cabinet. The egg squares were then inoculated onto diet in honey jars. Once the larvae, which hatched from the eggs, had developed and pupated, levels of fungal and viral contamination were recorded. Jars and their stoppers, containing pupae, were placed into emergence boxes and emerged moths were counted.

Results and discussion

In the first trial, the number of hatched eggs were counted for each treatment (two egg squares per treatment), and observations of fungal contamination were made (also two egg squares per treatment) (Table 3.2.4.2).

Table 3.2.4.2. Efficacy of formalin treatments in controlling fungal growth and the safety of these treatments to FCM

Treatment no	Formalin concentration	Treatment duration (minutes)	Total eggs	Egg mortality (%)	Fungal on PDA from egg squares
1	Untreated control	-	105	6.7	Heavily contaminated, mostly black, some green
2	2	2	124	5.6	Mostly black, more green than the control
3	2	5	64	7.8	Mostly green fungus with a little black
4	2	10	93	5.4	Some blue, mostly green
5	5	2	92	7.6	Moderate, mostly green, one spot of blue
6	5	5	133	8.3	Moderate, green only
7	5	10	81	9.9	One sheet green fungus, the other clean
8	10	2	104	7.7	One sheet green fungus, the other clean
9	10	5	107	13.1	No fungal contamination
10	10	10	82	12.2	One blue spot on one sheet

The lower concentrations only controlled the black fungus (Table 3.2.4.2, Fig. 3.2.4.1 & 3.2.4.2). Five minutes in 10% formalin, controlled all fungi (Fig 3.2.4.3). There was also only a small spot of blue fungal development in treatment 10 (10% formalin for 10 minutes) when the observations were made after a week. The photographs were taken a day later and the fungus had been spread by emerging larvae (Fig.3.2.4.3). It is also relevant to note that the blue fungus does not appear as a contaminant in FCM cultures, and its presence in some of the treatments might therefore be considered to be of negligible importance.

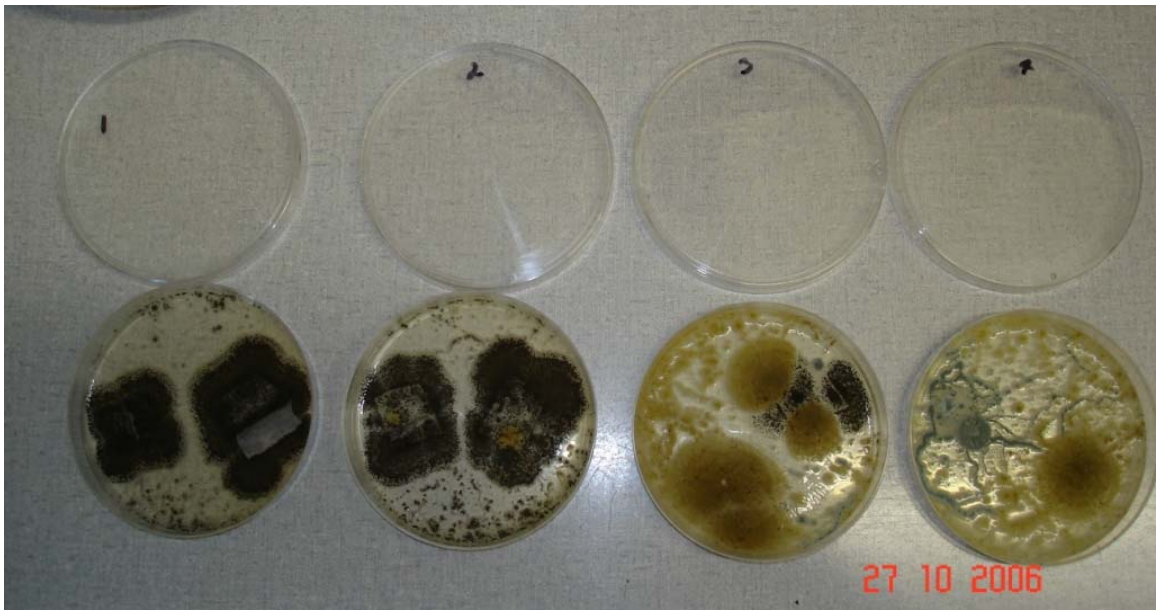

Fig 3.2.4.1. Fungal growth on PDA from FCM egg squares, after being subjected to treatments 1, 2, 3 and 4 (Table 3.2.4.1 & 2).

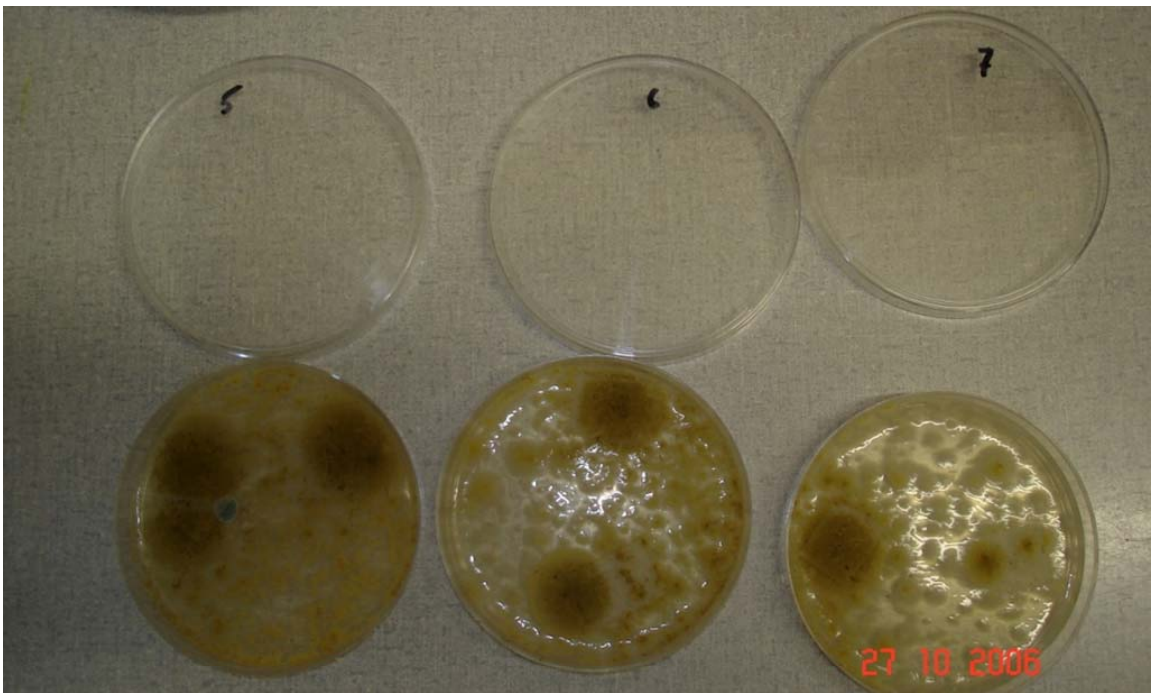

Fig 3.2.4.2. Fungal growth on PDA from FCM egg squares, after being subjected to treatments 5, 6 and 7 (Table 3.2.4.1 & 2).

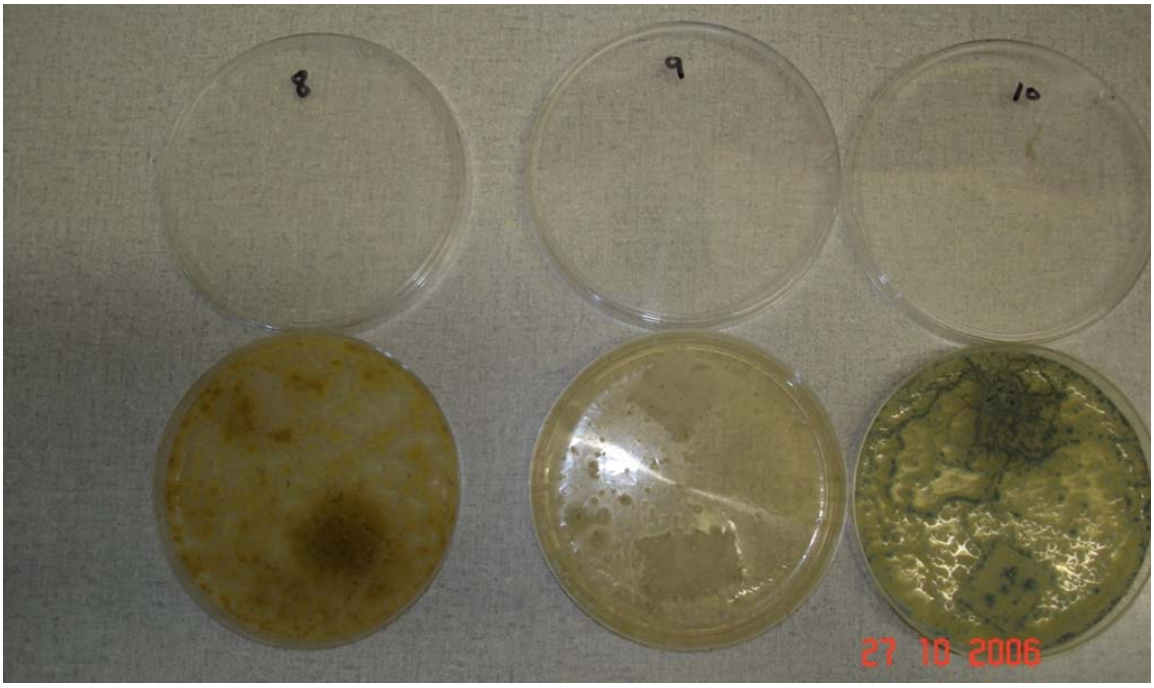

Fig 3.2.4.3. Fungal growth on PDA from FCM egg squares, after being subjected to treatments 8, 9, and 10 (Table 3.2.4.1 & 2).

Table 3.2.4.3. Development of larvae in diet after different egg treatments with formalin and associated fungal and viral contamination

Treatment no	Concentration of formalin (dipped for 10 min)	No of rearing jars	Mean no of moths per jar	Contamination
1	Untreated	6	Very few	One jar virus, 3 jars fungus and virus, 2 jars fungus
2	5%	8	206.6	2 jars fungus, jars well populated
3	10%	8	241.5	No fungus or virus, jars well populated
4	15%	8	No count due to escape	No fungus or virus, jars slightly less populated.

In the second trial, all the jars in the untreated control developed either viral or fungal contamination or both. Very few moths emerged from these jars. When 5% formalin was used, 25% of the jars developed fungal contamination (Table 3.2.4.3). The mean number of moths emerging per jar from this treatment was acceptable. However, the level of fungal contamination would not be acceptable in a commercial environment. When 15% formalin was used, conspicuously fewer larvae developed. Unfortunately moths escaped from the emergence box, so counts of emerged moths could not be conducted. From this trial it would appear that the best option would be to dip the egg squares in 10% formalin for 10 minutes. The mean number of moths per rearing jar was 241.5 and no fungal or viral contamination developed.

Another interesting observation was the high incidence of fungal and viral contamination where egg squares were not surface-sterilised (Fig 3.2.4.4). All the rearing jars developed fungal or viral contamination or both. There was also virtually no larval development and moth emergence. This highlighted the critical importance of surface-sterilisation of egg sheets in the mass rearing process.

Fig 3.2.4.4. Rearing jars where no egg sheet sterilization took place.

Conclusion

Surface-sterilisation of egg sheets is an imperative step in the process of mass rearing FCM. Formalin is a highly effective option for surface-sterilisation of FCM eggs. A 10% solution of 37% active ingredient formalin appeared to be the most effective treatment, both in reducing fungal and viral contamination and in not compromising FCM survival.

Future research

No further work is planned on this experiment.

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3.2.5 Understanding and improving biological control of false codling moth larvae

Experiment 690 by Kierryn Gendall (RU), Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

Die doel van hierdie proef was om die doeltreffendheid van VKM-larweparasitoïede te ondersoek en indien moontlik, om hulle vir die beheer van VKM te gebruik. In totaal is 7100 nawellemoene van Desember 2005 tot Junie 2006 versamel en vir VKM-larwes ondersoek. Larwebesmetting is in 32.91% van die lemoene opgemerk. Die hoogste getalle parasitoïede is in derde en vierde instar larwes gekry. Die hoogste vlakke van parasitisme is laat in die seisoen, van laat-April tot Juniemaand, opgemerk. 'n Gemiddeld van 9.99% larwes is oor die volle tydperk van die opname geparasiteer. Met massateling van *Agathis bishopi* is meer mannetjies as wyfies gekry. VKM-larwes wat in die dieët gepupeer het, het meer wyfieparasitoïede geproduseer. VKM-larwes wat in watteproppe gepupeer het, het meer mannetjieparasitoïede geproduseer. Ongelyke geslagsverhoudings (1W:2M en 2W:1M) in die parasitoïedouers het meer wyfienageslag per dieëtfles geproduseer. Meer mannetjies is per teelfles geproduseer toe die aantal parasitoïedouerpare van een tot twee per fles verhoog is. Drie groot probleme is teëgekom met die massateling van *A. bishopi*. Eerstens het die uitbroeiïng van wyfie-en mannetjieparasitoïede uit larwes wat van vrugte gekry is, nie saamgeval nie. Dit het veroorsaak dat baie wyfies nie bevrug is nie en daarom is net mannetjies in die nageslag gekry. Tweedens is 'n hoë vlak van viruskontaminasie in die kultuur gekry. Derdens en die belangrikste, is 'n hoë vlak van *Aspergillus* sp. swamkontaminasie in die kultuur gekry. Navorsing in die toekoms sal fokus op die voltooiïng van studies op die biologie van *A. bishopi*, die verbetering van massateeltegnieke en boordvrylating van parasitoïede.

Introduction

Much emphasis has been placed on studying and exploiting the egg parasitoid of FCM, *Trichogrammatoidea cryptophlebiae*. The next step in advancing the biological control of FCM is to examine the potential for improvement of control of the larval stage. A total of nine larval or egg-larval parasitoids have been identified from FCM on citrus in southern Africa. Six of these species occur in South Africa. Larval parasitoids of FCM have been discussed by Ullyett (1939), CIBC (1984) and Prinsloo (1984). They speculated that, perhaps due to the inaccessibility of the host, they do not seem to be important mortality factors. Ullyett (1939) found that many of the larval parasitoids were poorly distributed and suggested the exchange of parasitoids between the different provinces of South Africa. However, the distribution, seasonal occurrence and effectiveness of these parasitoids are not sufficiently clear. Knowledge of the natural enemies of a pest species and the control they exert is important when considering commercial control measures. Such a survey may lead to the translocation of one or more species of parasitoid from one area to another or a parasitoid augmentation programme.

A preliminary survey on FCM larval parasitoids was conducted in the Eastern Cape, Western Cape and Mpumalanga from December 2001 to May 2002 (Sishuba *et al*., 2002; Sishuba, 2003). Two parasitoids were reared from FCM larvae in this study: *Agathis bishopi* and *Apophua leucotretae*. *Agathis bishopi* was

the more abundant of the two and appeared to be a valuable parasitoid of FCM on citrus, but was only found in the Eastern Cape. *Agathis bishopi* and *T. cryptophlebiae* seemed to compliment each other. *Agathis bishopi* exhibited high parasitism rates early in the season, at a time when *T. cryptophlebiae* was either absent or at very low levels. Egg parasitism increased in the latter part of the season when the larval parasitoid was at low levels. It is interesting, therefore, to speculate on the effect of releasing large numbers of the larval parasitoids in the latter part of the season and the egg parasitoid in the early part of the season, when wild populations of the parasitoids are often low. Because these surveys were only conducted monthly, during the 2003/04 season weekly surveys of larval parasitoids were conducted in an Eastern Cape navel orange orchard with consistently high levels of FCM activity (Moore & Kirkman, 2003 & 2004). This survey confirmed the total dominance of *A. bishopi* in the Eastern Cape. Subsequently, mass collection and rearing of *A. bishopi* was conducted, accompanied by the initiation of studies on the biology of the parasitoid (Moore & Kirkman, 2005). Further and more detailed work on these aspects is recounted in this report. The bulk of this work has been conducted by the senior author, towards her MSc thesis.

Materials and methods

Parasitoid collection

To obtain a culture of *A. bishopi* wasps, it was necessary to collect parasitoids from the field. To achieve this, navel oranges were collected from farms in the Sunday's River Valley area from January 2006 to June 2006, on a weekly basis. They were collected both from the ground and picked off trees. Fruit collected off the trees were only picked if the entrance hole and discolouring around the hole was obvious, thereby indicating that the fruit was infested. The fruit were placed in crates and transported back to the laboratory.

Diet was prepared for rearing the larvae to adulthood. The diet contained the following: maize meal (2000 g), wheat germ (200 g), Brewers yeast (100 g), milk powder (36.5 g), nipagin (15 g) and sorbic acid (6.5 g) (Moore, 2002). A total of 200 g of the diet was placed in a glass dish with 200 ml of double distilled (dd)H_2O. The dish was then covered with foil and baked in an oven at 180°C for 25 minutes, and then placed in a laminar flow hood to cool. The medium was transferred to a glass vial by placing the vial upside down in the diet, forming a plug (40 g) of diet. The plug was then pushed to the bottom of the vial.

Fig. 3.2.5.1. Dissecting a navel orange looking for FCM larvae (Photo: K. Gendall).

Fruit were dissected by cutting thin slices around the point of entrance (Fig. 3.2.5.1). Once the larvae could be seen the fruit was forced open and the larva was removed to a glass vial containing the diet. A cotton wool plug was inserted into the mouth of the vial. The cotton wool plug was used as the pupation substrate for larvae (Fig. 3.2.5.2).

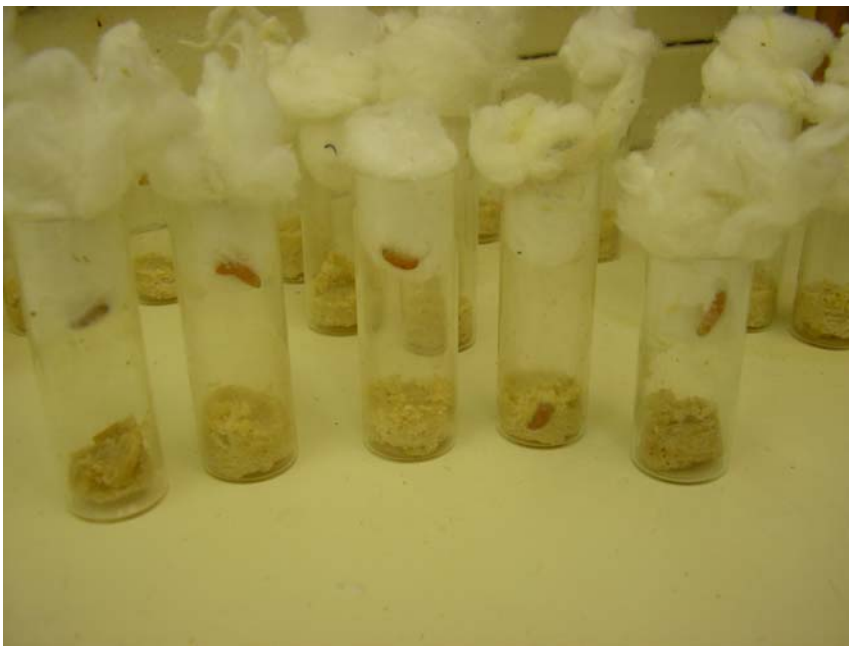

Fig. 3.2.5.2. The FCM larvae in the glass vials with the diet and cotton wool plugs (Photo: K. Gendall).

The following were recorded: the instar from which the parasitoids emerged, the percentage of larvae parasitized, and the ratio of female to male parasitoids.

Rearing and biology of *A. bishopi*

Diet (20 g) plus ddH_2O (20 ml) was placed in a honey jar and autoclaved at 120^oC for 20 minutes. Sixteen jars were prepared at a time.

Sheets of FCM eggs were cut into smaller pieces containing approximately 100 FCM eggs each. The sheets were then sterilized by placing them in 15% Sporekill (didecyldimethylammonium chloride) for 15 minutes and then dipping them in 25% formalin (37% a.i.) for 3 seconds. One piece was placed into each honey jar and placed at 27°C.

The vials were checked daily for the emergence of parasitoids. Emerged parasitoids were removed to a honey jar, containing diet and neonate larvae (approximately 100). At the beginning of the mass rearing process one female was placed with one male in the honey jar.

The parasitoids were left in the jars for 3-5 days, and were then transferred to a new jar, containing diet and larvae as before. When the parasitoids were removed, the lid was replaced with a cotton wool plug. When the larvae had pupated in the cotton wool plug (Fig. 3.2.5.3) they were removed from the jar, placed in an emergence jar and fclosed with a lid with holes. After 10-14 days the emergence jars and the honey jars were checked daily for the emergence of parasitoids.

Fig. 3.2.5.3. FCM larvae pupating in a cotton wool plug.

When the numbers of parasitoids in the culture increased, the newly-emerged parasitoids were placed in mating jars before being placed in the honey jar. This was to ensure that the females had mated before they began parasitizing the FCM larvae. Once mated the parasitoids were placed in the honey jars with varying parent ratios (1:1, 2:2, 1:2 and 2:1).

The following was recorded: the sex of the progeny, the numbers of emerged male and female parasitoids and the substrate in which the host larva was pupating when the parasitoid emerged. The host could pupate in either the cotton wool plug or in the artificial diet.

Results and discussion

Parasitoid collection

In total, 7100 navel oranges were collected and dissected for FCM larvae. A total of 2337 larvae were obtained (Table 3.2.5.1). This means that 32.91% of the oranges were infested with FCM larvae.

Table 3.2.5.1. FCM infestation of navel oranges collected from the Sundays River Valley

Collection month	Number of fruit collected	Fruit infested (%)	Number of each larval instar					Total number of larvae
			1	2	3	4	5	
26/01/2006	347	23	0	8	22	18	31	79
02/02/2006	204	33	0	2	12	18	36	68
09/02/2006	238	32	0	5	22	23	26	76
23/02/2006	678	48	2	55	104	95	68	324
02/03/2006	326	39	0	35	38	37	18	128
09/03/2006	803	43	0	56	90	106	93	345
16/03/2006	475	31	0	36	39	45	27	147
30/03/2006	476	45	0	29	52	63	70	214
06/04/2006	866	32	0	20	80	72	99	271
13/04/2006	631	37	0	10	61	69	94	234
20/04/2006	490	38	0	26	60	57	44	187
11/05/2006	404	37	0	35	41	47	28	151
18/05/2006	486	23	0	12	37	24	40	113
25/05/2006	382	37	13	24	53	32	21	143
08/06/2006	294	51	12	27	62	20	29	150

The majority of the larvae were in instars 3, 4 and 5 (Fig. 3.2.5.4). The instars were estimated according to the size (length) and colour of the larvae. The low number of first instar larvae recorded may have been due to greater difficulty in finding such small and inconspicuous larvae.

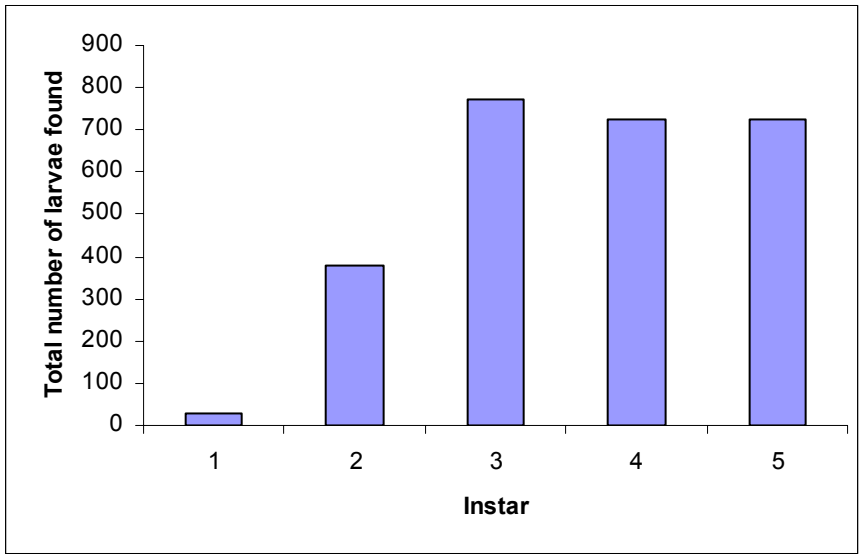

Fig. 3.2.5.4. The number of FCM larvae or each instar found in navel oranges.

Instars 3 and 4 yielded the highest number of parasitoids; instars 1 and 2 yielded very few and instar 5 yielded no parasitoids (Fig. 3.2.5.5). There was no emergence of parasitoids from larvae older than the 4th instar. The reason for this could be that the parasitoid retards host development so that larvae appear younger than they are. Stressed or threatened larvae (including parasitized larvae) are also known to pupate earlier than usual, even if this means omitting the final instar. From these results it can be said that it is not necessary to consider 5th instar larvae in a survey or quest for larval parasitoids of FCM.

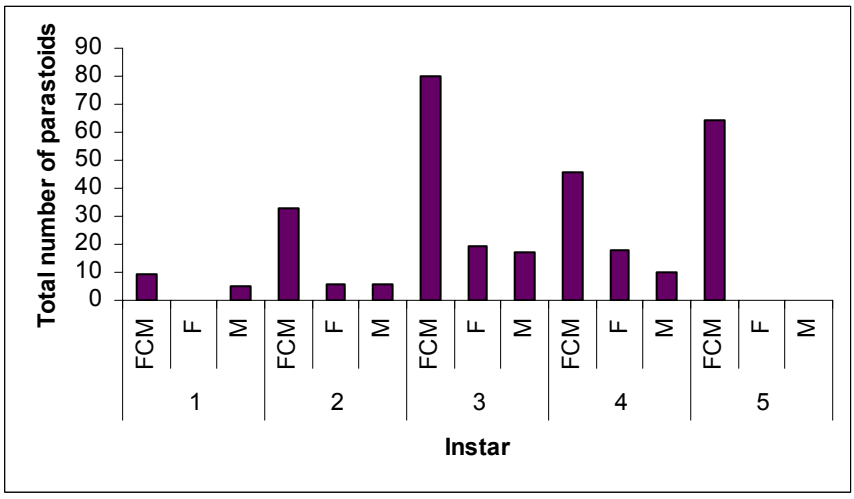

Fig. 3.2.5.5. The total number of parasitoids found at each larval instar.

The highest parasitism rates seemed to have occurred in the latter half of the season (late April to June) with an average of 9.99% of the larvae from all collections, being parasitized (Fig 3.2.5.6). The reason for the variation in parasitism may be due to fluctuations in the FCM population, changes in environmental conditions (rain, temperature and humidity), application of chemical sprays or orchard sanitation in the early part of the season, which can have an adverse effect on parasitoids (Ullyett 1939). Total parasitism recorded could have been underestimated, due to premature mortality of parasitized larvae, preventing parasitoids from completing their development.

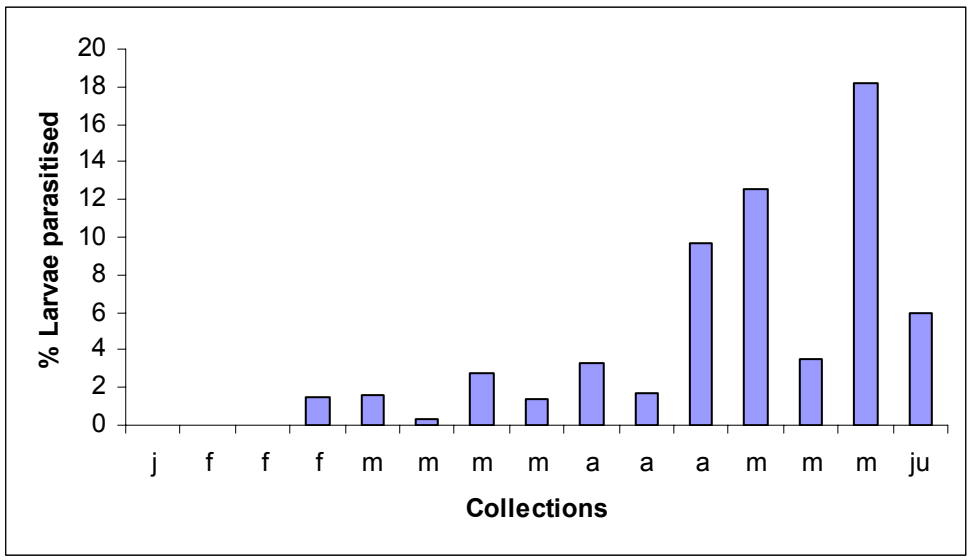

Fig. 3.2.5.6. Percentage of larvae parasitized over all the collections from 26 January to 8 June 2006.

From the parasitoids yielded from the collection of fruit, 39% were females and 41% were males. This gives a natural ratio of females to males of 1:0.95 – close to unity.

Rearing and biology of *A. bishopi*

A culture of *A. bishopi* was started from the parasitoids collected. In general, more male than female parasitoids emerged from parasitized larvae. However, there was no statistical difference between the two ($p>0.05$) (Fig 3.2.5.7). This indicates that this wasp has a male biased sex ratio, but it could also be due to some females having not mated before parasitizing the larvae – therefore reproducing pathenogenetically, meaning that only males would be produced.

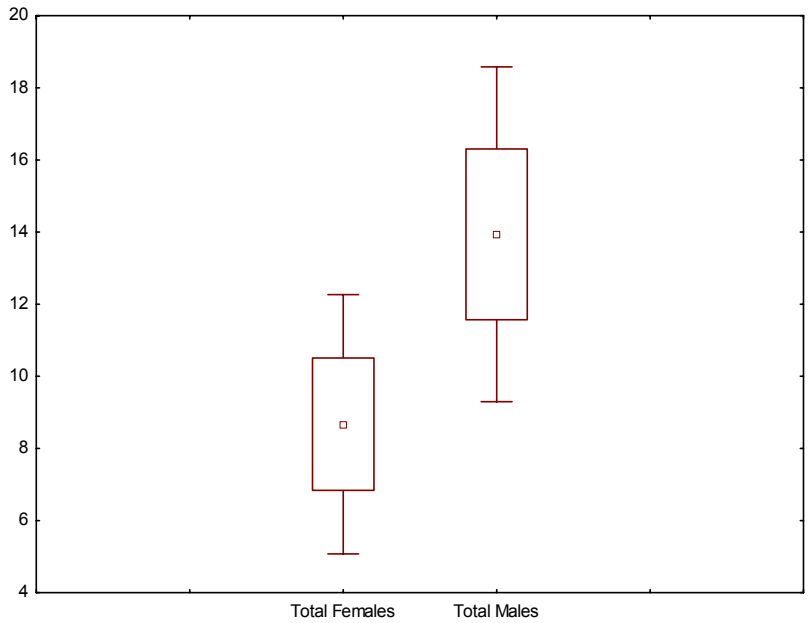

Fig. 3.2.5.7. The average number of female and male parasitoids produced per honey jar.

Varying ratios of female and male parasitoids emerged from hosts pupating in cotton wool and artificial diet. On average, more females emerged from the diet but there is no statistical difference ($P>0.05$) between the emergence of females from the diet compared to the cotton wool. Observations show that more females emerge from the diet. This could be due to the female parasitoids taking longer to develop and by then the cotton wool plug has been removed and so they pupate in the diet. Significantly more males ($P<0.05$) emerge from the cotton wool (Fig. 3.2.5.8). This could be due to the fact that they require a shorter time to develop therefore they pupate in the cotton wool plug before it is removed.

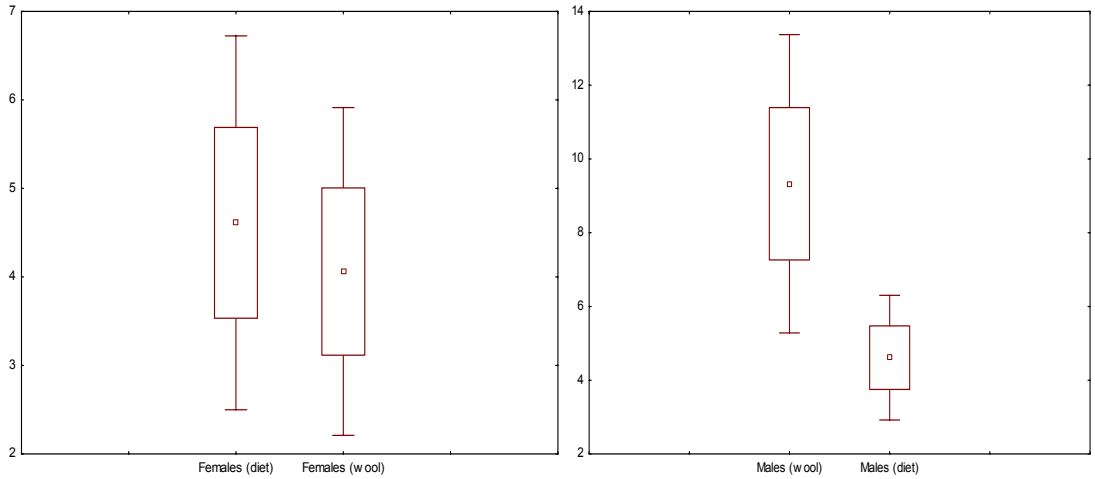

Fig. 3.2.5.8. The average number of females and males emerging from the cotton wool verses the diet.

In rearing trials with the parasitoids, four parent sex ratios (F:M) were used, 1:1, 2:2, 1:2 and 2:1. The ratios 1F:2M and 2F:1M gave the best results for a mass rearing situation by producing more females than males. The ratio 1F:1M produced the least favourable results (Fig 3.2.5.9). In a mass-rearing situation, a female-biased sex ratio is preferable, particularly where field releases of parasitoids are being conducted.

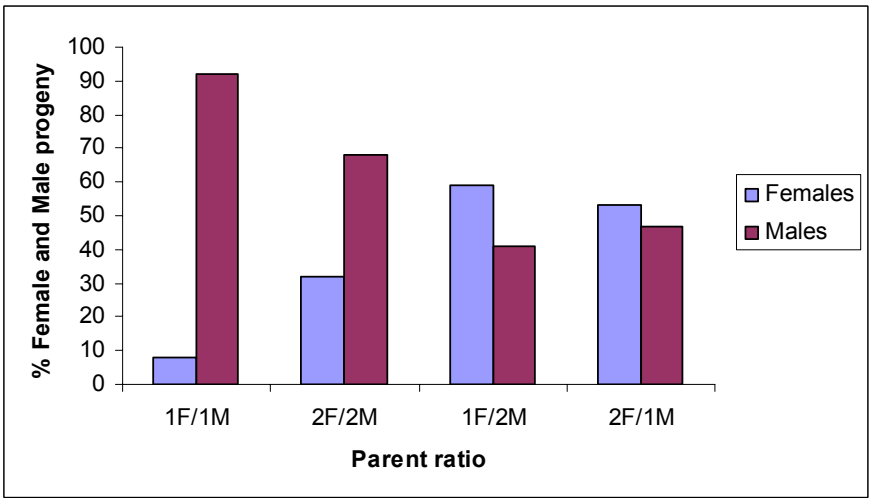

Fig. 3.2.5.9. The percentage of female and male progeny yielded by the various parent ratios.

There was no statistical difference between the number of female and male progeny for the parent ratios 1F:1M (P = 0.07), 1F:2M (P = 0.27) and 2F:1M (P = 0.43). For the parent ratio 2F:2M there is a statistical difference, with more male progeny being produced (P = 0.03). When looking at a graphic display of the results (Fig. 3.2.5.9) one would expect there to be a statistical difference between the number of female and male progeny for the parent ratio 1F:1M. The raw data shows that there are only three points where there is a major difference in the number of female and male progeny. This variation must explain why there is no statistically significant difference between the numbers of males and females produced by the 1F:1M parent sex ratio. The reason for these three points of high difference between numbers of male and female offspring, could be that at the start of the study the females had not mated. Therefore, only male progeny were produced. If these three points are removed due to this explanation, a far smaller difference in the sex ratio of the offspring is observed (Fig. 3.2.5.10).

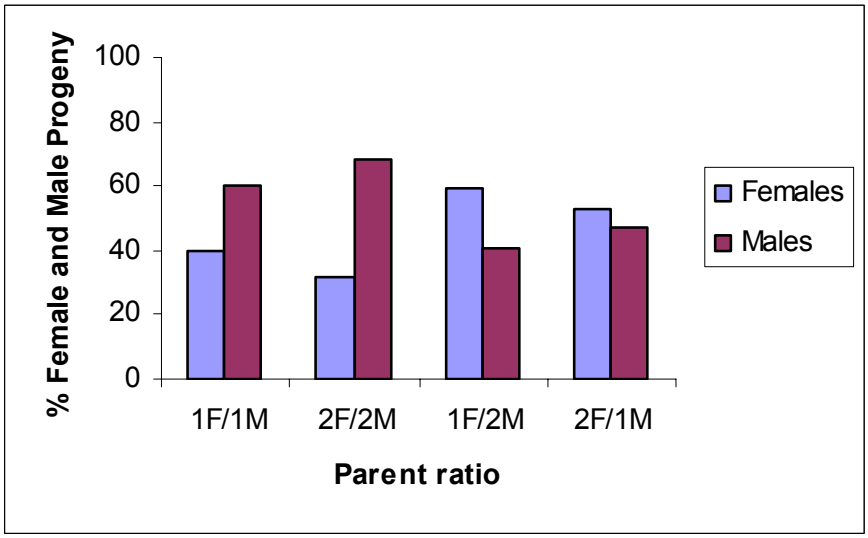

Fig. 3.2.5.10. The percentage of female and male progeny yielded by the various parent ratios, with the three most extreme data points from 1F:1M being removed from the data set.

The graph shows a closer relationship between the number of female and male progeny for the parent ratio 1F:1M (Fig. 3.2.5.10). There is still no statistical difference between numbers of male and female offspring (P = 0.68). However, this is now expected.

Three major problems were encountered in the mass rearing of *A. bishopi*. Firstly, the emergence of females and males from the larvae found in the fruit was not synchronised. Males emerged a few days before females, resulting in many of the females not being mated and therefore producing only male progeny. Females also emerged a few days after males emerged.

Secondly, a virus infected the culture. The virus is presumed to have been the *Cryptophlebia leucotreta* granulovirus, which affects the FCM larvae, killing them before the parasitoids can emerge. Insect viruses cause the insect to lose its appetite and become flaccid, which causes eventual death (Madigan *et al* 2003). The virus killed the majority of the larvae and affected approximately two out of three bottles. This reduced the size of the culture by two thirds, making the mass rearing process slow. This was eventually overcome by improved egg sterilization. This did not eradicate the virus, but did reduce its occurrence to about one out of 15 bottles, therefore having little effect overall.

Thirdly, the biggest problem was an *Aspergillus* fungus affecting the culture. It becomes obvious in the bottles just prior to pupation, and has disastrous affects on the parasitoid population. It mainly affected the diet, killing larvae and pupae. The effect of this was that no parasitoids emerged from the diet. This had a major effect on the female parasitoids numbers, as females emerged mainly from the diet, where the fungus was most prolific. This caused a rapid decline in the parasitoids population as the fungus affected almost one out of every two bottles. Approximately 10-15 jars were lost daily to the fungus contamination. From the time the fungus was first observed, it took approximately 1-2 days before the entire diet was affected, so preventing emergence of parasitoids and moths.

It was observed that fungal growth started on the bodies of larvae, which had been killed by virus. The larval cadavers were removed daily. However, some larvae died within the diet and so could not be observed or reached and therefore gave rise to the fungus. *Aspergillus* species belong to the group Deuteromycetes and their habitat is soil, decaying plant material and surfaces of animal bodies (Madigan, *et al.* 1999). It is therefore possible that the larvae could be carrying the fungus, in a dormant state. This needs investigation. It is also possible that the honey and water solution, provided to the parasitoids as food, could be a substrate for fungal growth. Other food sources (*i.e*. glucose, sugar solution, glycerol) will be investigated to see if they are adequately nutritious and if any fungal contamination occurs in these jars. Sugar alcohols, such as glycerol, are known to suppress microbes.

Conclusion

The parasitoid, *A. bishopi*, has potential to be a successful biological control agent. In the Sundays River Valley, it appears to be aiding in reducing FCM populations in citrus orchards. *A. bishopi* has the potential to be mass reared on a commercial scale, once hurdles such as viral and fungal contamination have been

resolved. Studies on the biology of the parasitoid will enable us to more effectively rear *A. bishopi* on a commercial scale and consequently exploit it as a biocontrol agent in certain citrus production areas.

Future research

Future research involves completing the biological studies on the parasitoid and finding a more effective method of mass rearing it. Once this is achieved, mass releases will be carried out in the Citrusdal area and in Mpumalanga. Establishment of the wasp will be assessed. Non-target effect tests against other lepidopteran species (which are also commercial pests), such as *Helicoverpa armigera, Cydia pomonella, Plutella xylostella* can also be executed. Scanning electron microscope studies will also be conducted to determine egg loads of gravid female parasitoids and development rates of parasitoids.

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3.2.6 Investigation of alternative hosts for FCM

Experiment 743 by Sean Moore and Wayne Kirkman (CRI)

Opsomming

Dit is onseker watter invloed alternatiewe gasheerplante aangrensend aan sitrusboorde op VKM-bevolkings in daardie boorde het. Opnames is op 'n gereelde basis in drie streke in die Oos-Kaap uitgevoer. Vrugte, galle en vlesige gedeeltes van moontlike alternatiewe gashere vir VKM is versamel. Monsters van elk is vir tekens van VKM ondersoek. Ander monsters is aan dagoue VKM-larwes blootgestel en twee weke later vir tekens van besmetting, indringing en larwale ontwikkeling ondersoek. VKM-larwes is in *Crassula ovata*,

Ricinus communis, *Schotia afra, Opuntia ficus-indica, Asparagus crassicladus, Solanum tomentosum, Passiflora caerulea, en Albuca sp.* gevind. Opnames is gedurende 2005 klaargemaak, maar beheermaatreels vir dié plante is ondersoek en word saam met die resultate in hierdie verslag bespreek. Die studie is nou voltooi.

Introduction

The false codling moth (FCM), *Thaumatotibia* (=*Cryptophlebia) leucotreta*, is an important pest of citrus, stone fruit, macadamias, avocados, peppers and various other agricultural crops. FCM causes an estimated loss of around R100 million to the southern African citrus industry annually.

Much emphasis is placed on the importance of orchard sanitation to keep FCM under control. However, little is known of alternative hosts for FCM. In the presence of alternative hosts, even diligent orchard sanitation might only have a limited impact on reducing FCM levels. A recent survey in the Western Cape revealed very few alternative hosts (Honiball, 2004). This might not be the case in the Eastern Cape, as FCM infestation reaches its highest peak relatively early in the season (early December) (Moore *et al*., 2005), indicating a build-up on other hosts before citrus fruits are available in meaningful quantities. The discovery of alternative hosts could lead to better management of these hosts, where they occur within or adjacent to citrus-producing areas. Stofberg (1939), Schwartz (1981) and Venette *et al.* (2003) reported a number of cultivated hosts (other than citrus) (Table 3.2.6.1) and wild hosts (Honiball, 2004) (Table 3.2.6.2) for FCM.

Table 3.2.6.1. Records of cultivated hosts for FCM (Stofberg, 1939; Schwartz, 1981; Venette *et al*., 2003)

Common name	Scientific name
Avocado	*Persea americana*
Apricot	*Prunus armeniciata*
Banana	*Musa paradisiacal*
Bean	*Phaseolus spp.*
Cacao	*Theobroma cacao*
Citrus	*Citrus sinensis, Citrus spp.*
Coffee	*Coffea arabica, Coffea spp.*
Cola	*Cola nitida*
Corn	*Zea mays*
Cotton	*Gossypium hirsutum*
Grape	*Vitis spp.*
Guava	*Psidium guajava*
Litchi	*Litchi chinensis*
Loquat	*Eribotrya japonica*
Macadamia nut	*Macadamia ternifolia*
Mango	*Mangifera indica*
Olive	*Olea europaea* subsp. *europaea*
Pepper/pimento	*Capsicum spp.*
Persimmon	*Diospyros spp*
Plum	*Prunus spp.*
Pineapple	*Ananas comosus*
Pomegranate	*Punica granatum*
Sorghum	*Sorghum spp.*
Tea	*Camellia sinensis*

Table 3.2.6.2. Records of wild hosts for FCM (Honiball, 2004; Stofberg, 1939; Schwartz, 1981; Venette *et al*., 2003)

Common name	Scientific name
Bur weed	*Triumfeta spp.*
Bluebush	*Diospyros lycoides*
Bloubos	*Royena pallens*
Boerboon	*Schotia afra*
Buffalo thorn	*Zizyphus mucronata*
Carambola	*Averrhoa carambola*
Castorbean	*Ricinus communis*
Chayote	*Sechium edule*

Common name	Scientific name
Cowpea	*Vigna unguiculata, Vigna spp.*
Custard apple	*Annona reticulate*
Elephant grass	*Pennisetum purpureum*
English Walnut	*Juglans regia*
Governors plum	*Flacourtia indica*
Indian mallow	*Abutilon hybridium*
Jakkalsbessie	*Diospyros mespiliformis*
Jujube	*Zizyphus jujube*
Jute	*Abutilon spp.*
Kapok/copal	*Ceiba pentranda*
Kei apple	*Dovyalis caffra*
Khat	*Catha edulis*
Kudu-berry	*Psuedolachnostylis maprouneifolia*
Lima bean	*Phaseolus lunatus*
Mallow	*Hibiscus spp.*
Mangosteen	*Garcinia mangostana*
Marula	*Sclerocarya caffra, S. birrea*
Monkey pod	*Cassia petersiana*
Oak	*Quercus spp.*
Okra	*Ablemoschus esculentus*
Peacock flower	*Caesalpinia pulcherrima*
Port Jackson	*Acacia longifolia*
Pride of De Kaap	*Bauhinia galpini*
Raasblaar	*Combretum zeyheri*
Red milkwood	*Mimusops zeyheri*
Rooibos / Bushwillow	*Combretum apiculatum*
Sida	*Sida spp*
Snot apple	*Azanza garckeana*
Stamvrugte	*Chrysophyllum palismontanum*
Sodom apple	*Calotropis procera*
Soursop	*Annona muricata*
Stemfruit	*Englerophytum magaliesmontanum*
Surinam cherry	*Eugenia uniflora*
Suurpruim / large sour plum	*Ximenia caffra*
Water-bessie	*Syzygium cordatum*
Wag'n'bietjie	*Capparis tomentosa*
Weeping boerboon	*Scotia brachypetala*
Wild almond	*Brabejum stellatifolium*
Wild fig	*Ficus capensis*
Wild medlar	*Vangueria infausta*
Wild plum	*Harpephyllum caffrum*
Wing bean	*Xeroderris stuhlmannii*
Yellow-wood berries	*Podocarpus falcatus*
Yellow-wood, real	*Podocarpus latifolius*

Insufficient information exists on these alternative wild hosts (Table 3.2.6.2). It is not clear to what extent they may host FCM and consequently, to what extent they could exacerbate an FCM problem, if they occur in or adjacent to citrus orchards. It is possible that some of these hosts may play an important role in some areas in the build-up of FCM populations before susceptible citrus fruit are present in meaningful numbers. Once citrus fruit are available, there is then a substantial inoculum of FCM from alternative hosts. Alternative hosts, which are present throughout the season, might also augment FCM numbers in orchards during the season.

It was therefore decided to conduct a survey of possible alternative wild hosts in the Eastern Cape, by examining vegetation directly adjacent to citrus orchards. This projected ended in March 2005, and no further surveys were conducted during 2006, but further literature research was done to find more alternative hosts. Further investigation was done on the plant species which were shown to host FCM during this study, as well as possible control and management strategies for them.

In studies by Moore and Kirkman in 2004/5, FCM larval infestation was recorded in the following species: *Ricinus communis*, *Opuntia ficus-indica, Schotia afra, Crassula ovata, Asparagus crassicladus, Solanum tomentosum, Passiflora sp.* and *Albuca sp.*

Materials and methods

At regular intervals from June 2004 to July 2005, nine surveys were conducted within areas of wild vegetation (both on and adjacent to citrus farms), at three sites in the Eastern Cape (Table 3.2.6.3). Fruiting, gall forming and succulent plants were noted. Fleshy plant parts and parts bearing fruit and galls were collected. A minimum of 10 such parts were collected for each plant species at each survey. If present, a species was collected during more than one survey. Each species could therefore have been surveyed several times. Five of the fruit and galls collected, were dissected to check for FCM infestation, while at least five other seemingly healthy fruit and galls were exposed to 4-6 neonate FCM larvae each. These fruits and galls were kept at approximately 27ºC and then dissected two weeks after exposure and inspected for the presence of FCM larvae or pupae. If these were found, an attempt was made to rear them to adulthood for identification. Adult moths were sent to either the Plant Protection Research Institute or the Transvaal Museum for identification. Plant species, where possible, were identified with the use of the following references: Gledhill (1981), Shearing (1994), Urton (1993), Vanderplank (1999), Van Wyk *et al.* (1998), Von Breitenbach (1985), G Kerley and M Landman (personal communication).

Table 3.2.6.3. Details of the three sites used for investigating alternative hosts for FCM

Area	Farm	GPS Coordinates
Kirkwood, Sundays River Valley	Welgelegen	S 33º 25' 15.2" E 25º 24' 33.3"
Sunland, Sundays River Valley	Woodridge	S 33º 28' 43.4" E 25º 41' 38.1"
Uitenhage	Citrus Foundation Block	S 33º 46' 26.9" E 25º 19' 36.7"

Results and discussion

Between June 2004 and July 2005, over 150 plants, representing some 81 different plant species, were collected. Three plant species, namely the Syringa, *Melia azerdarach*, the wild fig, *Ficus capensis*, and *Clausena anisata* showed signs of penetration, but no FCM larvae were found infesting any of them. Eight of the different species tested in the laboratory, were confirmed to host FCM larvae (Table 3.2.6.4), as they were each infested with live FCM larvae two weeks after neonate larvae were placed superficially onto them.

Table 3.2.6.4. Plant species confirmed to host FCM in laboratory trials

Scientific name	Common name
Albuca sp	
Crassula ovata	Kerkeibos
Asparagus crassicladus	
Passiflora caerulea	Wild granadilla, passion flower
Solanum tomentosum	Slangappelbos
Schotia afra	Karooboerboon
Opuntia ficus-indica	Prickly pear
Ricinus communis	Castor oil plant

Fig. 3.2.6.1. *Albuca* sp.

The *Albuca sp*. (Fig. 3.2.6.1) has a weak flowering stem and pendulous white and green flowers and is fairly common in the Eastern Cape. Certain species have a high conservation status, and should therefore not be removed.

Fig. 3.2.6.2. *Crassula ovata*, Kerkeibos (Photo: Craig Chambers)

One 5th instar FCM larva was found in a hollowed stem of *Crassula ovata*, Kerkeibos (Fig. 3.2.6.2). However, FCM is known as a fruit and nut feeder. Therefore, the stem boring of this species might be viewed as aberrant behaviour. It is considered unlikely that this plant would be a preferred or important host for FCM.

Fig. 3.2.6.3. *Asparagus crassicladus*.

Three larvae were recovered from the fruit of *Asparagus crassicladus* (Fig. 3.2.6.3), two of which were positively identified as FCM. This *Asparagus* species is a prickly shrub with cream-coloured sweetly scented flowers and red berries, and has a high conservation status – being rated Albany Centre Endemic – and should legally not be removed.

Fig. 3.2.6.4. *Passiflora caerulea*, wild granadilla (Photo: Thys Du Toit).

In September 2004, signs of penetration were found on the fruit of *Passiflora caerulea,* wild granadilla (Fig. 3.2.6.4), but no larvae were recovered. In May 2005, four penetration marks were recorded on fruit, and one 4th instar larva was recovered, reared to adulthood and identified as FCM. These *Passiflora* species are creeping weeds, native to South America, and should therefore be removed where possible. They did not appear to be abundant during the surveys conducted: a scattered distribution was noted at the Citrus Foundation Block site. They have been recorded sporadically in the Eastern Cape, the Swellendam area of the southern Cape and in the northern provinces of the country.

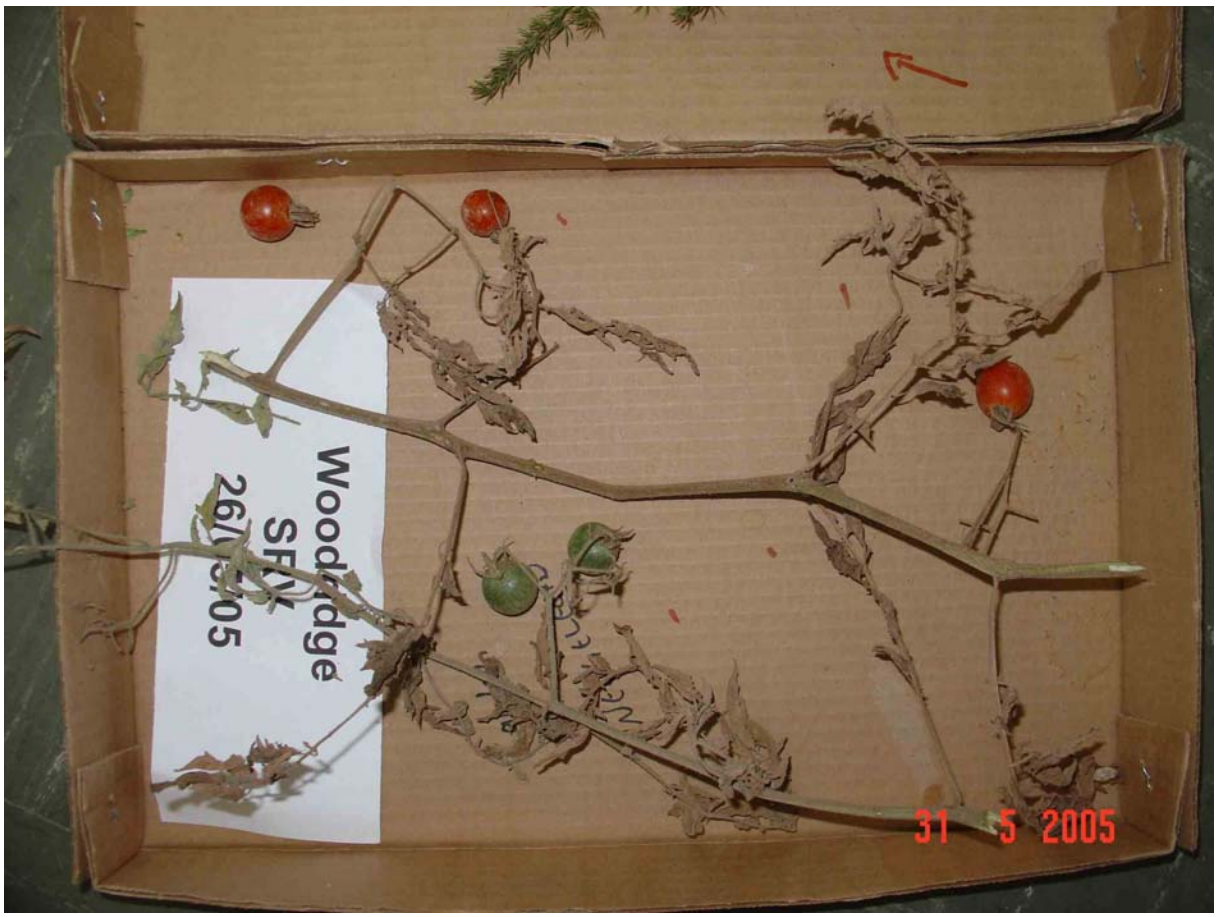

Fig. 3.2.6.5. *Solanum tomentosum*, slangappelbos.

In two of the early surveys, fruit of *Solanum tomentosum*, the slangappelbos (Fig. 3.2.6.5), showed signs of penetration, but no larvae were recovered. However, during a later collection in May 2005, one fifth instar and three third instar larvae were recovered from the fruit. Three of these were reared to adulthood and identified as FCM. This represented a 60% infestation of the fruit inoculated. These particular fruit were larger and riper than the previous collections, which could account for the high infestation rate. *Solanum tomentosum* is widespread throughout southern Africa. It belongs to the same genus as the bug weed, *Solanum mauritianum*, a highly problematic invader, which should therefore also be considered as a possible host for FCM.

Fig 3.2.6.6. *Schotia afra* pod infested by a FCM larva (Photo – Craig Chambers).

In June 2004, five larvae were recovered from the kernels inside the pods of *Schotia afra*, Karooboerboon (Fig. 3.2.6.6). These were identified as *Cryptophlebia peltastica*, the litchi moth. As FCM is closely related to this moth, and as FCM has previously been recorded attacking *Schotia brachypetala,* the huilboerboon (Schwartz ,1981), it was deemed quite possible that FCM could also infest *Schotia afra*. Further samples were therefore collected and further signs of penetration were noted. However, no larvae were recovered. In May 2005, an FCM larva and an FCM pre-pupa were found in *Schotia afra* pods. The pre-pupa was too advanced to have originated from a neonate larvae introduced in the laboratory. This infestation must therefore have occurred naturally in the field, before the pods were collected. *Schotia afra* is endemic to the Eastern and Western Cape Provinces, and should therefore not be removed.

Fig 3.2.6.7. *Opuntia ficus-indica*, prickly pear.

In June 2004, a larva closely resembling a fourth instar FCM larva was discovered in the fruit of *Opuntia ficus-indica*, the prickly pear (Fig. 3.2.6.7), but it could not be reared to adulthood. In January 2005, seven penetration marks were found on prickly pear fruit, but no larvae were recovered. In May 2005, seven larvae were recovered from inoculated fruit, six of which were reared to adulthood and positively identified as FCM. These larvae were recovered from very mature fruit, which appear more susceptible to FCM infestation than do green fruit. The prickly pear plant flowers during August and September and fruit is set soon thereafter. It is therefore possible that this plant could host FCM and enable the build up of numbers leading to the typical peak in FCM activity in citrus, recorded during December (Moore *et al*., 2005). They could also augment FCM populations later in the season, when the prickly pear fruit ripens and becomes more attractive and suitable as an FCM host. This plant occurs in abundance in the Uitenhage, Addo and Kirkwood areas. The prickly pear is classified as a Category 1 weed, which spreads rapidly in disturbed areas. Growers would be advised to clear these plants if they occur in close proximity to their orchards. Working for Water use a stem injection treatment to control prickly pears. A hole is made in the stem of the plant, through which 2 ml of a 33% MSMA (Methanearsonic acid) 720 solution is injected (Tony Bold, personal communication).

Opuntia ficus-indica is also under biological control through firstly, the prickly pear moth, *Cactoblastis cactorum*, the larvae of which feed on the pads of the plant, and secondly, the prickly pear cochineal, *Dactylopius opuntiae.* The cochineal must be applied to individual plants, as the nymphs are wind-dispersed and in the thick bush where the prickly pear often occurs, this movement is hampered. Working for Water suggests the use of a herbicide for isolated plants and biological control for dense populations where the biocontrol agents can spread easily (Abbey Heunis, personal communication). Cochineal has been used more successfully in the drier Karoo areas, as the higher rainfall along the coastal belt tends to affect the cochineal's protective waxy layer, making them more susceptible to predation.

Neither of these biological control agents is currently mass reared by Working for Water. However, farmers can contact them to obtain field-collected natural enemies. Farmers can then rear and release their own insect cultures.

Fig. 3.2.6.8. *Ricinus communis* (Photo: Craig Chambers).

FCM larvae were twice found in the seeds of *Ricinus communis*, the castor oil plant (Fig. 3.2.6.8), which has been reported as a host for FCM in Israel, where it is planted commercially (Hamburger *et al*., 2000). In South Africa it is classified as a Category 2 weed. This category includes plants which have commercial use, but are proven invaders under uncontrolled conditions. This weed is very fast growing, and could contribute to FCM infestations in citrus orchards. The castor oil plant originated from tropical Africa, and is high on Working for Water's list of plants to be eradicated. It is interesting that the seeds are extremely toxic to humans and animals, but a suitable host for FCM.

Working for Water recommends a stem chop, followed by application of Confront 360 SL, a clopyralid, onto the cut stump (Tony Bold, personal communication). More attention should be paid by growers to eradicating this weed in and around their orchards. Growers should be careful to not clear bush too long before planting of citrus takes place, as this weed thrives in these disturbed conditions.

Conclusion

Only *Schotia afra* was found to be infested with FCM prior to collection. However, the samples collected in the field were fairly small (5-10 plants per species per survey). Even for a favourable host, like citrus, a random sample of this size would hold very little chance of revealing FCM infestation. The other seven species (apart from *S. afra*) were infested under laboratory conditions. Although this study does not prove that FCM attacks these species in the wild, it gives a strong indication that this could well occur. The castor oil plant has previously been recorded as a host for FCM.

Wild alternative hosts for FCM could well create an inoculum of FCM at times when there are very few citrus fruits around. Not many moths are required to build up a substantial population. One female moth can lay more than 400 eggs (Daiber, 1980). If we speculate that only 20% survive, this leaves 80, of which around 40 would be females. Extrapolating this further: the following generation would deliver 1600 females, the next 64 000, and the following generation over 2.5 million females. Given that it takes the interception of only one live larva to result in the rejection of a consignment of fruit for certain markets, management of these alternative hosts should be seriously considered.

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3.2.7 **Investigating and improving field persistence of Cryptogran**

Experiment 791 by Wayne Kirkman and Sean Moore (CRI)

Opsomming

Die doel van hierdie navorsing was om probleme met die nawerking van Cryptogran te identifiseer, te kwantifiseer en op te los en om die nawerking van Cryptogran te verbeter deur formulasie van die produk. 'n Lugbespuiting van Cryptogran kon geen meetbare onderdrukking van VKM uitoefen nie. In 'n boordproef is verbeterde werking van Cryptogran saam met twee bymiddels, naamlik lignien en Wetcit, gekry. Wetcit en lignien het onderskeidelik die uitklopaksie en die nawerking van Cryptogran verbeter, laasgenoemde waarskynlik as 'n UV-beskermingsmiddel. In 'n tweede boordproef het die byvoeging van BP Medium olie die werking van Cryptogran verbeter. Dit is ook gewys dat Cryptogran en Acarol as 'n bespuiting mengbaar is. Die werking van Cryptex teen VKM is merkbaar swakker as dié van Cryptogran. Cryptex is die enigste behandeling wat nie 'n betekenisvolle afname in VKM-besmetting veroorsaak het nie. In 'n verdere proef het kunsmatige reënval nie die werking van Cryptogran verswak nie. In biotoetse met eerste instar VKM-larwes het Cryptogran na 30 minute se blootstelling aan UV-bestraling begin afbreek. In biotoetse om moontlike UV-beskermers te toets, het die byvoeging van lignien die werking van Cryptogran verbeter. Dit het voorgekom asof molasse nie UV-beskerming vir Cryptogran bied nie.

Navorsing sal voortgesit word om die vermoë van moontlike UV-beskermers in laboratorium- en boordproewe te toets. Nagemaakte reënvalstudies en proewe om uit te vind of die nawelent van nawellemoene enige beskerming van die virus teen UV-bestraling verskaf, sal herhaal word. Die nawelentproewe is in die vorige navorsingssiklus geïnisieër. Laastens sal proewe met Cryptogran uitgevoer word in 'n poging om die geregistreerde raklewe van die produk te verleng.

Introduction

Field trials have been conducted with Cryptogran since the year 2000. Cryptogran is also in its third year of commercial use. Results from both field trials and commercial use have shown varying degrees of field persistence. A principal disadvantage of the use of baculoviruses in the field is their short residual activity due to their inactivation by UV irradiation (Huber, 1990; Shapiro, 1995). This has also been demonstrated for CrleGV (Moore, 2002), the virus in Cryptogran. A prerequisite for the success of Cryptogran as a means of controlling false codling moth is to understand all of the factors affecting field persistence of the virus (not only UV irradiation) and to find ways to improve it. Environmental persistence can be improved by ensuring rain fastness and UV protection (Most & Quinlan, 1986). This experiment – in its second year – aims to identify, quantify and resolve persistence problems and to improve persistence through formulation.

Materials and methods

Aerial spray trial

A trial was conducted at Moosrivier Farm (Schoeman Boerdery) near Marble Hall in the Loskop Valley (Mpumalanga Province). Three blocks of Palmer navel oranges of between 10 and 20 ha were used for the three treatments, which were an aerial spray, a conventional ground spray and an untreated control. Block P42, with a total of 6252 trees was sprayed with a Cessna Husky fixed-wing aircraft using Micronair AU5000 atomisers (four per wing i.e. a total of eight). The aircraft flew at 190 km/h. The rotation speed of the atomiser was 3800 rpm and consequently the droplet size ranged from 170-250 μm, with an average size of 200 μm. Thirty to 37 droplets were applied per cm^2, in a swath of 21 m. A total of 25 L was applied per hectare. This spray was applied on 30 November 2005 from 05h30 – 06h00. In total, 500 L of spray mix was applied to this 20.32 ha block, consisting of a mixture of 21 L of Cryptogran and 35 L of molasses in water.

Block P43 was treated with Cryptogran from the ground, using an Eagle oscillating tower mistblower. The block consisted of 4774 trees and was 14.84 ha in size. Cryptogran was applied at 10 ml/100 ℓ water, with molasses at 500 ml/100 ℓ water.

Block P44 was used as an untreated control.

After sprays were applied, six data stations, consisting of five trees each, were marked in each block. Weekly, from 21 December 2005 until 22 February 2006, fruit which had dropped under data trees were collected and inspected for cause of drop. Mean FCM infestation per tree per treatment was statistically compared over the evaluation period, using ANOVA and the Bonferroni multiple range test, using Statgraphics Plus for Windows Version 5.1 (Manugistics, Inc., Rockville, Maryland, USA).

Adjuvant field trial: Allandale Farm

A trial was conducted to test a range of different adjuvants with Cryptogran (Table 3.2.7.1), to determine whether any of them could improve the performance of Cryptogran. The trial was applied in an orchard of Lane Late navel oranges on Allandale farm in the Sundays River Valley. The orchard, in which trees were spaced at 6 m x 3 m (rows x trees), was planted in 1998. The trial was laid out in a single-tree, random block formation, replicated 10 times. On 12, 13 and 14 December 2005, an average of 21 L of spray mix was applied per tree. An untreated control was retained.

After application, the trial was evaluated in the following manner. Fruit drop from data trees was evaluated from three weeks after application, until there was a substantial decline in efficacy. Dropped fruit from each tree was collected weekly, and analysed separately. Evaluations were done by inspecting and dissecting fruit in order to determine the cause of drop. FCM infestation was determined by the presence of the larva or its frass. Mean numbers of FCM infested fruit per tree per week were compared using ANOVA and the Bonferroni multiple range test, using Statgraphics Plus for Windows Version 5.1 (Manugistics, Inc., Rockville, Maryland, USA).

Table 3.2.7.1. Treatments applied on 12, 13 & 14 December 2005 for the control of FCM on Lane Late navel oranges on Allandale Farm

Treatment		Dosage in 100 ℓ water
1	Untreated control	
2	Cryptogran + molasses + Raynox	10 ml + 500 ml + 250 ml
3	Cryptogran + molasses + Lignin carrier	10 ml + 500 ml + 200 ml
4	Cryptogran + molasses + Wetcit	10 ml + 500 ml + 200 ml
5	Cryptogran + molasses + Nufilm 17	10 ml + 500 ml + 20 ml
6	Cryptogran + molasses	10 ml + 500 ml
7	Cryptogran + molasses + Agral 90	10 ml + 250 ml + 18 ml

Adjuvant field trial: Carden Farm

A second field trial was conducted to test the effect of various other adjuvants when applied with Cryptogran (Table 3.2.7.2). Simultaneously, Cryptogran was applied with Acarol, to test for compatibility. Cryptex, another FCM virus product, which may shortly be registered for use on citrus, was also tested. The efficacy of Ultracide against FCM was also tested. This trial was applied on an orchard of Autumn Gold navel

oranges on Carden farm in the Sundays River Valley. The orchard, in which trees were spaced at 6 m x 2 m (rows x trees), was planted in 1999. The trial was laid out in a double-tree, random block formation, replicated 10 times. On 29 and 30 March 2006, an average of 27 L of spray mix was applied per double-tree treatment. An untreated control was retained.
After application, the trial was evaluated in a similar manner to that described for the trial on Allandale Farm. As two trees were sprayed per replicate, fruit drop between the trunks of the two trees was evaluated from three weeks after application, therefore being representative of fruit drop from a single tree. The double-tree layout therefore created two half-tree buffers between each treatment. Statistical analyses were also conducted as previously described.

Table 3.2.7.2. Treatements applied on 29 & 30 March 2006 for the control of FCM on Autumn Gold navels at Carden farm

Treatment		Dosage in 100 ℓ water
1	Untreated control	
2	Cryptogran + molasses + Agral 90	10 ml + 250 ml + 18 ml
3	Cryptogran + molasses + Agral 90 + Acarol	10 ml + 250 ml + 18 ml + 30ml
4	Cryptogran + molasses + Agral 90 + Borax	10 ml + 250 ml + 18 ml 300g
5	Cryptogran + molasses + Clementine oil	10 ml + 250 ml + 300 ml
6	Cryptogran + molasses +BP Medium spray oil	10 ml + 250 ml + 300 ml
7	Ultracide + Agral 90	150 ml + 18 ml
8	Cryptogran + molasses + Break-thru	10 ml + 250 ml + 5 ml
9	Cryptex + molasses	5 ml + 500 ml

Timing field trial

A third field trial was applied to test the efficacy of Cryptogran when applied at various times of the year, coinciding with peaks in FCM trap catches and between peaks (Table 3.2.7.3). This trial was applied on an orchard of Lina navel oranges on Junkyard farm in the Sundays River Valley. The orchard, in which trees were spaced at 6 m x 2 m (rows x trees), was planted in 1999. The trial was laid out in a semi-commercial block format, replicated twice. Each block consisted of about 60 trees. The treatments were applied using an oscillating-tower mist-blower, and was operated one-sided. Sprays were applied on 7 December 2005, 10 January 2006, 9 February 2006 and 14 March 2006. An average of 14 L of spray mix was applied per tree. Untreated control blocks were retained.

After application, the trial was evaluated and analysed as described for the previous two trials, except that seven data trees were used in the centre of each block. There were therefore a total of 14 data trees per treatment.

Table 3.2.7.3. Treatements for the control of FCM on Lina navels at Junkyarde farm

Treatment		Dosage in 100 ℓ water	Date of application
1	Untreated control		
2	Cryptogran + molasses + Agral 90	10 ml + 250 ml + 18 ml	07/12/2005
3	Cryptogran + molasses + Agral 90	10 ml + 250 ml + 18 ml	10/01/2006
4	Cryptogran + molasses + Agral 90	10 ml + 250 ml + 18 ml	09/02/2006
5	Cryptogran + molasses + Agral 90	10 ml + 250 ml + 18 ml	14/03/2006

Rainfastness bioassays

A previous trial showed Cryptogran to be rainfast (Moore *et al*., 2004b). This concurred with experiences in field trials, where Cryptogran remained effective in controlling FCM after rainfall had occurred. Previously, treated fruit were dipped into water, so as to simulate a worst case scenario of rain, and then exposed to neonate FCM larvae. In this trial, an attempt was made to simulate rainfall more accurately by using a rain simulation machine (Hattingh, 1998). Ninety Autumn Gold navel oranges were harvested from the Citrus Foundation Block. Sixty of these fruit were treated with Cryptogran, by dipping the fruit in the registered concentration of Cryptogran, molasses and Agral 90 (Moore *et al*., 2004a) (Table 3.2.7.4). Fruit were then allowed to dry for 24 h. Half the number of fruit were then exposed to simulated rainfall, while the other half were not. Thirty fruit were retained untreated, as a control. The rainfall was applied at 36mm of rain in 5 minutes, which would be classified as a cloudburst (Aaron *et al*, 1986). The amount of rainfall was determined by placing a rain gauge under the simulated rain shower. The fruit were then left to dry, after

which they were inoculated with four neonate FCM larvae each. If the product proved rainfast under these extreme conditions, no further testing would be considered necessary.

Table 3.2.7.4. Treatments on Autumn Gold navel oranges to test the rainfastness of Cryptogran

Treatment		Dosage in 100 ℓ water	Rainfall
1	Untreated control		None
2	Cryptogran + molasses + Agral 90	10 ml + 250 ml + 18 ml	None
3	Cryptogran + molasses + Agral 90	10 ml + 250 ml + 18 ml	36 mm in 5 minutes

The fruit were then left for two weeks, after which they were inspected for penetration marks and the presence of FCM larvae. Treatments were compared using ANOVA and the Bonferroni multiple range test, using Statgraphics Plus for Windows Version 5.1 (Manugistics, Inc., Rockville, Maryland, USA).

Adjuvant Bioassays

All bioassays were conducted according to the protocol described by Moore (2002) for neonate FCM larvae. Where possible, probit analyses were conducted in order to establish a dose-response relationship between the treatments and neonate FCM larvae. LD_{50} values (i.e. the dosage at which 50% of the individuals in a sample are killed by a particular treatment) were calculated where probit analyses were successfully performed.

The first bioassay was conducted with Wettcitt, as in a field trial, the addition of Wetcit to Cryptogran appeared to improve its efficacy. Firstly, the effect of Wetcit on its own against FCM was tested. Wetcit was tested at the following concentrations (all per 100 ℓ water): 25 ml, 50 ml, 100 ml, 200 ml and 400 ml. There were therefore five concentrations of Wetcit, prepared as a series of two-fold dilutions. A distilled water control was also used.

A second bioassay was conducted in a similar way to test the effect of Boric acid on FCM. It was initially intended to test Borax, but this substance would not be dissolved in water, even when heated. Similar problems had been recorded during field trials, when the borax used had blocked the outlet pipes of the spray machine. Boric acid was bioassayed against neonate FCM larvae at the following concentrations (all per 100 ℓ water): 0.25%, 0.5%, 1%, 2% and 4% - therefore, again a two-fold dilution series. A distilled water control was also used.

In a third bioassay, a combination of Wetcit and Cryptogran were tested against neonate FCM larvae. Cryptogran was prepared in a five-fold series dilution (Moore, 2002), while the Wetcit concentration was kept constant at 200 ml per 100 ℓ water.

UV-protectant bioassays

As a precursor to test the UV protectiveness of additives, a bioassay was conducted to test the effect of UV on Cryptogran. Where possible, probit analyses were conducted in order to establish a relationship between the different exposure times to UV-irradiation and survival of neonate FCM larvae. SD_{50} values (i.e. the dosage at which 50% of the individuals in a sample survive a particular treatment) were calculated where probit analyses were successfully conducted. A Cryptogran suspension of a 1.34×10^5 OBs/ml concentration was prepared. A total of 15 ml of the suspension was placed into each of four Petri-dishes and exposed to a germicidal UV lamp for periods ranging from 30 minutes to 240 minutes. After exposure the suspensions were inoculated onto artificial diet and bioassayed against neonate FCM larvae. A distilled water treated control was used and a positive control was included i.e. a suspension of Cryptogran which was not exposed to UV.

A second bioassay was conducted in a similar way to the previous one, but the Cryptogran solutions were exposed to natural sunlight instead of the germicidal UV lamp.

A third bioassay was conducted to determine the effectiveness of a lignin sulphate carrier as a UV protectant. A suspension of Cryptogran was again prepared at 1.34×10^5 OBs/ml and 15 ml was inoculated into each of eight Petri-dishes. The lignin carrier (200 ml/100 ℓ water; source and formulation proprietary) was added to four of the eight Petri-dishes. These Petri-dishes were then exposed to a germicidal UV lamp for periods ranging from 30 minutes to 240 minutes. After exposure the suspensions were inoculated onto artificial diet and bioassayed against neonate FCM larvae. Two distilled water treated controls were used and one control of each of Cryptogran and Cryptogran with lignin, which were not exposed to UV. Two replicates of this bioassay were conducted.

A fourth bioassay was conducted, identical to the previous one, except that exposure times ranged from 60 minutes to 360 minutes.

A fifth bioassay was conducted to determine the effectiveness of an optical brightener, Tinopol DMS-X, and of Silica, as UV protectants. The Silica product used was Nontox-Silica, marketed and distributed by Plant Bio Regulators. A Cryptogran suspension of 1.34 x 10^5 OBs/ml was prepared and inoculated into each of 12 Petri-dishes at 15 ml per Petri-dish. Tinopol (1%) was added to four of these; Silica was added to four; and nothing was added to the final four. A stock solution was made up by dissolving 400 g of the product in 1 L of water. This stock solution was then added to the Cryptogran solutions at a rate of 200 ml per 100 ℓ water. These Petri-dishes were exposed to a germicidal UV lamp for periods ranging from 60 minutes to 360 minutes. After exposure the suspensions were inoculated onto artificial diet and bioassayed against neonate FCM larvae. Three distilled water treated controls were used, as well as one suspension of each type, which was not exposed to UV.

Cryptogran has been registered to be applied with molasses, due to the notable improvement which it gives to the efficacy of Cryptogran. A sixth bioassay was conducted in a similar way to the previously described ones, in order to determine if its effect on Cryptogran was one of UV protection. Cryptogran (with and without molasses) was bioassayed against neonate larvae, after exposure to UV irradiation for varying periods. The molasses was added to the Cryptogran suspension at a rate of 250 ml/100 ℓ water.

Bioassay improvement

At one stage variable results were being recorded with bioassays. Many of the Sterilin 25-cell bioassay trays were old and scratched, and had been soaked in sodium hypochlorite for long periods. It was thought some sodium hypochlorite may have been absorbed into these trays over time and that this may have increased the mortality of neonate larvae.

A bioassay was conducted to compare new bioassay trays with old trays. Cryptogran was prepared in a five-fold series dilution, and inoculated onto artificial diet in old and new trays. These were then bioassayed against neonate FCM larvae.

Results and discussion

Aerial spray trial

From 21 December 2005 to 22 February 2006, the average FCM infested fruit drop per tree per week for the three treatments was: aerial Cryptogran treatment – 0.91; ground applied Cryptogran treatment – 0.88; untreated control – 0.83. There was no statistically significant difference in FCM infestation between any of these three treatments ($P>0.05$; Bonferonni LSD multiple range test). It is possible that the aerial treatment did not have much impact on FCM infestation, as coverage may have been inadequate to achieve control. This appeared to be the case after observing droplet penetration on water sensitive paper placed at various depths within trees. However, the efficacy of Cryptogran as a ground applied treatment is well documented, including on this very farm (Moore *et al*., 2003; 2004b). The lack of discernable efficacy must therefore have been attributable to inherent differences in FCM levels between the respective blocks.

Adjuvant field trial: Allandale Farm

FCM infestation of fruit in the Allandale trial orchard, was extremely high. At the first evaluation, an average of almost 10 infested fruit per tree was recorded for the untreated control (Table 3.2.7.5). Over the full eleven week evaluation period, infestation in the untreated control averaged 3.9 fruit per tree per week. This was probably higher than had previously been recorded in any other trial in the region. Cryptogran (the registered treatment) worked reasonably well against this high level of infestation for the first five weeks of evaluation, reducing infestation by 50.2%. However, thereafter efficacy declined. It is very probably that the Cryptogran treatment would have remained effective for a lot longer until commercial conditions i.e. where blocks of trees, rather than single trees, were sprayed. It was previously confirmed that a Cryptogran treatment applied to blocks of 60 trees remained effective for 11 weeks longer than the same treatment applied to single trees (Moore *et al*., 2004b). All treatments significantly reduced FCM infestation (Table 3.2.7.5). However, there were no significant differences between any of the treatments. Despite this, Cryptogran with lignin and Cryptogran with Wetcit, produced the best results. Wetcit appeared to improve the knockdown of Cryptogran, whereas lignin appeared to improve its persistence. Lignin was used as a potential UV-protectant and it appears that its effect might have been exactly that (Hunter-Fujita *et al*., 1998).

Table 3.2.7.5. FCM infestation of Lane Late navel oranges on Allandale farm, subjected to various treatments (in combination with Cryptogran) applied 12-14 December 2005

Treatment	Fruit from data trees infested with FCM										Reduction in infestation (%)
	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT	9 WAT	10 WAT	11 WAT	Mean/ Tree/ week	
Untreated control	97	74	22	20	14	32	43	38	16	3.9a	-
Cryptogran + molasses + Raynox	42	24	11	13	13	23	40	21	15	2.2b	43.3
Cryptogran + molasses + Lignin	49	27	7	7	3	21	29	19	12	1.9b	51.1
Cryptogran + molasses + Wetcit	34	19	4	7	7	21	39	22	15	1.9b	52.8
Cryptogran + molasses + Nufilm	50	30	8	9	9	27	38	24	11	2.3b	42.1
Cryptogran + molasses	47	31	13	10	12	26	40	29	13	2.5b	37.9
Cryptogran + molasses + Agral 90	38	29	5	11	6	21	37	31	14	2.1b	46.1

Adjuvant field trial: Carden Farm

In the Carden trial, all treatments except Cryptex significantly reduced FCM infestation. As there were indications in the previous trial that Wetcit might improve the efficacy, Cryptogran was tested here with the two publicised ingredients of Wetcit i.e. Borax and citrus oil. Each was used separately with Cryptogran, in order to try to determine which one might be the best synergist. Unfortunately, neither made a significant difference (Table 3.2.7.6). Results with Break-Thru (a polyether trisiloxane wetter and spreader), in the place of the registered alkylated phenol-ethylene oxide wetter, indicated that this group of wetters can be used too. Cryptogran and molasses with BP Medium oil resulted in the greatest reduction in FCM infestation – although not significantly so.

Table 3.2.7.6. FCM infestation of Autumn Gold navel oranges on Carden farm, subjected to various treatments (in combination with Cryptogran) applied 29-30 March 2006

Treatment	Fruit infested with FCM								Reduction in infestation (%)
	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT	9 WAT	Mean/ tree/ week	
Untreated control	8	13	8	7	9	3	3	0.76a	-
Cryptogran + molasses + Agral 90	4	4	1	7	8	2	1	0.39bc	47.1
Cryptogran + molasses + Agral 90 + Acarol	3	5	4	5	3	1	1	0.31bc	56.9
Cryptogran + molasses	4	3	5	3	9	0	5	0.41bc	43.1

Treatment	Fruit infested with FCM								Reduction in infestation (%)
	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT	9 WAT	Mean/ tree/ week	
+ Agral 90 + Borax									
Cryptogran + molasses + Clementine oil	1	4	2	3	6	2	4	0.31bc	56.9
Cryptogran + molasses +BP Medium	3	2	4	4	5	0	1	0.27c	62.7
Ultracide + Agral 90	7	5	3	3	5	0	1	0.33bc	52.9
Cryptogran + molasses + Break-Thru	3	2	5	6	6	0	1	0.33bc	54.9
Cryptex + molasses	7	5	7	6	8	2	4	0.56ab	23.5

There was no indication that Acarol might be incompatible with Cryptogran (Table 3.2.7.6). Ultracide caused a significant reduction in FCM infestation – not dissimilar to that with Cryptogran.

Timing field trial

Treatments 2 (December), 4 (February) and 5 (March) were applied shortly after peaks in FCM flight activity – determined by Lorelei pheromone trap catches. Treatment 2 (January) was applied between flight peaks i.e. between FCM generations. FCM infestation was monitored in each of the treatments, until the treatment appeared to be having little or no effect (Table 3.2.7.7). The December treatment was monitored for 15 weeks, the January treatment for 11 weeks, the February treatment for seven weeks and the March treatment for only three weeks – until harvest.

Table 3.2.7.7. FCM infestation of Lina navel oranges on Junkyard farm, sprayed with Cryptogran at various times of the year

Date	Fruit infested with FCM				
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
	Untreated control	Applied 07/12/05	Applied 10/01/06	Applied 09/02/06	Applied 14/03/06
04/01/06	40	15	-	-	-
10/01/06	20	13	-	-	-
18/01/06	14	2	-	-	-
25/01/06	2	1	-	-	-
02/02/06	2	1	2	-	-
09/02/06	11	4	4	-	-
17/02/06	10	5	5	-	-
24/02/06	7	2	4	-	-
03/03/06	2	1	2	0	-
10/03/06	1	0	2	1	-
17/03/06	3	2	3	0	-
24/03/06	6	4	3	5	-
30/03/06	5	2	5	5	-
07/04/06	16	7	8	4	5

13/04/06	5	4	4	5	1
20/04/06	14	-	-	-	10

Over the periods monitored, the treatments applied shortly after flight peaks (approximately one week) reduced infestation by 58.5%, 42.9% and 45.7%, whereas the treatment applied between peaks reduced infestation by 38.2% (Table 3.2.7.8). This strongly indicated that there may be value in the theory that Cryptogran sprays should be applied shortly after flight peaks. Cryptogran is only effective against the larvae and must be ingested by them. The only stage at which larvae can be exposed to this possibility is between egg hatching and penetration into fruit. This is therefore a very small window of opportunity, which will reach a peak shortly after a peak in flight activity.

Table 3.2.7.8. FCM infestation of Lina navel oranges on Junkyard farm, per treatment – summarised results – treated with Cryptogran at various times of the year

Treatment		Mean/Tree/Week	Reduction in infestation (%)
1	Untreated control	0.62	-
2	Applied 07/12/05	0.26	58.46

Treatment		Mean/Tree/Week	Reduction in infestation (%)
1	Untreated control	0.41	-
3	Applied 10/01/06	0.25	38.24

Treatment		Mean/Tree/Week	Reduction in infestation (%)
1	Untreated control	0.39	-
4	Applied 09/02/06	0.22	42.86

Treatment		Mean/Tree/Week	Reduction in infestation (%)
1	Untreated control	0.78	-
5	Applied 14/03/06	0.42	45.71

A summary of all Cryptogran field trials conducted between 2000 and 2006, demonstrated that results with November/December applications were consistently superior (Moore *et al*., 2005). This was again demonstrated in this trial (Table 3.2.7.8). Results with February applications have been consistently the poorest – probably due to a combination of high levels of UV-irradiation and an increasing difficulty to achieve good coverage and penetration with sprays as trees become denser. Despite this, even the February application in this case – which was applied shortly after a flight peak, was more effective than the January application – which was applied between flight peaks (Table 3.2.7.8).

Rainfastness

Clearly, the simulated rainfall did not reduce the efficacy of Cryptogran (Table 3.2.7.9). Infestation of fruit exposed to rainfall, was even lower than that of fruit which were not exposed to rainfall. Unfortunately neither of the Cryptogran treatments worked very well, although the Cryptogran plus rainfall treatment did result in a statistically significant reduction in mean number of larvae infesting fruit. It was not clear why Cryptogran was not more effective in this trial, as it had been in the initial rainfastness trial – reducing infestation by more than 60% (Moore et al., 2004b). Nevertheless, for this reason, it will be necessary to repeat this trial.

Table 3.2.7.9. Damage to and infestation of Autumn Gold navel oranges treated with distilled water, Cryptogran or Cryptogran with simulated rainfall. Four neonate larvae were placed per fruit; 30 fruit per treatment

Treatment	Fruit infested (%)	Mean no of larvae per fruit
Control	88.3	1.23a
Cryptogran	76.7	1.10ab
Cryptogran + rain	60.0	0.73b

Adjuvant bioassays

Wetcit caused larval mortality of up to 28% (Table 3.2.7.10). However, there was no dose response. It was therefore not possible to conduct a probit analysis. It was particularly peculiar that larval mortality was lowest with the highest concentration of Wetcit. As no higher than 28% mortality was recorded, it is unlikely that Wetcit will be adequately effective to be used as a stand-alone product for control of FCM.

Table 3.2.7.10. Mortality of neonate FCM larvae when bioassayed against a dilution series of Wetcit

Treatment (concentrations of Wetcit in ml/100 ℓ water)		Larval mortality (%) (corrected for control mortality)
1	Distilled water	-
2	25	20
3	50	24
4	100	16
5	200	28
6	400	8

In the second bioassay, using Boric acid instead, again no dose response was recorded. It was therefore again not possible to conduct a probit analysis (Table 3.2.7.11). However, Boric acid did cause a notably higher level of mortality than did Wetcit.

Table 3.2.7.11. Mortality of neonate FCM larvae when bioassayed against a dilution series of boric acid

Treatments (concentrations of boric acid (%)		Larval mortality (%) (corrected for control mortality)
1	Distilled water	-
2	0.25	44
3	0.50	56
4	1.00	32
5	2.00	44
6	4.00	44

*Larval mortality corrected for control mortality

In the third bioassay, mortality of larvae was relatively low, indicating that bioassays will have to be repeated in order to obtain reliable results (Table 3.2.7.12). Despite this, a dose-response relationship was noted for Cryptogran. It appears from the LD_{50} values, calculated from probit analysis, that the addition of Wetcit reduced the pathogenicity of the virus. Some wetters contain detergents, such as Sodium Dodecyl Sulphate (SDS) which can be detrimental to baculoviruses. (Lua *et al*, 2003). It is not known if this is the case with Wetcit.

Table 3.2.7.12. Mortality of neonate FCM larvae when bioassayed against a dilution series of Cryptogran, with and without the addition of Wetcit

Treatments	Concentration of Cryptogran (OBs/ml)	Corrected larval mortality (%) with Wetcit (200 ml/100 ℓ)	Corrected larval mortality (%) without Wetcit (200 ml/100 ℓ)
1	Control	-	-
2	1.22×10^2	12	0
3	6.10×10^2	16	4
4	3.05×10^3	0	8
5	1.53×10^4	8	12
6	7.63×10^4	24	24
LD50 value (OBs/ml)		1.25×10^9	1.56×10^4 *

* Where Wetcit was not added, larval mortality in the untreated control was reduced from 20% to 12% in order to be able to conduct a probit analysis.

<u>UV-protectant bioassays</u>

In the bioassay to test the effect of UV-irradiation (generated by a germicidal lamp) on the virus, some breakdown became apparent even after 30 minutes of exposure to the germicidal UV lamp (Table 3.2.7.13). (In similar bioassays previously conducted, breakdown was only detected after 60 minutes exposure to UV-

irradiation (Moore *et al*., 2005).) Through probit analysis, an SD_{50} value of 437 minutes was calculated. This indicated that it took 437 minutes of exposure to the particular specifications of the UV-irradiation used, in order for the virus to lose 50% of its activity. After this time there was 50% of the original activity remaining (OAR) of the virus. However, because the dose-mortality relationship is sigmoid, rather than linear, the calculation of OAR provides a misleading guide to the actual extent of virus inactivation (Hunter-Fujita *et al.*, 1998).

Table 3.2.7.13. Impact of UV-irradiation (germicidal UV lamp) on Cryptogran (1.34 x 10^5 OBs/ml) measured by mortality of neonate FCM larvae in a dose-response bioassay

Treatments		Time of exposure to UV (min)	Mortality of neonate FCM larvae (%)
1	Distilled water	0	16
2	Cryptogran	0	68
3	Cryptogran	30	44
4	Cryptogran	60	48
5	Cryptogran	120	40
6	Cryptogran	240	36

In the second bioassay to test the effect of UV-irradiation on the virus – this time natural sunlight, the virus again showed signs of reduced efficacy after 30 minutes of exposure to irradiation (Table 3.2.7.14). Through probit analysis, a SD_{50} value of 463 minutes was calculated. This indicated that it took 463 minutes of exposure to the particular specifications of the UV-irradiation used, in order for the virus to lose 50% of its activity. This SD_{50} value is similar to that of the previous bioassay where a germicidal lamp was used as the source of UV-irradiation, rather than sunlight – as in this case. This indicated that the germicidal lamp was a fair substitute for sunlight, at the time and under the conditions that the trial was conducted. It is possible that in treatment 6 – Cryptogran exposed to UV-irradiation for 240 minutes – some evaporation of water from the Cryptogran suspension may have occurred from the Petri dish during the long period of exposure. This would result in the suspension being more concentrated than initially measured and hence the higher larval mortality than expected.

Table 3.2.7.14. Impact of UV-irradiation (sunlight) on Cryptogran (1.34 x 10^5 OBs/ml) measured by mortality of neonate FCM larvae in a dose-response bioassay

Treatments		Time of exposure to UV (min)	Mortality of neonate FCM larvae (%)
1	Distilled water	0	12
2	Cryptogran	0	76
3	Cryptogran	30	72
4	Cryptogran	60	72
5	Cryptogran	120	52
6	Cryptogran	240	60

In the next bioassay, the addition of lignin appeared to improve the efficacy of the virus. This was seen in the higher levels of larval mortality recorded after longer periods of exposure to UV-irradiation, where lignin was added compared to where it was not added. This was probably due to UV-protection provided by lignin, which reduced the rate of breakdown of the virus (Table 3.2.7.15). Unfortunately there was no clear dose-response, so it was not possible to conduct a probit analysis. The toxicity of lignin alone to FCM larvae should also be tested, although there was very little difference in larval mortality between the distilled water control treatment and the lignin (with distilled water) treatment.

Table 3.2.7.15. Impact of UV-irradiation (germicidal lamp) on Cryptogran (1.34 x 10^5 OBs/ml), with and without lignin, measured by mortality of neonate FCM larvae in a dose-response bioassay

Treatments		Time of exposure to UV (min)	Mortality of neonate FCM larvae (%) (with lignin)	Mortality of neonate FCM larvae (%) (without lignin)
1	Distilled water	0	28	24
2	Cryptogran	0	60	72
3	Cryptogran	30	68	72
4	Cryptogran	60	88	68

5	Cryptogran	120	68	56
6	Cryptogran	240	68	60

In the next bioassay, the addition of lignin once again appeared to improve the persistence of the virus under UV-irradiation (Table 3.2.7.16). Unfortunately there was no clear dose-response, so it was not possible to conduct a probit analysis. Again there was very little difference in larval mortality between the distilled water control treatments and the lignin control treatment.

Table 3.2.7.16. Impact of UV-irradiation (germicidal lamp) on Cryptogran (1.34 x 10^5 OBs/ml), with and without lignin, measured by mortality of neonate FCM larvae in a dose-response bioassay

Treatments		Time of exposure to UV (min)	Mortality of neonate FCM larvae (%) (with Lignin)	Mortality of neonate FCM larvae (%) (without Lignin)
1	Distilled water	0	20	16
2	Cryptogran	0	84	80
3	Cryptogran	30	84	80
4	Cryptogran	60	76	68
5	Cryptogran	120	92	68
6	Cryptogran	240	80	68

In the third bioassay to be conducted with Cryptogran and lignin, sunlight was used as the source of UV-irradiation and the exposure times were lengthened. Once again, the addition of lignin appeared to improve the efficacy of Cryptogran after the longer periods of exposure to UV-irradiation (Table 3.2.7.17). Again there was very little difference in larval mortality between the distilled water control treatments, with and without lignin. This would indicate that the higher larval mortality where lignin was added was not due to a synergistic effect between lignin and the virus, but due to UV-protection provided by lignin, which reduced the rate of breakdown of the virus. Clearly there was no dose-response, so it was not possible to conduct a probit analysis.

Table 3.2.7.17. Impact of UV-irradiation (sunlight) on Cryptogran (1.34 x 10^5 OBs/ml), with and without lignin, measured by mortality of neonate FCM larvae in a dose-response bioassay

Treatments		Time of exposure to UV (min)	Mortality of neonate FCM larvae (%) (with lignin)	Mortality of neonate FCM larvae (%) (without lignin)
1	Distilled water	0	16	8
2	Cryptogran	0	48	28
3	Cryptogran	60	56	44
4	Cryptogran	120	60	56
5	Cryptogran	240	52	44
6	Cryptogran	360	68	44

Control mortality in the Tinopol and Silica bioassays was unacceptably high (Table 3.2.7.18). This bioassay must therefore be repeated. In all three cases the suspension which was exposed to UV-irradiation for the longest period (360 minutes) caused higher mortality than most of the treatments which were exposed to UV-irradiation for shorter periods of time. This may have been due to evaporation from the Petri dishes. As previously explained, this would have resulted in a more concentrated virus solution than that calculated, causing a higher than expected level of mortality.

Table 3.2.7.18. Impact of UV-irradiation (germicidal lamp) on Cryptogran (1.34 x 10^5 OBs/ml), with and without Tinopol and Silica, measured by mortality of neonate FCM larvae in a dose-response bioassay

Treatments		Time of exposure to UV (min)	Mortality of neonate FCM larvae (%) (without Tinopol or Silica)	Mortality of neonate FCM larvae (%) (with Tinopol)	Mortality of neonate FCM larvae (%) (with Silica)
1	Distilled water	0	32	56	52
2	Cryptogran	0	76	88	96

Treatments		Time of exposure to UV (min)	Mortality of neonate FCM larvae (%) (without Tinopol or Silica)	Mortality of neonate FCM larvae (%) (with Tinopol)	Mortality of neonate FCM larvae (%) (with Silica)
3	Cryptogran	30	92	76	92
4	Cryptogran	60	72	84	84
5	Cryptogran	120	56	64	72
6	Cryptogran	240	76	88	92

Even without molasses and after 360 minutes of exposure to the germicidal lamp, there appeared to be little breakdown of the virus (Table 3.2.7.19). It is possible that this again might have been due to evaporation of water from the Cryptogran suspension. If this is the case, then this shortcoming with the methodology must be remedied. It will be possible to test and remedy this, by measuring the volume of the suspension after exposure to UV-irradiation and if the volume is lower than it should be, then to top it up with sterile distilled water. Despite the low level of mortality, it did not appear that molasses provided any UV protection for the virus (Table 3.2.7.19). Clearly there was no dose-response, so it was not possible to conduct a probit analysis. In field application, the addition of molasses results in a substantial improvement in the efficacy of Cryptogran. This benefit may be from molasses being a feeding attractant and a sticker, rather than a UV-protectant.

Table 3.2.7.19. Impact of UV-irradiation (germicidal lamp) on Cryptogran (1.34 x 10^5 OBs/ml), with and without molasses (250 ml/100 ℓ water), measured by mortality of neonate FCM larvae in a dose-response bioassay

Treatments		Time of exposure to UV (min)	Mortality of neonate FCM larvae (%) (With molasses)	Mortality of neonate FCM larvae (%) (Without molasses)
1	Distilled water	0	28	16
2	Cryptogran	0	80	68
3	Cryptogran	60	92	92
4	Cryptogran	120	88	84
5	Cryptogran	240	80	72
6	Cryptogran	360	88	92

Bioassay improvement

There was no obvious difference in the mortality of larvae bioassayed in the new and old trays (Table 3.2.7.20) The LC_{50} value was higher where the new trays were used. This was probably not significant. However, the result did not support the theory that old trays were impregnated with sodium hypochlorite residues, leading to higher larval mortality. If this was so, the LC_{50} value for the old trays would have been higher.

Table 3.2.7.20. Mortality of neonate FCM larvae when bioassayed against a series dilution of Cryptogran, in new and old Sterilin 25-cell bioassay trays

Treatments	Concentration of Cryptogran (Ob's/ml)	Corrected* larval mortality (%) new trays	Corrected* larval mortality (%) old trays
1	Control	-	-
2	1.22 x 10^2	0	0
3	6.10 x 10^2	8	8
4	3.05 x 10^3	4	24
5	1.53 x 10^4	20	32
6	7.63 x 10^4	52	48
LC_{50} values (OBs/ml)		3.20 x 10^{4**}	1.52 x 10^{4**}

*Larval mortality corrected for control mortality

** Mortality in both untreated controls was increased by 4 % in order to be able to conduct probit analysis.

Conclusion

An aerial application of Cryptogran did not appear to be effective. Bioassays confirmed that Cryptogran, or CrleGV, is degraded by UV-irradiation. Both laboratory bioassays and field trials provided a strong indication that lignin could be an effective UV-protectant for Cryptogran, increasing the field persistence of the product. Although the addition of molasses to Cryptogran has been shown to improve the efficacy of the product, the benefit of molasses is unlikely to be that of UV-protection. In field trials Wetcit and BP Medium oil also resulted in a greater reduction in FCM infestation than the registered Cryptogran treatment. Cryptogran and Acarol were shown to be compatible as a tank-mix. Cryptex was notably weaker than Cryptogran, in being the only treatment not to significantly reduce FCM infestation relative to the untreated control. Simulated rainfall did not reduce the efficacy of Cryptogran.

Future research

Research will be continued with further tests of potential UV-protectants, both in laboratory bioassays and in field trials. Simulated rainfall trials will be repeated, as will trials to examine whether the navel end of navel oranges provides any protection of the virus against UV irradiation. The latter trials were initiated during the previous research cycle. Finally, shelf-life trials will be conducted with Cryptogran in an attempt to extend the registered shelf-life of the product.

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3.2.8 Entomopathogenic nematodes for control of FCM

Experiment 793 by Antoinette P. Malan (SU) and Sean Moore (CRI)

Opsomming

Sitrusgrondmonsters vanuit drie provinsies (Wes-Kaap, Oos-Kaap en Mpumalanga) is vir die voorkoms van entomopatogeniese nematodes (EPNs) ontleed. ’n Totaal van 135 monsters is ontleed, waarvan 18 (13%) positief vir die voorkoms van EPNs gevind is. Die nematodes word lewend gehou vir verdere navorsing en identifikasie. Isolate gevind in sitrusboorde behoort aan die genus *Heterorhabditis*, behalwe vir een wat identifiseer is as *Steinernema khoisanae*. ’n Totaal van 48 lokaal-beskikbare EPN-isolate is getoets met ’n

laboratorium biotoets. Die biotoetse is gedoen onder ideale toestande en mortaliteit van die insek is gebruik as hoof biologiese indikator vir die potensiaal van EPN-isolate teen VKM 5'de instar larwes, sowel as ten volle verharde papies. Daar is gevind dat die mortaliteit van VKM-larwes oor die algemeen vir die verskillende EPN-isolate hoog en vir papies laag is. Uit hierdie biotoetse is isolate gekies waarvan die mortaliteit vir larwes 100% en vir papies bo 40% was. Van die isolate is onder natuurliker toestande getoets deur die papies in sand te begrawe waar infektiewe larwes aktief moes soek na die gasheer. Met die uitgesoekte isolate is daar in die sandbiotoetse tot 96% mortaliteit van papies en motte verkry. Hiervan is vyf EPN-isolate selekteer wat gebruik gaan word in verdere glashuis- en boordtoetse.

Introduction

Realization of, *inter alia*, the environmental damage caused by chemical control of insects stimulated a worldwide newfound interest in entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae as biological control agents of insects. The infective juvenile (IJ), the only free living stage, found naturally in most soils around the world, are obligate parasites of a wide range of insects (Homonick, 2002). These nematodes, together with their symbiotic bacteria, have the potential to be incorporated into integrated pest management (IPM) programmes. They can be used in combination with other biological control agents, or can reduce or replace the reliance on chemical control of certain insect pests (Shapiro-Ilan *et al*., 2002).

Current control programmes used against false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick), include chemicals, mating disruption and biological control by application of a granulovirus, in an IPM setup (Moore *et al*., 2004). Not one of these control measures is targeted against the pupal stage of FCM in the soil. Soil is the natural habitat for EPNs. FCM larvae leave the fruit, burrow into the soil and pupate, emerging later as moths. This offers a window period for EPNs to be used as biological control agents against FCM in an IPM programme.

The ability of different EPN strains to control a specific pest can vary greatly (Shapiro-Ilan *et al*., 2002). The key to achieving success with an EPN as a biological control agent against a specific pest, is to obtain a good nematode match for the target insect. The aim of this study was to obtain new EPNs strains locally adapted to citrus orchards, by conducting a survey in the different citrus producing areas. Since mortality of FCM was the most important biological aspect selected for in the EPNs, a fast screening method with all available isolates was used to select EPNs with the potential to give high mortality of both larvae and pupae. Since pupae will be the main targeted life stage of FCM, a sand bioassay was used for selected EPN isolates in a more natural environment.

Materials and methods

Survey for EPNs from citrus orchard

During 2005/6 soil samples were collected from citrus orchards by taking sub samples from four quadrants in an orchard and combining the soil in one composite sample of approximately 1 kg. The soil from the sample was divided in 250 ml plastic containers and five larvae of the greater wax moth (*Galleria mellonella* L.) were added to each container and closed with a lid (Bedding & Akhurst, 1975). The containers were kept in the dark for seven days. During this period dead larvae were removed and placed on White's traps (White, 1927).

Nematode identification

Hermaphrodites or females of the first generation were preserved in 95% alcohol for molecular characterisation. Nematodes were identified by sequencing of the ITS regions and alignment with EPN sequences deposited in Genbank (Nguyen *et al*., 2004). Molecular identification of species was supported by the morphology of the first generation male, female and hermaphrodite and morphometrics of the IJ.

Source of nematodes and insects

The IJs used were obtained during the survey of citrus orchards and from the University of Stellenbosch EPN collection (Malan *et al*., 2006). *Galleria mellonella* larvae were cultured in the laboratory on a diet of baby food, glycerine, bran, wheat germ, yeast and bee combs. False codling moth was obtained from the River Bioscience rearing facility in Addo, South Africa.

Inoculum preparation

Nematodes were maintained by recycling through *G. mellonella* larvae (Dutky *et al*., 1964) every three months. The IJs were stored in horizontally placed, vented culture flasks, containing 150 ml of filtered tap water, and kept at 25°C. For each bioassay the nematode inoculum was freshly prepared and harvested within the first week of emergence from White's traps (White, 1927) and used within one month of storage. Concentrations were calculated according to the technique of Navon & Ascher (2000).

Screening of available isolates for mortality

Twelve wells of two 24-well bioassay plates for each nematode isolate and for both pupae and larvae were used. After 48 hours exposure at 25°C, mortality was determined by looking at the colour change of larvae and movement of pupae. To confirm whether mortality had been caused by nematodes, the insects were kept for another two days and dissected to determine the presence of developing nematodes.

Sand bioassay for pupae

Glass Petri dishes, 15 cm in diameter, were filled with 100 ml dry sterile sand, 10 ml of water added and kept at 25°C overnight for even moisture distribution. Twenty pupae were arranged on the sand in the outer circle of the petri dish and covered with 40 ml of dry sand. Nematodes were inoculated by pipetting 1 ml of IJs in the centre of the soil to give 200 IJs for each pupae. For each EPN isolate five Petri dishes were used and placed in a closed plastic bag for moisture control. After a period of six days at 25°C the mortality was assessed by counting the dead pupae and emerged moths. Dead pupae and moths were kept in petri dishes with moistened filter paper at 25°C for another two days and dissected to determine the presence of developing nematodes.

Results and discussion

A total of 135 soil samples from citrus orchards were collected and trapped for the presence of EPNs. Nematodes were found in 18 (13%) samples (Table 3.2.8.1). Only one *Steinernema* species, *S. khoisanae,* was found during the survey. This was previously described as a new species from South Africa (Nguyen *et al*., 2006). *Heterorhabditis* was the most common genus while *Steinernema* was found to be rare in citrus orchards. According to these results and those from a previous survey (Malan *et al*., 2006), *Heterorhabditis* is the most common genus found in agricultural soils in South Africa, including soil from citrus orchards.

Table 3.2.8.1. Soil samples from citrus orchards from three provinces in South Africa analyzed for the presence of EPNs

Area/Province	No. of samples	No. of farms	Positive samples	Genus/Species
Western Cape				
Ashton	2	1	0	
Citrusdal	13	13	3	*Heterorhabditis* sp. (42-C; 113-C; 111-C)
Clanwilliam	1	1	0	
Eendekuil	1	1	0	
Hermanus	3	1	0	
Knysna	2	1	1	*Heterorhabditis* sp. (56-C)
Montagu	3	1	0	
Moreesburg	2	1	1	*Heterorhabditis* sp. (130-C)
Paarl	6	2	0	
Piketberg	3	2	0	
Porterville	8	1	2	*Heterorhabditis* sp. (104-C); *Steinernema khoisanae* (106-C)
Robertson	19	4	0	
Stellenbosch	2	1	0	
Swellendam	7	4	0	
Villiersdorp	1	1	0	
Wellington	1	1	1	*Heterorhabditis* sp. (26-C)
Wolsely	1	1	0	
Total:	**75**	**37**	**8**	
Mpumalanga				

Area/Province	No. of samples	No. of farms	Positive samples	Genus/Species
Nelspruit	7	2	4	*Heterorhabditis* sp. (63-C; 65-C; 66-C; 67-C)
Origstad	5	0	0	
Total	**12**	**2**	**4**	
Eastern Cape				
Addo	13	5	1	*Heterorhabditis* sp. (29-C)
Ford Beaufort	2	1	0	
Kirkwood	9	4	2	*Heterorhabditis* spp. (51-C; 20-C)
Ladismith	1	1	0	
Patensie	7	2	2	*Heterorhabditis* sp. (89-C; 136-C))
Sunday River Valley	16	8	1	*Heterorhabditis* sp. (17-C)
Total:	**48**	**21**	**6**	

A total of 46 EPN isolates were tested by means of laboratory bioassay (fast screening method). FCM mortality was used as the main biological indicator to select between different EPN isolates for their ability to kill larvae and pupae under ideal conditions. Differences in mortality of larvae (Fig. 3.2.8.1) and pupae (Fig. 3.2.8.2) were found with different isolates of EPNs. It was found that larvae experience a high level of mortality of between 80-100% and for fully formed hardened pupae a reduced mortality of between 30-40%. From these results EPN isolates were chosen for the sand bioassays, simulating field conditions.

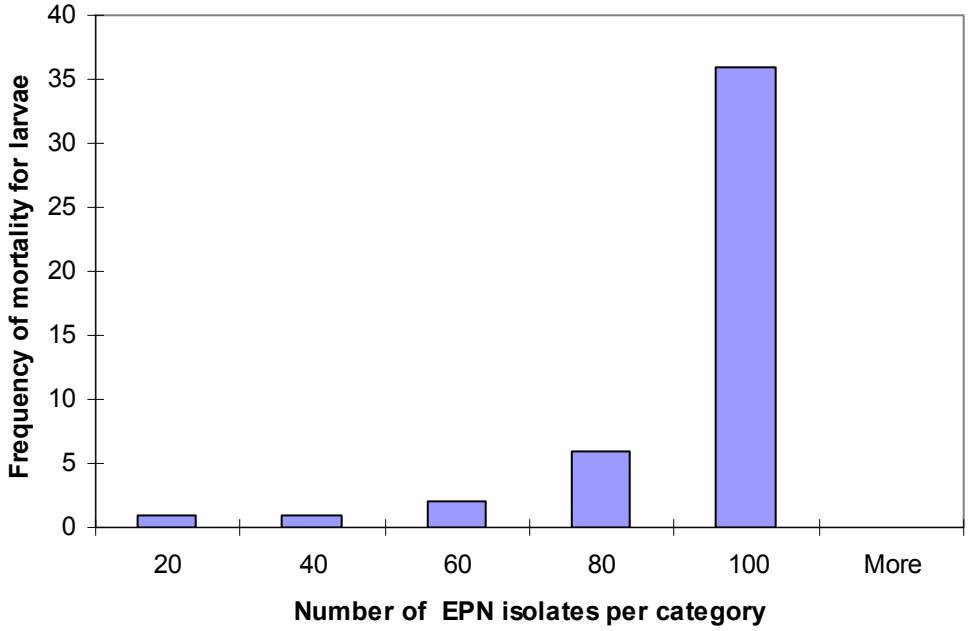

Fig. 3.2.8.1. Results of fast screening of available EPN isolates for mortality of FCM larvae in a laboratory bioassay.

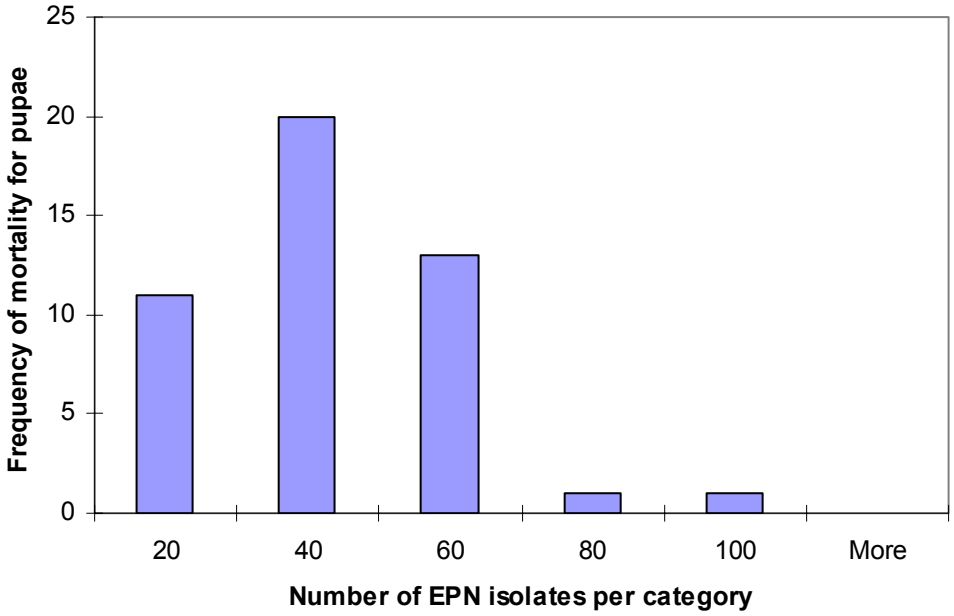

Fig. 3.2.8.2. Results of fast screening of available EPN isolates for mortality of FCM pupae in a laboratory bioassay.

Species and isolates which caused 100% mortality of larvae and above 40% mortality of pupae, were selected from the fast screening bioassays. These isolates were tested in the sand bioassays (Table 3.2.8.2). It was found that pupae, as well as moths that emerged from the sand during the test period, were infected with nematodes. Some of the isolates gave a combined percentage mortality for pupae and moths of up to 96%. The overall mortality was used to select 5 isolates to be used against FCM in greenhouse and field tests.

Table 3.2.8.2. Combined percentage mortality of pupae and emerged moths

Species	EPN Isolate	Combined % mortality for pupae and emerged moths	% Mortality of pupae	% Mortality of emerged moths
Heterorhabditis bacteriophora	SF351*	93	13	82
H. bacteriophora	SF10	70	23	47
H. bacteriophora	SF134	62	41	20
H. bacteriophora	SF1	58	19	39
H. zealandica	SF41*	78	40	37
Heterorhabditis sp	SF379*	89	20	74
Heterorhabditis sp	67-C*	87	38	52
Heterorhabditis sp	17-C	73	16	60
Heterorhabditis sp.	SF593*	96	33	66
Steinernema khoisanae	SF87	57	34	23

*Final selected EPN isolates for FCM control

Conclusion

During the survey of citrus orchards for EPNs, a low percentage of EPNs was recovered from analysed samples. All isolates, except for one, belonged to the genus *Heterorhabditis*. Species of *Steinernema* in other countries were found to be good biological control agents of Lepidoptera. In South Africa *Heterorhabditis* seems to be well adapted to local conditions and an effective biological control agent against FCM may be found from this genus.

The fast screening for mortality of FCM larvae and pupae eliminates isolates at an early stage that are not effective against FCM larvae and pupae, and will be used in the future for the screening of new isolates

found. Nematodes performed exceptionally well in the sand bioassay, because of the long exposure period and the inability of emerged moths to escape contact with the nematodes.

Future research

In order to obtain EPN species proven to be effective in other countries and suitable for use against Lepidoptera, further samples from citrus orchards and surrounding natural vegetation should be taken. All isolates of EPNs found should be cryopreserved to prevent loss of genetic traits, accidental loss and contamination. Simulated field conditions with larvae, pupae and emerged moths in soil should be used for the selection of two final EPN isolates. These two isolates will be mass cultured *in vivo* for field trails in citrus orchards.

Acknowledgements

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3.2.9 **Globale verspreiding van valskodlingmot**
Proef 805 deur M de Villiers (SU)

Summary

False codling moth is a pest of international phytosanitary concern. Therefore, certain phytosanitary restrictions are placed on international citrus trade. If the current distribution of the pest is determined, it will be possible to model its potential to invade other parts of the world, thereby providing a scientific basis to evaluate the relevance of both present and future phytosanitary restrictions. The goal of the latter is to reduce the risk of introducing FCM into importing countries. Only through obtaining reliable, scientific information, can phytosanitary restrictions on citrus trade be contested. It is therefore considered critical to obtain such information in order to gain and maintain market access.

In order to model the potential global distribution of the pest, detailed distribution data need to be obtained. Information on the biology of the pest is also important. A literature search was therefore undertaken. Museums and other researchers were also contacted to obtain further information regarding its distribution and biology. To get the best results from modelling, more detailed distribution and abundance data is needed. Since such information is not currently available, surveys are required. The objective of the surveys is to determine the relative abundance of the pest in southern Africa. To meet these objectives, traps for FCM were placed in Stellenbosch, Citrusdal, Swellendam, Knysna, Onseepkans, Keimoes, Britstown, Jan Kempdorp, Addo, King William's Town, Bloemfontein, Pietermaritzburg, Nkwalini,

Groblersdal/Marble Hall, Nelspruit, Komatipoort, Tshipise, Tom Burke, Tzaneen, Rustenburg, as well as Harare (Zimbabwe). FCM traps will probably also be placed in Alexander Bay during January 2007. Three delta traps with Chempac pheromone were used per locality. Monitoring started during August, September or October. Traps were serviced on a monthly basis.

No FCM samples or data were obtained from Tom Burke. FCM samples or data were obtained from all other areas. For Harare, samples for December have not been received. For Gariep Dam both November and December samples are still lacking. FCM was absent from Onseepkans and Britstown. Counts were lowest in Swellendam, Knysna, Jan Kempdorp, Addo, Pietermaritzburg, Tshipise and Tzaneen, with counts of less than 10 moths per trap per month. Counts were slightly higher in Komatipoort and Harare, mostly with monthly averages of 10 to 19.9 moths per trap. High FCM counts (20 to 49.9 moths per trap) were observed during certain months in Citrusdal, King William's Town, Bloemfontein, Nkwalini, Groblersdal/Marble Hall, Nelspruit and Rustenburg and high to very high counts (more than 50 moths per trap per month) in Stellenbosch and Keimoes. A full report will be given upon completion of the study.

Opsomming

Valskodlingmot is 'n plaag van internasionale fitosanitêre belang, met die gevolg dat sekere fitosanitêre beperkings op internasionale sitrushandel geplaas word. Indien die huidige verspreiding van dié plaag bepaal word, sal dit moontlik wees om die potensiaal daarvan om ander wêrelddele binne te dring, te modelleer. Sodoende word 'n wetenskaplike basis verskaf om die relevansie van beide huidige en toekomstige fitosanitêre beperkings te evalueer. Die doel van laasgenoemde is om die risiko van binnedringing van VKM in invoerende lande te verminder. Slegs deur betroubare, wetenskaplike inligting te verkry, kan fitosanitêre beperkings op sitrushandel beveg word. Dit word dus as krities beskou dat sulke inligting bekom word ten einde marktoegang te verkry en te handhaaf.

Ten einde die potensiële globale verspreiding van VKM te modelleer, moet volledige verspreidingsdata verkry word. Inligting oor die biologie van die plaag is ook noodsaaklik. 'n Literatuurstudie is derhalwe uitgevoer. Museums en ander navorsers is ook gekontak om verdere verspreidings- en biologiese data te verkry. Om die beste resultate vanuit modellering te verkry, is vollediger data rakende die verspreiding en volopheid van die plaag nodig. Aangesien sulke inligting nie tans beskikbaar is nie, moet opnames gedoen word. Die doel van die opnames is om die relatiewe volopheid van die plaag in suidelike Afrika te bepaal. Om hierdie doelwitte te bereik, is VKM-lokvalle in Stellenbosch, Citrusdal, Swellendam, Knysna, Onseepkans, Keimoes, Britstown, Jan-Kempdorp, Addo, King William's Town, Bloemfontein, Pietermaritzburg, Nkwalini, Groblersdal/Marble Hall, Nelspruit, Komatipoort, Tshipise, Tom Burke, Tzaneen, Rustenburg, asook Harare (Zimbabwe) uitgeplaas. VKM-lokvalle behoort gedurende Januarie 2007 ook in Alexanderbaai gehang te word. Drie deltavalle met Chempac-feromoon is per lokaliteit gebruik. Monitering het gedurende Augustus, September of Oktober begin. Valle is maandeliks nagegaan.

Geen insekmonsters of data is vanaf Tom Burke verkry nie. VKM-monsters of -data is vanaf alle ander areas verkry. Vir Harare is monsters vir Desember nog uitstaande. Vir Gariepdam is beide November en Desember monsters nog uitstaande. VKM was afwesig in Onseepkans en Britstown. VKM-getalle was die laagste in Swellendam, Knysna, Jan-Kempdorp, Addo, Pietermaritzburg, Tshipise en Tzaneen, met getalle van minder as 10 motte per val per maand. Getalle was ietwat groter in Komatipoort en Harare, meestal met maandelikse gemiddeldes van 10 tot 19.9 motte per val. Groot getalle (20 to 49.9 motte per val) is gedurende sekere maande in Citrusdal, King William's Town, Bloemfontein, Nkwalini, Groblersdal/Marble Hall, Nelspruit en Rustenburg waargeneem en groot tot baie groot getalle (meer as 50 motte per val per maand) in Stellenbosch en Keimoes. 'n Volledige verslag sal met voltooing van die studie geskryf word.

3.2.10 **The host status of lemons for FCM**
Experiment 828 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

Die Chinese mark het onlangs vir uitvoere van Suid-Afrikaanse sitrusvrugte beskikbaar geraak. Die uitvoerprotokol vereis dat vrugte op pad na China kouebehandel moet word. Kouedisinfestasie van suurlemoene is egter nie moontlik nie omdat die vrugte deur die voorgeskrewe kouebehandeling beskadig word. Dié proef het ten doel gehad om die geskiktheid van suurlemoene in verskillende stadia van kleurontwikkeling as gashere vir VKM te bepaal. Die mikpunt is om die Chinese beamptes te voorsien met data wat bewys dat suurlemoene van sekere rypheidsgrade nie gashere vir VKM kan wees nie. Suurlemoene van 'n reeks kleure op bome is in nette saam met pare motte ingehok. Drie weke later het evaluasie getoon dat eierlegging voldoende was, maar geen besmetting van suurlemoene en net 'n lae vlak van besmetting van ryp lemoene (as 'n kontrole) is gekry. In 'n daaropvolgende proef, waar vrugte vyf weke

lank aan motte blootgestel was, is ’n gemiddelde besmettingsvlak van 36.7% gekry. Dit was duidelik dat die proefbenadering toepaslik was. Omdat die proewe in die winter uitgevoer is, was die besmettingsondersoek moontlik deur stadige larwe-ontwikkeling en lae larwe-oorlewing benadeel. Die proef sal in die somer herhaal word. Genoeg herhalings sal gebruik word om data te kry wat vir publikasie in ’n internasionale wetenskaplike joernaal geskik is. Dit sal moontlik help om die Chinese owerheid (en moontlik ook dié van die VSA) van die niegasheerstatus van VKM op suurlemoene te oortuig.

Introduction

The Chinese market has recently opened for exports of South African citrus fruits. Originally, the relevant protocol stated that only fruit from FCM-free orchards was admissible. This protocol in effect means that if any FCM adults are caught in pheromone traps in an orchard, even if the fruit is not infested, then the fruit from that orchard cannot be packed for China. The Chinese market has now accepted an alternative protocol of cold disinfestation of fruit in transit. This treatment is not a feasible option as lemons damage easily at the low temperatures required. It is known that lemons are an unsuitable host for FCM (Newton, 1998). Consequently, trials to examine the host status of lemons for FCM were initiated a year ago (Moore *et al*., 2005). Season-long monitoring of pheromone traps confirmed that lemon orchards are not free of FCM. Laboratory trials revealed notable levels of infestation of detached lemons of all degrees of colour. However, this was associated with a high level of fruit decay. This experiment proposes to determine the suitability of lemons, at various stages of development, as hosts for FCM.

Materials and methods

On 6 June 2006, a mature Eureka lemon orchard on The Outback Farm in Sundays River Valley (33° 39.986’ S, 25° 41.897’ E), with fruit ranging from T2-T6 in colour (Anonymous, 1995) was used for a trial. Branches holding between 15-30 lemons were bagged with mosquito netting. Eight such nets were enclosed over fruit on three adjacent trees. Four pairs of mating FCM adults were released into each bag. Approximately 60 Cara-cara navel oranges (T2 colour), in an orchard on the Citrus Foundation Block near Uitenhage, were subjected to the same protocol, as a positive control. A week later, bags of fruit were opened and the colour of each fruit was categorised and marked on the fruit with an indelible pen. Observations were conducted to confirm that FCM eggs had been laid. Bags were again sealed. After another two weeks, on 27 June, fruit was collected and inspected in the laboratory for eggs, penetration marks and larval infestation.

To further test the protocol – this time only with control fruit – a second trial was conducted on Autumn Gold navel oranges at the Citrus Foundation Block on 4 July 2006. The protocol used in this trial was as described for the first trial, except that the fruit was only harvested on 9 August 2006 – five weeks after introduction of the moths. In the previous trial, the fruit had been harvested after three weeks.

Results and discussion

Inspections conducted one week after enclosure of moths in nets, confirmed the presence of FCM eggs on fruit and leaves. A number of moths were still alive at this time. Bags were again sealed. After another two weeks, inspections conducted in the laboratory on all 204 lemons collected from the nets, no larval infestation was found (Table 3.2.10.1). However, three incidents of attempted penetration were recorded. Unfortunately, infestation of the control oranges was not much higher. Only two live larvae were recorded in the fruit, with a total of three penetration marks observed. The trial protocol appeared to be suitable, as an average of 2.45 eggs were recorded per lemon fruit. It was postulated that the low level of infestation of control fruit (oranges) was due to cool winter temperatures and that if such a trial was conducted during the cooler months, fruit should be left in the nets for longer than three weeks.

Table 3.2.10.1. FCM egg laying, penetration and infestation of lemons on The Outback Farm and oranges on the Citrus Foundation Block, three weeks after being netted with four pairs of FCM adults per net, in June

Cultivar	Colour plate	Total fruit	Total eggs	Mean eggs/fruit	Fruit with penetration marks	Fruit infested with larvae
Lemons	1	2	4	2.00	0	0
	2	12	5	0.42	0	0
	3	22	96	4.36	0	0
	4	41	100	2.44	0	0
	5	79	143	1.81	0	0

	6	40	139	3.48	1	0
	7	8	16	2.00	2	0
Cara-cara navel oranges	2	57	84	1.47	3	2

In the second netted orange (control) trial, infestation of oranges ranged from 5.6 – 61.5% in the three nets (Table 3.2.10.2). This was an average of 36.7% fruit infested, or 54.5%, if fruit from only the two most well infested nets were considered. Larvae were very small – little more than neonate size. It could therefore have been quite easy to miss larvae when dissecting fruit collected from the nets. This indicated that even though fruit had been left hanging for two weeks longer than in the previous trial, this was still inadequate. Development of FCM in July was clearly longer than had been anticipated. If the trial is repeated in winter, fruit should be kept in the nets for even longer, after exposure to moths. However, it would be more appropriate to conduct the trial during summer, when survival of larvae will be higher and their development more rapid. Nevertheless, the results were sufficient indication that the trial protocol is sound.

Table 3.2.10.2. FCM egg laying, penetration and infestation of Autumn Gold oranges on the Citrus Foundation Block, five weeks after being netted with four pairs of FCM adults per net, in July

	Total fruit	**Total eggs**	**Eggs/fruit**	**Fruit with penetration marks**	**Total larvae**	**Fruit infested (%)**
Bag 1	18	9	0.5	1	2	5.6
Bag 2	13	114	8.8	8	21	61.5
Bag 3	18	75	4.2	11	27	50.0
Total	49	198	4.0	20	50	36.7

Conclusion

Entry of lemons into the Chinese market cannot easily be achieved through the current protocol. Lemons cannot be cold disinfested in transit due to their relatively high sensitivity to cold and consequent high blemish and waste levels, which would occur. Orchard trials conducted with netted fruit and moths, indicate that this is an appropriate protocol for testing the host status of FCM. However, these trials should be conducted again in summer.

Future research

Netted fruit and moth trials will be repeated in summer. The objective will be to accumulate sufficient data to be publishable in a peer-reviewed international scientific journal and therefore to convince the Chinese and USA authorities of the host-status of lemons for FCM.

Acknowledgements

The owner, Hannes Joubert, and manager, Gary Webb, of The Outback Farm in Sundays River Valley are thanked for the use of their farm.

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3.2.11 **Improvement of cold treatment conditions for the disinfestation of False Codling Moth in citrus fruit, using a potentiating CO_2 shock treatment**

Experiment 858 by Chirene Jelbert (SU)

Opsomming

Die valskodlingmot (VKM), *Thaumatotibia leucotreta* (Meyrick), is 'n inheemse plaag in Suid-Afika en word as 'n kwarantynplaag deur verskeie uitvoermarkte beskou. 'n Twee en twintig-dae lange kouebehandeling teen -0.6°C is tans die enigste na-oes disinfestasiebehandeling vir VKM in sitrus. Die behandeling belemmer egter vruggehalte en nuwe markte vereis nou ook VKM-disinfestasie prosedures. Daar is dus 'n groot behoefte vir die ontwikkeling van 'n alternatiewe na-oes disinfestasiebehandeling wat minder skadelik vir vrugkwaliteit is en verenigbaarder is met markte met kort verskepingstye.

'n Moontlike alternatiewe na-oes disinfestasiemetode is geïdentifiseer toe Spaanse navorsers sensitiwiteit in *Ceratitis capitata* (Wiedemann) in "Fortune"-mandaryne gevind het na CO_2-skokbehandelings. Die kouebestandste ontwikkelingstadia van VKM-larwes, die 4'de en 5'de instars, is aan verskeie CO_2-skok- en daaropvolgende kouebehandelings blootgestel. 'n Aansienlike toename in persentasie mortaliteit is in dié larwes opgemerk. Die doeltreffendste behandeling is geïdentifiseer vir verdere navorsing op vrugte om die uitwerking van sulke behandelings op vrugkwaliteit en larwesensitiwiteit in die vrugte, te ondersoek.

Introduction

The false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick), is an indigenous pest to South Africa, and thus considered a quarantine pest by foreign markets. A 22-day cold treatment at -0.6°C is currently the only post-harvest disinfestation treatment for FCM in citrus. This treatment is, however, detrimental to fruit quality, yet new markets also require FCM disinfestation procedures. There is thus an urgent need to develop alternative post-harvest disinfestation treatments which are less detrimental to fruit quality and more compatible with markets that have short shipping times.

Recent work by Spanish researchers (Alonso *et al.,* 2006*),* found that CO_2 diminishes cold tolerance of third-instar larvae of *Ceratitis capitata* (Wiedemann) in 'Fortune' mandarin. This could be because CO_2 causes the insect's spiracle muscles to open, causing uncontrolled water loss and dehydration (Lehmann *et al*., 2000). This can impact citrus quarantine treatments and prompted a South African research project to look at a new practical post harvest disinfestation treatment, using a combination of CO_2 and cold temperature. Potentially the required 22-day cold treatment at -0.6°C could be reduced and the detrimental effects on fruit quality eliminated.

Materials and methods

The initial research process involved rearing FCM in jars to the most cold tolerant 4^{th} to 5^{th} larval instars. The rearing process was investigated through a literature study and a visit to the CRI offices in Port Elizabeth, where the rearing technique was demonstrated to the author (Moore and Kirkman, pers. com.). The technique was adopted, and FCM larvae were reared to 4-5^{th} instars from eggs supplied by CRI PE.

Prepared jars of mature 4-5^{th} instar larvae were then exposed to different CO_2 concentrations of 95%, 75%, 50% and 25%, for 8, 16, 20, and 24 hours at both 20°C and 25°C. Larvae were then subjected to either 1°C or -0.5°C for consecutive lengths of either 10, 12, 14, 16, 18, 20, and 22 days and assessed for percentage mortality/time. Data were to be plotted on a Probit regression model. Lemon fruit were also subjected to the same treatments and assessed for fruit quality before and after treatments.

Initial results with the above-mentioned treatments indicated an average mortality of 97.2%. However, due to the largest percentage of jars presenting 100% mortality rate, which cannot be used in the intended Probit analysis, the experiment needed to be changed.

A closer look was given to the rearing process itself which was presenting some problems, such as a inconsistency in the number of larvae per jar, fungal infections and a tendency for larvae to crawl out of the diet when exposed to CO_2 and when exposed to cold possibly dying more quickly due to being outside the diet and more exposed to cold temperatures. This was a critical problem in the initial experiment as only one jar was being tested per combination of variables. Despite the experiment being repeated 6 times, the results still showed too much variation.

To make the experiment more manageable, a number of co-variables were removed from the initial experiment. Co-variables were removed on the basis of a number of critical observations that were made in

the original experiment. Lemon fruit were put through the same treatments as the jars with 4-5th instar larvae. The lemon fruit showed an extreme breakdown in flesh firmness with the high concentrations of CO_2, especially 95% CO_2. Thus the treatment co-variable of 95% CO_2, was removed from the experiment. From the remaining CO_2 treatments it was decided to keep the two most extreme points to try and show significant difference between treatments. Carbon dioxide is normally present in the atmosphere at a concentration of 0.03% (Wong 1992). Thus for the control, ambient air (0.03% CO_2) was also included. Where the prepared jars of 4-5th instar larvae are exposed to the CO_2 gas treatments for varying hours, the 8 and 24 hours co-variables were used. 25˚C was chosen as the temperature at which the CO_2 gas treatments were applied. The cold treatment temperature selected was -0.5°C, as this is the standard required temperature in export disinfestation treatments. Exposure times to cold treatments were also shortened to 6, 7.8, 10.14, 13.18, 17.13 days at -0.5°C to better fit a Probit regression model.

It was also noted that there were a number of larvae that had not achieved the most cold tolerant 4th and 5th instar stage, due to the normally variable developmental rates of eggs and larvae. These 2-3rd instars were less cold tolerant and showed higher percentage mortality within both the control and other treatments. To allow for a more accurate representation of the most cold tolerant instars only, a distinction was made between 2-3rd instars and 4-5th instars through obvious visual aspects of size and colour. These aspects were compared and confirmed by a literature study before being implemented.

The adapted experiment was repeated three times after the first two experiments showed some problems in the form of variance in diet and number of larvae per treated jars. These initial variances were due to a large number of jars being affected by fungal infection and dried out diet. These problems were solved when a faulty laminar flow bench filter was replaced and the diet and liquid were increased to better suit the cold temperature exposure times that the jars with larvae are exposed to. It was found that the diet tended to harden and dry out more readily within these prolonged periods of time kept in the cold and were possibly the cause of death in larvae, instead of the combination of CO_2 and cold treatments.

The final experiment showed the desired results for the prepared jars with 4-5th instar larvae. The experimentally obtained data have been analyzed for normality and to obtain the most significant and effective treatment. The data around the treatment will be analyzed further to obtain the required Probit analysis models.

Results and discussion

Data were analyzed with a three way ANOVA model, to incorporate all the different treatments. The different treatments were then compared to the dependent variable of mortality in 4th and 5th instar larvae, the most resistant larval instars to cold treatments. The data were tested for normality, as can be seen in Fig. 3.2.11.1, and it was found that the data can be considered to be normally distributed.

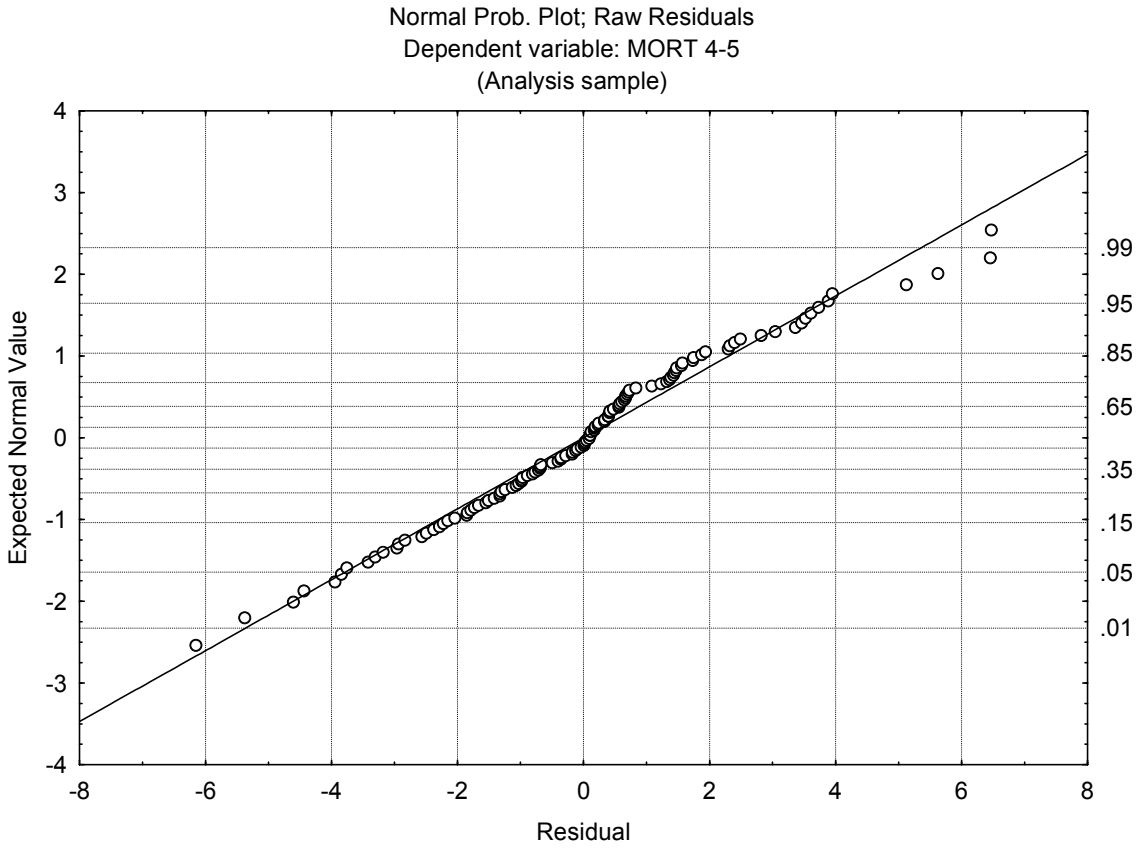

Fig. 3.2.11.1. The normal probability plot for the mortality of 4-5th Instar larvae exposed to the revised experimental CO_2 treatments of ambient (0.03% CO_2), 25% CO_2 and 75% CO_2; and cold treatments at -0.5°C of 6, 7.8, 10.14,13.18 and 17.13 days.

There was a significant difference between the ambient control treatment and the other treatments of 25% CO_2 and 75% CO_2 at the different cold exposure time treatments at -0.5° C, 6, 7.8, 10.14,1318 and 17.13 days (Fig. 3.2.11.2). The significant difference indicates a positive response to the CO_2 treatments of both 25% CO_2 and 75% CO_2 at both the 8 and 24 hour exposure time to the varying cold treatments. This suggests that the increased exposure of carbon dioxide is making the cold hardy 4-5th instar larvae more sensitive to cold temperatures and causing increased mortality in shorter periods of cold treatments. However, no significant difference was statistically found between the CO_2 treatments of 25% and 75% CO_2 as it seems that at 6 days the surviving larvae had already passed the dose response point. No treatment can thus be classified as being the most effective.

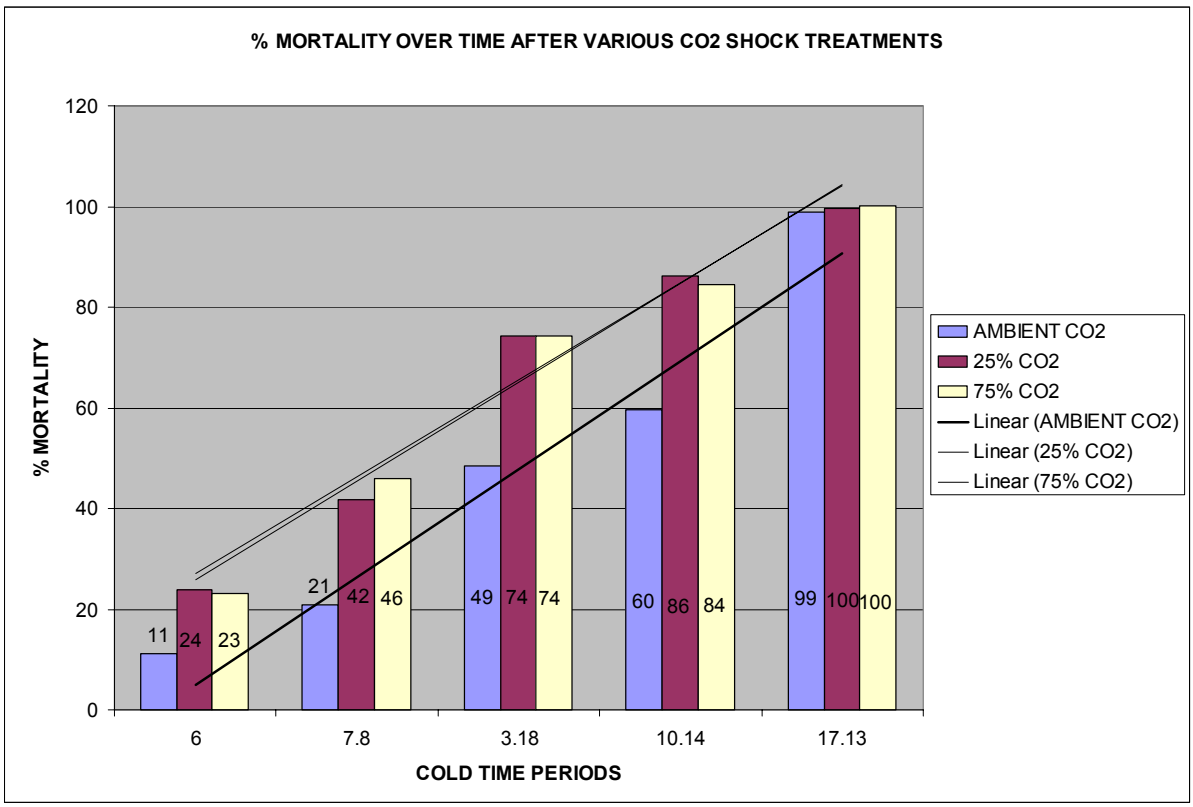

Fig. 3.2.11.2. Mortality percentage in 4-5th instar larvae exposed to carbon dioxide shock treatments of ambient (0.03% CO_2), 25% CO_2 and 75% CO_2 and exposed to a cold temperature of -0.5°C for varying amounts of time, namely 6, 7.8, 10.14, 13.18 and 17.13 days.

Conclusion

The treatments have been considered at different levels to motivate the use of a specific treatment for future experiments. A major consideration for the treatments that should be used is the health effects associated with exposure to carbon dioxide. Menn *et al*. (1970) states that even a 'minimal' concentration of 2,8% carbon dioxide can cause dyspnoea in humans after a mere 30-minute exposure. In exposures ranging from 17% to 30% Wong (1992) found that within 1 minute there could be a loss of controlled and purposeful activity, unconsciousness, coma, convulsions, and death.

It has been decided to use the potentially least harmful CO_2 treatments for human safety and the treatment which could potentially have the least damaging effect on fruit. This combination of treatments is namely 25% CO_2 (CO2TREAT2) at 8 hours (CO2 TIME: 1), at -0.5° C for varying amount of time, namely 6 (COLD TIME: 1), 7.8 (COLD TIME: 2), 10.14(COLD TIME: 3), 13.18 (COLD TIME: 4), and 17.13 (COLD TIME: 5) days. The data used to construct the Probit analysis model will be from the combination of these treatments. Future experiments on the fruit to assess physiological affects and the possible fruit damage and decay that can be caused, will also be conducted with this combination of treatments.

Future research

Future work for 2007 will commence in April and include bioassays with field-collected FCM infested fruit, using a selection of treatment conditions that have shown potential in the preceding bioassays and proved to be the safest for human safety and potentially the least damaging to fruit. It will be observed whether these CO_2 shock treatment increases sensitivity of FCM larvae inside citrus fruit to subsequent cold treatment. If so, the reduced duration, or increased temperature, conditions for the subsequent cold treatment, as a potential new disinfestation treatment will be determined.

Further the effect of such a combination treatment on fruit quality will be evaluated. If the study indicates that such a combination treatment may suffice as a prospective new disinfestation treatment for FCM in citrus, a further series of development trials will be required to establish the requisite treatment conditions in order to meet the Probit-9 efficacy level. Likewise, subsequent large scale fruit quality trials would also ensue to establish the practical applicability of the treatment. Such development work would then be

followed by official bi-lateral negotiations with SA's trading partners, before the treatment could potentially be adopted as an alternative disinfestation treatment. However, the potential advantages of an alternative disinfestation treatment that is less damaging to fruit quality, add a minimal of extra costs and increase the reliability of standard protocols for exports and access to export markets, would be enormous, making it imperative that all such potential opportunities be fully investigated.

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3.2.12 **An investigation into the details of FCM population dynamics across time and space**

Experiment 859 by Rob Stotter (SU)

Opsomming

'n Ondersoek word alreeds 11 maande lank uitgevoer om die gebieds- en seisoensgebonde verspreiding van valskodlingmot, *Thaumatotibia leucotreta*, in die Citrusdalgebied te ondersoek. Die opnames behels lokvalmonitering op 6 dwarssneë deur die Olifantsriviervallei in beide sitrusboorde en inheems fynbos op wisselende afstande van die boorde af.

Uitbreide monstering van moontlike gasheerplante word ook bykomend twee-weekliks uitgevoer. Slegs akkers en koejawels is sover as alternatiewe gasheerplante vir VKM in Citrusdal gevind, alhoewel vele inheems plantspesies versamel is. Alhoewel VKM in akkers voorkom, word laasgenoemde hoofsaaklik deur 'n Phycaetid-motspesie besmet. Waar alternatiewe gasheerplante nie in die veld aanwesig is nie, is VKM-getalle baie klein of nul.

Feromoonlokvalle wys oortuigend dat VKM-mannetjies in die veld voorkom, maar dat hul getalle beduidend met afstand en hoogte van die naaste sitrusboorde af, afneem. Boordlokvalle die naaste aan inheemse veld vang meer mannetjies as dié verste van die veld af, wat op 'n sterk interaksie en moontlike beweging van die mannetjies tussen boorde en die nabygeleë inheemse fynbos dui.

Alhoewel bestrydingsmaatreëls toegepas word, word daar nog steeds meer mannetjies in sitrusboorde as in fynbos gevang. Dit lyk onwaarskynlik dat inheemse fynbosspesies 'n beduidende rol in VKM-besmettings in sitrusboorde speel. Dit is moontlik dat die mannetjies slegs skuiling in nabygeleë fynbos soek, aangesien geen VKM-besmette inheemse vrugte opgespoor kon word nie.

Daar is ook waargeneem dat mannetjiemotte baie in Valenciaboorde voorkom, wat dikwels nie teen VKM behandel word nie omdat hulle nie as betekenisvolle gashere beskou word nie. Dit kan wel so wees indien dit met nawels vergelyk word, maar daar kan beduidende migrasie tussen boorde plaasvind. In baie gevalle word meer mannetjies in Valencia- as in nawelboorde gevang en somtyds ver meer as die ekonomiese drempelvlak vir bestryding.

Introduction

In the past, population dynamics of FCM and its egg parasitoids have been studied within the citrus orchard, while very little attention has been given to population studies outside the orchard system. An agroecosystem comprises not only of cropping systems but also indigenous plant patches (Fig. 3.2.12.1).

FCM is known to feed on various alternate host plants besides citrus, allowing it to be active and pose a threat throughout the year. The succession and composition of alternate cultivated and wild host plants may have an important influence on FCM population fluctuations within the citrus orchard. It has long been suggested that initial FCM infestations in citrus orchards stem from alternate host plants in the vicinity of citrus, and that these could significantly increase population levels throughout the year.

Hence, this project seeks to investigate what population numbers are sustained outside the orchards, and how these change throughout the season. It also seeks to determine the capability of FCM to utilize a succession of alternative host plants, and their capability to migrate back into citrus orchards and cause a re-infestation early in the season.

This study has important implications for the success of the planned mass Sterile Insect Releases of FCM as a control practice in the near future.

Fig. 3.2.12.1. Illustrating a typical land mosaic close to Citrusdal, with citrus orchards on the valley floor and indigenous fynbos on mountain slopes.

Materials and methods

Pheromone trapping

Yellow Delta traps were used as they are light and easy to carry, and also readily visible (Fig. 3.2.12.2). These, along with sticky pads, were provided by Chempak. The pheromone lure used is the Lorelei pheromone, chosen due to its long-lasting activity, and the fact that thresholds are based on this pheromone lure. Traps are inspected biweekly.

Fig. 3.2.12.2. Yellow delta trap with sticky pad and Lorelei pheromone lure, as set up in a wild-growing guava tree at The Baths.

Six transects were identified, dissecting the Olifantsrivier valley around Citrusdal. Each transect runs from a mountain range, across a valley, comprising mostly of citrus orchards and up to another mountain range. Transects are spaced between 5 and 10 km apart, so the area covered is approximately 60 km along the Olifantsrivier. Within each transect, 7 traps were placed in citrus orchards, comprising either navel oranges, Valencias, or mixed orchards. In addition, 7 traps were placed at set distances from and heights above the nearest citrus orchard, in indigenous veld (Fig. 3.2.12.3), to the west, and to the east of citrus orchards, extending up into the mountains (Table 3.2.12.1).

Fig. 3.2.12.3. Pheromone trap in Sugar bush in veld.

Table 3.2.12.1. Veld trap constants showing trap placement in relation to nearest citrus orchard

VELD TRAP CONSTANTS		
TRAP 1	≤ 20 m elevation = ≤ 4000 DE (Distance*elevation units)	≤ 200 m distance from nearest orchard
TRAP 2	20 m – 50 m elev. = 4 000 – 20 000 DE units	200 m – 400 m dist.
TRAP 3	50 m – 70 m elev. = 20 000 – 42 000 DE units	400 m – 600 m dist.
TRAP 4	70 m – 100 m elev. = 42 000 – 90 000 DE units	600 m – 900 m dist.
TRAP 5	100 m – 150 m elev. = 90 000 – 180 000 DE units	900 m – 1 200 m dist.
TRAP 6	150 m – 200 m elev. = 180 000 – 300 000 DE units	1 200 m – 1 500 m dist.
TRAP 7	+200 m elevation = +300 000 DE units	+1 500 m distance

GPS co-ordinates of each trap are kept to facilitate easy finding of traps in the veld, which can sometimes be difficult.

Alternative host sampling

Every two weeks, possible alternative host plants are collected. Very few alternative hosts of FCM have been identified in the past (Stephan Honiball, 2004). Any plant bearing fruit is collected, particularly those known to be alternative host plants, such as the Real yellow wood *Podocarpus latifolius*, the Common oak *Quercus robur*, Guava*Psidium guajava*, wild almond *Brabejum stellatifolium*, and the wild olive *Oleae europea* subsp. Africana. Table 3.2.12.2 shows a list of plant species intensively sampled to date.

Table 3.2.12.2. Reported host plants intensively sampled

Latin name	Common name
Brabejum stellatifolium	Wild almond
Carya illinoinensis	Pecan nut
Diaspyros glabrata	
Eucalyptus sp.	Gum tree
Harpephyllum caffrum	Wild plum
Kigellaria africana	Wild peach
Mytenus oliodes	
Olea europeae subs. a*fricanum*	Wild olive
Phylica sp.	
Podocarpus latifolius	Real yellow wood
Populus simonii	Chinese poplar
Protasparagus sp.	
Psidium guajava	Guava
Quercus robur	Common oak
Solanum sp.	
Syringae sp.	Syringa

Results and discussion

Pheromone Trapping

Bi-weekly trapping data has been used to show the cumulated number of male FCM caught per trap in relation to its position in the transect, over an 11 month period. Fig. 3.2.12.4 represents this data, with blue bars showing cumulated trap counts in citrus orchards, and red and yellow bars showing cumulated trap counts in west and east veld traps respectively. Bear in mind that each bar represents the trap position in all 6 transects.

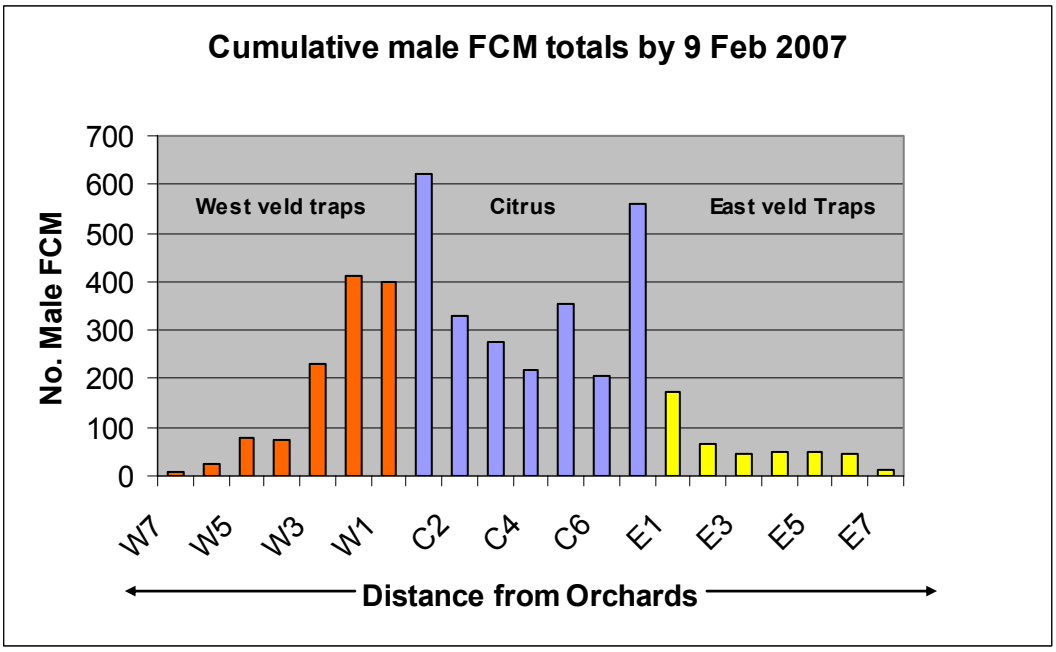

Fig. 3.2.12.4. Cumulative male FCM totals by Feb 9th, 2007.

From this graph, we get a clear indication of the presence of male FCM across transects. In the veld, FCM numbers are higher closest to citrus orchards and almost nil at 1,5 km distance from the nearest orchard. Citrus trap counts are highest in orchards nearest to the veld, possibly indicating migration of male FCM between orchards and veld plants.

While the graph appears skewed to the West side of the valley, this is largely due to the high presence of male FCM in one transect, at The Baths, in which veld traps run through a commercial campsite and up a kloof, in which numerous oak trees, wild olive trees and wildly growing guava trees occur. FCM larvae have been collected from both oak trees and guava trees within this kloof. Trap counts here are extremely high at certain times of the year, illustrating the possibility for FCM build-up in areas of high alternative host plant presence.

Fig. 3.2.12.5 shows the average number of male FCM per trap per week in citrus orchards. Again, bear in mind that each line represents the average of 6 traps, one from each transect. This graph shows the temporal (seasonal) distribution of male FCM within citrus orchards, with peaks occurring in June, early September, and early November. All of the navel orchards are treated with insecticides for FCM control, as are many of the Valencia orchards, so this graph does not necessarily give a completely accurate representation when comparing it to graphs of the veld traps, where no treatment takes place. None of the orchards involved in the surveys were treated with a mating disruption product, which would have disrupted trap catches to a greater extent.

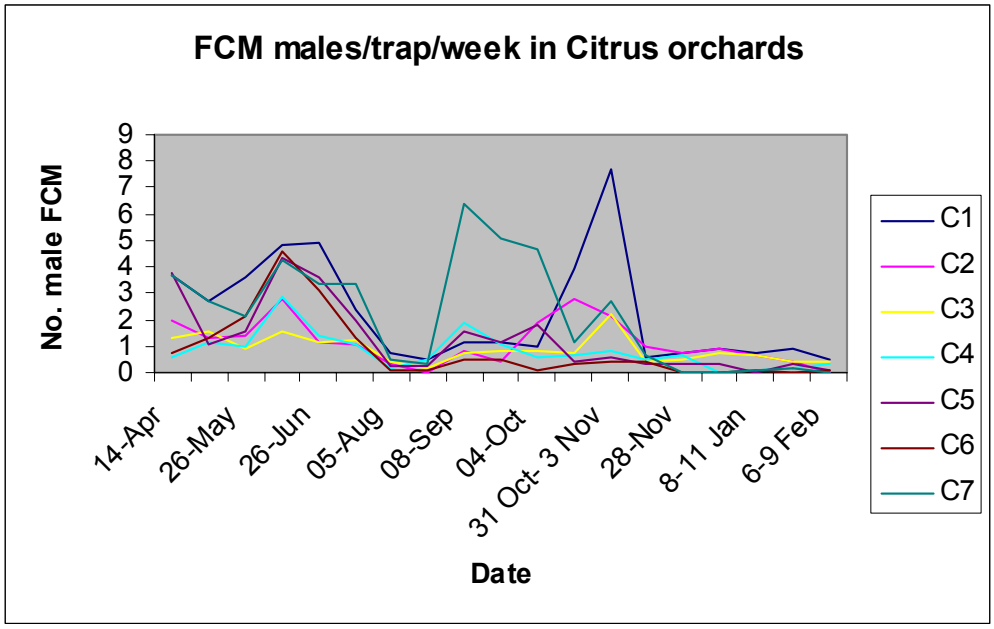

Fig. 3.2.12.5. Number of male FCM per trap per week in citrus orchards across transects.

It has been observed that many Valencia orchards are untreated, and in many cases, male FCM presence in these orchards is higher than their presence in navel orchards. In a few cases, trap counts in Valencia orchards have far exceeded the economic threshold value of ten moth per week. As Valencia fruit are often only picked as late as October-November, there exists the possibility that FCM populations can be maintained in these orchards until well into the navel fruiting season, offering an uninterrupted food supply for larvae all year round. Mixed orchards have higher numbers of male FCM than other orchards (Fig. 3.2.12.6).

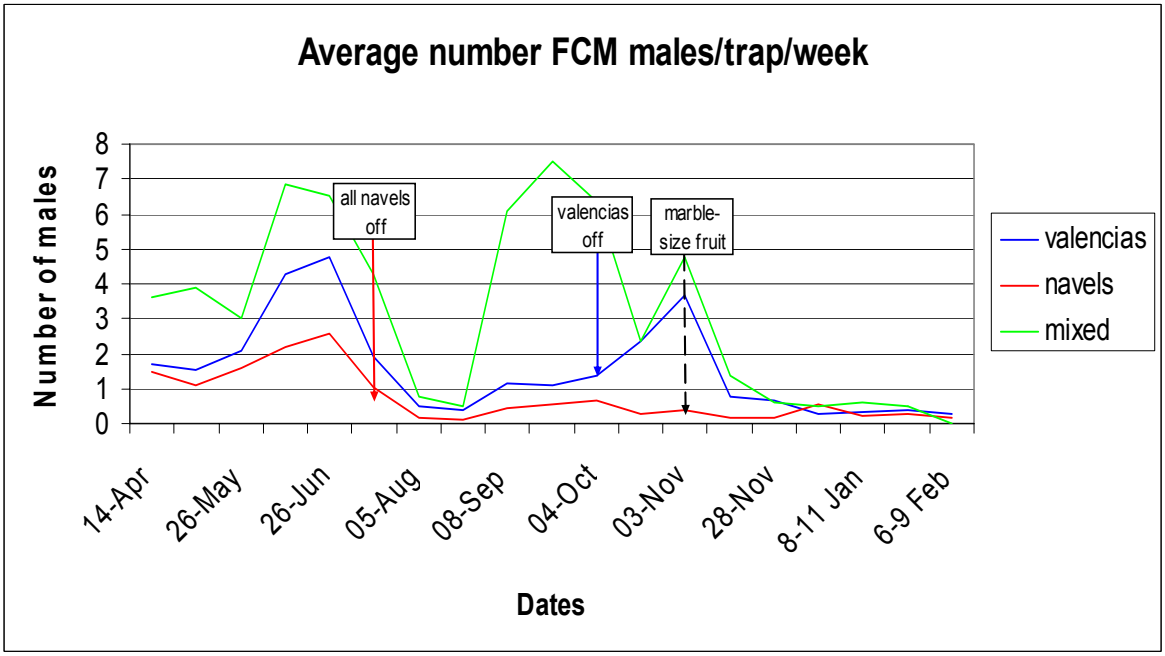

Fig. 3.2.12.6. Comparison of seasonal male FCM presence in navels, Valencias, and mixed orchards.

Graphical representation of veld trap catches is seen in Fig. 3.2.12.7 and Fig. 3.2.12.8. Each line again represents the average of 6 traps, and the average moth catches in citrus orchards at the same time has also been included for comparison.

From these graphs it is clearly seen that veld trap catches follow the same seasonal trend as citrus trap catches, indicating a strong interaction between veld and citrus catches, probably due to migration of moths between orchards and veld during times of high moth presence.

It can also be seen that veld traps closest to citrus orchards have higher numbers of male FCM throughout the season, than those furthest away.

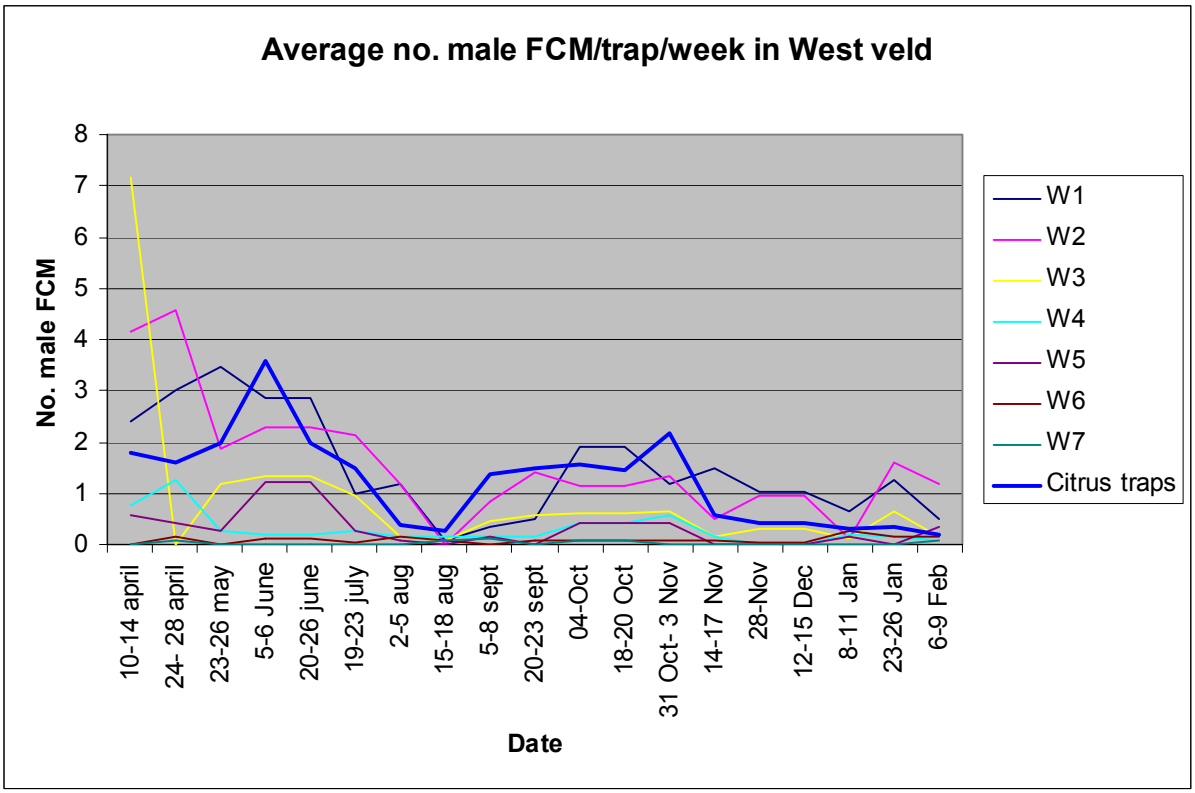

Fig. 3.2.12.7. Seasonal male FCM trap catches in west veld traps compared to average citrus trap counts.

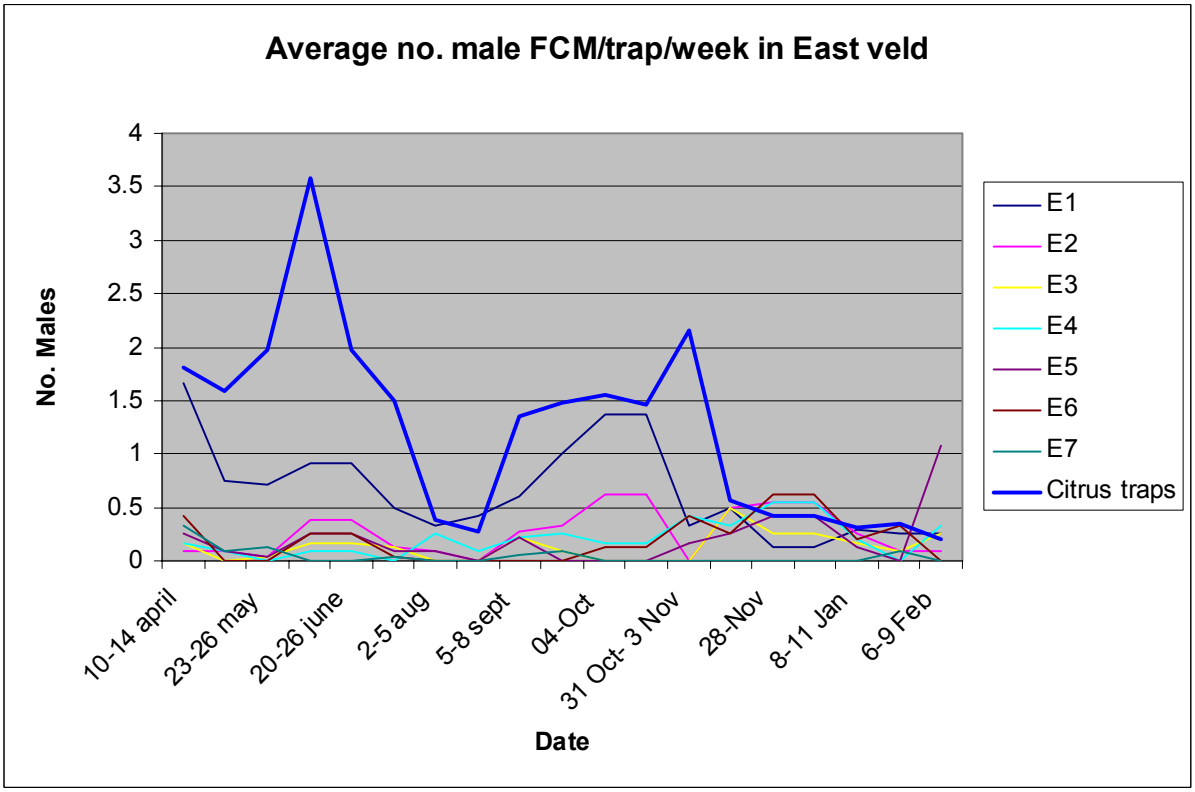

Fig.3.2.12.8. Seasonal male FCM trap catches in east veld traps compared to average citrus trap counts.

Alternative host plant sampling

So far, no indigenous plant species have been observed to be hosts of FCM in the Citrusdal area. This, despite references of Real yellow wood, wild olive, and wild almond all being possible alternative hosts of FCM. These plants are widespread in the Citrusdal area and have been sampled very intensively form various areas. This indicates the preference of FCM for citrus as a host.

Alien plant species have also been sampled intensively, including various reported host plants. So far, FCM has only been detected in acorns of the Common oak, and in guavas. Whilst acorns are heavily infested, it has been observed that most of these infestations are not due to FCM, but to a phycaetid moth, which widely attacks acorns, but is not yet known to attack citrus. However, some FCM have been collected from acorns (Fig. 3.2.12.9 and Fig. 3.2.12.10), and at the time of writing, I can report that all infested acorns being collected at the moment are infested by FCM, although infestation levels are very low. Perhaps the phycaetid moth only starts to infest acorns later in the season, possibly outcompeting FCM in the acorns. Time will tell.

Fig. 3.2.12.9. Infested acorns beneath an oak tree, close to a citrus orchard, showing a larva of a phycaetid moth species.

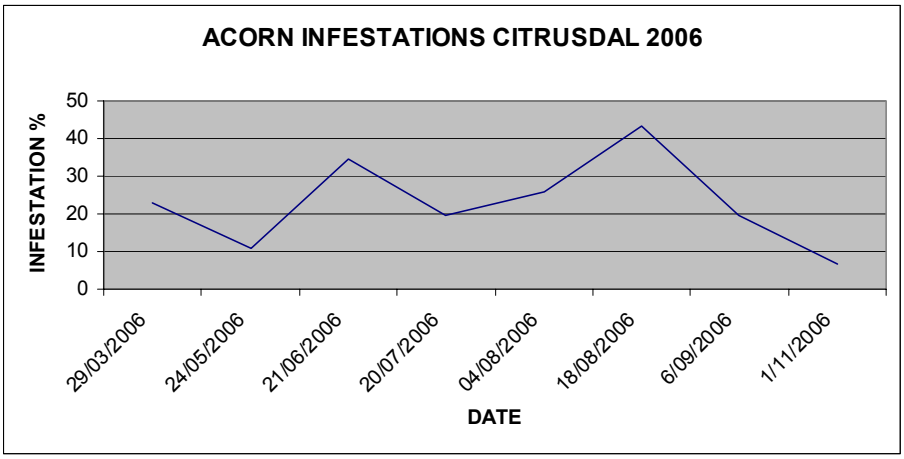

Fig. 3.2.12.10. Acorn infestations around Citrusdal, 2006. Mostly phycaetid moths.

Conclusions

FCM males are present outside orchards in the Citrusdal area, but their numbers decrease significantly with increasing distance from citrus orchards. Whilst they are present in the veld, no indigenous alternative host plants have been identified, despite extensive sampling taking place bi-weekly. This could mean that whilst

male FCM are present in the veld, FCM may not necessarily breed and increase in numbers in the veld, except in exceptional circumstances where numerous alien alternative host plant species occur. Indigenous plants, therefore, seem unlikely to contribute significantly, if at all, to successive FCM infestations in citrus orchards every year. Rather, it is more likely that late-hanging Valencia fruit may contribute to supporting FCM numbers in orchards throughout the year. Acorns and other alien alternative host plants may provide refuge to some FCM, but the extremely low infestation levels indicate that their preferred host plant is still citrus.

Future research

Sampling and trapping will continue as it has, until September 2007, which will give 18 months of data. This should hopefully culminate with and end in the MSc project, with the possibility of upgrading to a PhD project, in which the aspects of FCM ecology within and outside citrus orchards will be investigated in more detail.

Acknowledgements

Many thanks to CRI for funding the project, and to Prof. Vaughan Hattingh, Prof Michael Samways and Hendrik Hofmeyr for advice and guidance.

A big thank you to all the cooperating farmers around Citrusdal, who have been willing to help, and to let me onto their farms. A thank you also to Dr. Anton Paauw, for identifying plant species for me.

3.3 PROJECT: FRUIT FLY

Project Co-ordinator: Tim G. Grout (CRI)

3.3.1 Project summary

Fruit flies are becoming increasingly important as we seek to enter new markets and maintain traditional markets. The public is also becoming increasingly aware of their significance as new fruit flies appear in various parts of the world and they are exposed to eradication measures or infested fruit. CRI continues to rear the three species of *Ceratitis* that are economically important in southern Africa (3.3.2). These flies are used in many of the following experiments. A cold disinfestation, Phase 4 trial was conducted with oranges to determine whether 1.5°C for 16 days would be an acceptable treatment for the control of Medfly. Unfortunately, three flies out of 62 492 survived so this work will need to be repeated for the same period at 1°C (3.3.3). No progress was made in developing a means of evaluating slow-acting bait toxicants in the field without going to large-scale trials (3.3.4) and the English company contracted to develop a rapid diagnostic on-site test for Medfly larvae have also had little success (3.3.5). The developmental thresholds were successfully determined for all three *Ceratitis* species and show that Medfly and Marula fruit fly are very similar in their requirements and differ slightly from Natal fruit fly whose optimum rearing temperature is one degree lower. However, results for our Natal fruit fly differ to those for this species in Reunion which must be a different strain (3.3.6). Research on field control of fruit flies showed that the application of bait to the ground or ground cover to avoid fruit residues is ineffective. Determination of lure efficiency to known numbers of released fruit flies showed that Capilure and Ceratitislure were most effective for sexually mature flies whereas maturity was not important for Questlure (3.3.7). Natal fruit fly is being monitored throughout the year by various collaborators at various locations around southern Africa to provide us with more information on its requirements. Flies are being identified and recorded but it is too early to draw any conclusions (3.3.8). The tropical invader fruit fly, *Bactrocera invadens*, is present throughout West, Central and East Africa and may eventually threaten subtropical parts of southern Africa. Contact is being maintained with researchers in East and West Africa and our own Department of Agriculture to determine what proactive research may require funding.

Projekopsomming

Die toenemende belangrikheid van vrugtevlieë speel nie net ’n rol in die verkryging van toegang tot nuwe markte nie maar ook in die behoud van bestaande, tradisionele markte. Die publiek se bewustheid van hul belangrikheid neem ook toe soos nuwe vrugtevlieë ontdek word in verskeie dele van die wêreld en hul blootgestel word aan uitroeiingsaksies of besmette vrugte. ’n Telingsprogram van die drie ekonomies, belangrike *Ceratitis* spesies in suider Afrika word deur CRI instand gehou (3.3.2) en is in die meeste van die volgende proewe gebruik. ’n Fase 4, koue disinfesteringsproef is met lemoene uitgevoer om te bepaal of 1.5°C vir 16 dae ’n aanvaarbare behandeling sal wees vir die beheer van Medvlieg. Ongelukkig het 3 van die 62 492 vlieë oorleef en die werk sal dus herhaal moet word vir dieselfde periode by 1°C (3.3.3). Geen

vordering is gemaak in die ontwikkeling van 'n metode om lokmiddels met gifstowwe wat stadig-werkend is, in die veld te evalueer sonder die uitvoering van grootskaalse proewe nie (3.3.4). Die Engelse maatskappy wat gekontrakteer is om 'n vinnige diagnostiese toets vir Medvlieg larwes te ontwikkel het nie baie sukses gehad nie (3.3.5). Die ontwikkelingsdrempelwaardes is suksesvol bepaal vir al drie *Ceratitis* spesies. Die behoeftes van Med- en Maroela vlieg was baie dieselfde en het net effens verskil van die Natalse vrugtevlieg. Die Natalse vrugtevlieg se optimum telingstemperatuur was een graad laer. Resultate wat verkry is van ons Natalse vrugtevlieg verskil egter van die resultate van die spesie wat in Reunion voorkom wat daarop dui dat dit 'n ander ras moet wees (3.3.6). Navorsing op die beheer van vrugtevlieë in die veld het getoon dat die aanwending van lokmiddels op die grond of op 'n grondbedekker om residue op vrugte te voorkom, nie effektief is nie. Bepalings van die effektitwieit van lokmiddels op 'n bekende getal vrugtevlieë wat losgelaat is, het getoon dat Capilure en Ceratitislure die effektiefste was met geslags volwasse vlieë, terwyl volwassenheid nie belangrik was in die geval van Questlure nie (3.3.7). Verskeie medewerkers op verskillende plekke in suider Afrika vorm deel van 'n moniteringsaksie wat reg deur die jaar plaasvind om meer inligting in te win rakende die behoeftes van die Natalse vrugtevlieg. Die vlieë word geïdentifiseer en aangeteken maar die eksperiment is nog in 'n te vroeë stadium om enige gevolgtrekkings te maak (3.3.8). Die tropiese indringer vrugtevlieg, *Bactrocera invadens,* is teenwoordig in Wes-, Sentraal- en Oos-Afrika en mag moontlik ook die subtropiese dele van suider Afrika bedreig. Kontak vind op 'n voortdurende basis plaas met die navorsers in Oos- en Wes-Afrika asook met ons eie Departement van Landbou om te kan bepaal vir watter pro-aktiewe navorsing befondsing nodig sal wees.

3.3.2 **Fruit fly rearing**

Experiment 407 by John-Henry Daneel and Rooikie Beck (CRI)

Die drie ekonomies, belangrike *Ceratitis* spesies word voortdurend geteel by CRI-Nelspruit om te kan voorsien in 'n konstante bron van vlieë vir navorsingsdoeleindes. Gedurende 2006, is 300 000 Medvlieg eiers gebruik in koue disinfesteringsnavorsing (3.3.3). Vlieë is gebruik in die toetsing van stadig-werkende gifstowwe in veldhokke (3.3.4), vir die evaluasie van 'n prototipe van 'n vinnige diagnosties toets (3.3.5) en om kulture te begin vir ontwikkelingstudies (3.3.6). Daar is ook 81 000 vlieë losgelaat in die veld om die relatiewe effektiwiteit van verskillende lokval sisteme te vergelyk (3.3.7). 'n Totaal van 36 000 vlieë is aan Johan de Graaf (Westvalia Tegniese Dienste) voorsien vir navorsing en om sy eie kulture te vestig. Verskeie besendings papies van *C. capitat*a en *C. rosa* is ook aan Juanita Heunis by die Universiteit van Stellenbosch gestuur vir haar navorsing wat handel oor die gedrag en biologie van *C. rosa*. Die telingstegniek van die Natalse vrugtevlieg is met tyd verbeter en tydens die Sitrusnavorsing Simposium in Port Elizabeth is 'n plakkaat aangebied. 'n Beskrywing van die telingstegniek word in die volledige verslag gegee. Daar sal voortgegaan word met die teling van al drie *Ceratitis* spesies.

3.3.2.1 **Rearing of Natal Fruit fly *Ceratitis rosa* Karsch**

Experiment by John-Henry Daneel, Tony Ware, Rooikie Beck and Tim Grout (CRI)

Introduction

Natal fruit fly, *Ceratitis rosa* Karsch, is an important economical and phytosanitary pest on many crops in South Africa and occurs in many parts of the country. Due to the significance of this pest, there is a need to conduct mitigating treatments and other research that requires large numbers of these insects. The rearing technique commonly used for the related Mediterranean fruit fly *C. capitata* is not successful for Natal fruit fly for two reasons. Firstly, insectary-reared Mediterranean fruit fly oviposit through the gauze walls of the rearing cages and the eggs can be collected in a container filled with water placed below each cage. Natal fruit fly does not conform to this behaviour and in the laboratory they will only oviposit in apples or other fruit that has been previously pierced with a pin or similar device. However, this is a labour-intensive method and therefore not suitable for mass production. Secondly, the growth medium commonly-used for Mediterranean fruit fly larvae is not suitable for Natal fruit fly larvae. For these reasons the following technique was developed over a number of years and a comparison of larval diets conducted.

Rearing technique

Egg collection

After collecting the first wild Natal fruit flies in 1998 it was confirmed that the flies would not under any circumstances oviposit through the gauze walls of the cage, as the insectary-reared Mediterranean fruit flies did. First attempts to force adult flies to oviposit in various devices were not very successful. Even the use of attractants such as guava paste or apple pulp smeared on either the outside or inside of these devices did not give the required results.

Various fruit types were evaluated as oviposition substrates and it was found that if an apple was placed in a cage, the females would oviposit in it but the collection of the eggs was difficult because eggs were laid anywhere in the apple and especially in the stem and stylar ends. We therefore exploited the fact that fruit flies oviposit in fruit wounds (Papaj et al. 1989). A fruit-piercing device (with eight adjacent pins in a line) (Fig. 3.3.2.1.1) was made to prick the apples; thereby stimulating the females to oviposit in the holes. This also forced the flies to lay their eggs in a fixed area making the extraction of the eggs easier. Each apple was then pricked four times around its circumference to create holes for the egg-pockets. It was found that the best cultivar to use was Granny Smith, while apples like Golden Delicious were unsuitable because the flies still laid their eggs anywhere on the surface of the fruit, even when the fruit-piercing device was being used.

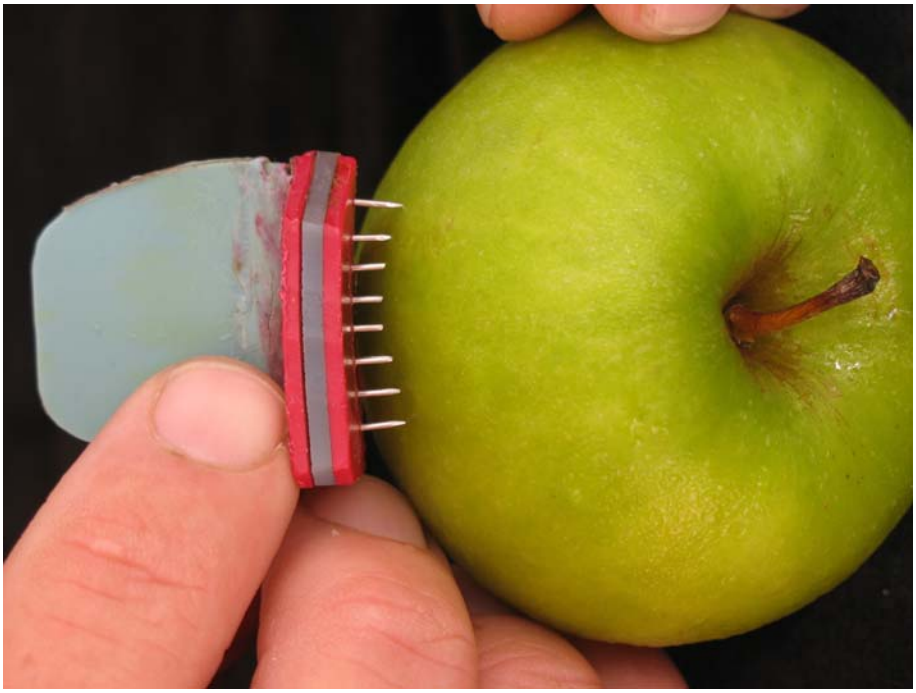

Figure 3.3.2.1.1. Fruit piercing device used to stimulate oviposition by Natal fruit fly

Eggs are collected by removing a longitudinal slice of apple including 5 mm of flesh below the skin containing the egg-pockets. The slice is then placed peel-side-down and a cut made through and along the row of eight holes, without cutting through the peel. The two sides of the slice can then be bent back to expose the egg-pockets (Fig. 3.3.2.1.2). A washbottle with a narrow spout is used to rinse the eggs from the flesh into a container. This method was successful but is extremely labour intensive.

Figure 3.3.2.1.2. Egg pockets just below the peel of the apple

As the culture increased and enough eggs were collected from the apples, apples were only placed in the most productive cages thereby depriving certain cages of oviposition substrates. With the absence of an oviposition substrate, it was noticed that the flies sometimes attempted to oviposit into the water dispensers in the cages. This observation led to the development of an egg-collection device consisting of two orange, plastic, honey-jar screw-on-lids attached to the top halves of two plastic honey-jars that were joined together with masking tape (Fig. 3.3.2.1.3). Small holes (1 mm diameter) were punched into the lids through which the females could oviposit. Paper towel, saturated with water, was placed in the centre section of each container to ensure that the eggs laid in the containers did not dry out before collection.

The lids are removed and rinsed with water to collect the eggs. Three of these containers are enough to maintain the culture and are usually placed in the three strongest egg-producing cages. It was noticed that once a population of fruit flies in a cage was exposed to these containers, the flies would no longer oviposit in apples. The containers are placed in the cages twice a week to collect eggs with eggs being harvested after 48 hours exposure.

Figure 3.3.2.1.3. Egg-collection device comprising the top halves of two plastic honey jars taped together with the lids perforated with small holes to stimulate oviposition

Larval media

Once the eggs are collected, a small pipette is used to suck up the eggs and two thousand eggs (by volume) are evenly distributed onto the media in one 11 cm-diameter Petri dish. Loosely fitted lids are placed on these containers for two days to prevent the media and eggs from desiccating.

The larval medium commonly used for the Mediterranean fruit fly is not optimal for the rearing of Natal fruit fly. After experimentation with different media, the first alternative mixture was used: Oats (200 g), granulated sugar (85 g), Torula yeast (55 g), dried carrot powder (50 g), sodium benzoate (1.8 g) dissolved in 100 ml water and 7 ml hydrochloric acid in 233 ml of water. First the dry mixture is mixed thoroughly then the water and hydrochloric acid are added until the medium is saturated and only then is the dissolved sodium benzoate added. Although fruit fly larvae were successfully reared from this medium it was not ideal as once the larvae were ready to jump, some larvae still remained moribund at the bottom of the containers. It was therefore necessary to tap the containers daily to ensure that all larvae jumped onto the sand to pupate. A more effective medium was therefore developed. This medium (called the Catyaan diet) comprised carrot powder (70 g), Torula yeast (35 g), ascorbic acid (0.32 g), nipagin (0.69 g) and 380 ml boiled water. It is very similar to the medium used by Etienne (1973) to collect Natal fruit fly eggs except that he used Brewers' yeast and he used two different media for larvae.

Pupal collection

Four of the 11 cm containers are placed in a plastic 10-litre cake box, which has openings in its walls covered with fine mesh to allow air movement through the container. This larger container is sealed to avoid contamination from *Drosophila* sp. flies and to protect the larvae from parasitoids. The bottom of the cake box is covered with a 1 to 2 mm deep layer of fine sand, to facilitate pupation of the larvae. When using the Catyaan diet the larvae start pupating after 7 days at 24^{o}C without tapping or movement of the containers. Once all the larvae have pupated and hardened the sand and pupae are separated by using a sieve. The pupae are then placed in a small paper bag and moved back to a clean, larger container until the adults start emerging; 18 days after the eggs have been placed on the media when held at 24°C. The paper bags are then moved to a new adult cage where a new generation of adults emerge.

Adult cages

The adult cultures are kept apart from the larval and pupal cultures in the laboratory. This ensures that there is a backup available should anything go wrong with the adult culture, reducing the possibility of a total collapse of the culture.

The adults need a natural day-night cycle with crepuscular conditions in order to produce eggs (Myburgh 1952) and are therefore kept in an outdoor insectary enclosed with steel gauze. Ten cages of 160 ℓ capacity are used in the insectary to maintain the adult fly culture and to produce extra flies for various trials. Egg production is negatively affected by low temperatures and therefore a larger culture is needed to ensure that enough eggs are produced during winter months.

Each cage is provided with two inverted honey jars that act as fresh water reservoirs. One is hung inside the cage and the other is placed on top of the cage on gauze through which the flies can reach the water. Small holes of 2.5 mm diameter are punched into the lids of each jar that is then covered with wet filter paper to maximize the surface area of water available to the flies, without any water dripping into the cage.

Adult flies in the cages are fed a dry mixture of granular sugar and yeast hydrolysate enzymatic in a ratio of 3:1. Because of the hygroscopic nature of the mixture it is important that the mixture is inspected regularly and replaced if it becomes wet.

Further developments required

In order to improve the above technique for large-scale rearing, further attention must be given to developing an even less labour-intensive method of collecting eggs and the creation of artificial crepuscular lighting conditions suitable for mating, so that rearing can continue at optimal temperatures during winter.

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3.3.3 Cold disinfestation of Medfly infested lemons, grapefruit, oranges and Clementines using temperatures above 0°C

Experiment 772 by Bruce Tate, Tim Grout, Peter Stephen and John-Henry Daneel (CRI)

In 'n poging om die temperature te verhoog vir die in-transit koue behandeling van alle sitrus wat na Japan uitgevoer word, is navorsing gedoen met temperature bo 0°C. Tydens die navorsing is 'n Fase 4 disinfesteringsproef uitgevoer met Valencia lemoene vir 'n tydperk van 16 dae by 1.5°C. Drie oorlewendes is egter gevind in die 62 492 larwes wat behandel is. Die persentasie mortaliteit van 99.9952 wat verkry is, is egter effens te laag as dit wat benodig word vir die probit 9 vlak en daarom sal die werk in 2007 herhaal moet word by 'n temperatuur van 1°C vir 'n 16-dag periode.

Introduction

Due to chilling injury problems when using the current cold disinfestation protocol for Japan research is being conducted in order to apply for a longer treatment period at a higher temperature as used by some other countries that export citrus to Japan. Earlier phases of this research have been conducted with various citrus cultivars. This research just required that the phase 4 evaluation be conducted with oranges at a core temperature of 1.5°C.

Materials and methods

Fruit type: Small Valencia oranges 70-80 mm in diameter were used. Fruit preparation entailed the removal of the calyces and dipping in a combination of Sporekill (100 ml/100 ℓ water) and guazatine (480 ml/100 ℓ water) for one minute to surface sterilize and control fungal growth in inoculated fruit. Two thousand five hundred fruit were treated in each of three replicates.

The work area: The entire work space of 60 m^2 and the surrounding area of ± 160 m^2 were sprayed with a high pressure sprayer using 100 ml/100 ℓ Sporekill to surface sterilize all working surfaces. A solution of guazatine is used for sterilizing all apparatus in contact with the fruit, and workers dipped their hands in this solution before handling fruit.

Test insects: Mediterranean fruit fly (*Ceratitis capitata* [Weidemann]) reared at the CRI facility, were used to produce the eggs for inoculating test fruit. A minimum of 30 000 larvae exposed to the cold treatment is required to determine whether there are any survivors.

Inoculation: Eggs were collected within 24 h of oviposition and placed in deionized water. The egg/water ratio is adjusted until the number of eggs per 0.025 ml aliquot removed using an automatic pipette, is ± 40 which are placed in a 5 mm diameter hole drilled ± 30 mm deep into the fruit from the calyx end. Before the eggs are inserted, a small quantity of yeast is placed in the fruit to act as a protein source for the developing larvae. The hole is plugged with cotton wool before it is sealed using molten wax. Two thousand five hundred Valencia oranges were inoculated with ± 40 eggs each on 19 July 2006. The second replicate was inoculated on 30 August and the third replicate was inoculated on 5 October 2006.

Incubation: The fruit is individually placed into brown paper bags and packed into plastic crates (lug boxes) at 50 per crate. All fruits are systematically packed into these crates by loading the total number of fruit (2 500) into 50 crates two at a time per crate until the total (50 fruit) is reached. This ensures that fruit are shared equally among the crates as they emerge from the inoculation process. A few extra fruit are always added per box to ensure that correct numbers of fruit are obtained. These crates are then placed in a temperature-controlled room at 26°C to boost larval development. This period lasts for 6 days at which stage the fruit is removed and 2 000 fruit (40 crates) are transferred to the cold disinfestation room and the remaining 500 are retained as controls and dissected in the laboratory to determine the number of live larvae per fruit (ideally an average of 5+ larvae per fruit). Approximately 20 000 larvae were used in each of three replicates. Two thermoprobes in fruit connected to a Grant Squirrel data logger were used to monitor the core temperature during this first incubation period.

Cold disinfestation: Thermoprobes are calibrated before each disinfestation session using the freezing point method, the probes are immersed in melting ice and the temperature recorded when they reach equilibrium. A thermometer immersed in the melting ice is used to record the temperature, this is repeated 3 times. Calibration is done immediately prior to any of the tests being conducted. The sensors are placed arbitrarily among the test fruit and their positions recorded. The temperature of the room is recorded at the inlet and outlet of the cooling coil, 14 probes are placed in the fruit and 2 in the room, readings are recorded hourly by a Grant Squirrel data logger. The 16-day cold period was deemed to have started once the mean core temperature reached 1.5°C. Fruit were removed 16 days later and transferred to another room held at 26°C.

Evaluation: After 48 h at 26°C the fruit are dissected and the numbers of live and dead larvae recorded. The dead larvae are readily seen because they turn dark brown so the actual numbers of insects are used rather than an estimate based on the numbers found in the control fruit.

Temperature data: Temperatures were recorded in two fruit in one replicate during the two incubation periods at 26°C to confirm that the fruit was being held at the correct temperature. During the cold disinfestation treatment, temperatures were recorded within 14 fruit as described above. The mean temperature of these 14 probes and the highest and lowest temperatures were plotted for each replicate.

Results and discussion

Temperatures in the fruit in the first incubation period levelled off after 48 h to between 25.2 and 26.5°C (Fig. 3.3.3.1). Similarly, when fruit was removed from the cold treatment, a temperature of 26°C was achieved after 24 h (Fig. 3.3.3.2). In the cold treatments, temperatures were more stable at 1.5°C than is usually the case at temperatures below zero (Figs. 3.3.3.3, 4, 5). However, a power failure towards the end of the second cold treatment resulted in a slight increase in temperature for a few hours (Fig. 3.3.3.4). Control mortality was maintained below 8% (Table 3.3.3.1) but unfortunately, three survivors were found in the third replicate resulting in an overall mortality level that was very slightly above that required for the Probit-9 standard. This work will therefore have to be repeated for a 16-day period at 1°C in 2007.

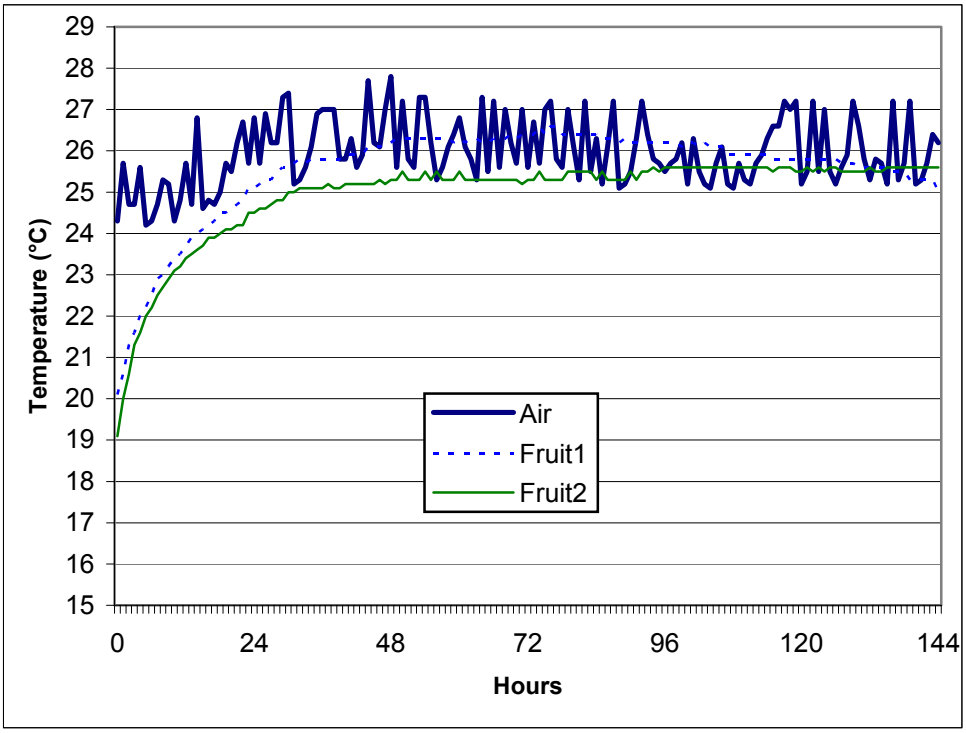

Figure 3.3.3.1. Temperatures in air and two fruit during the initial incubation period of the second replicate at 26°C when larvae are developing.

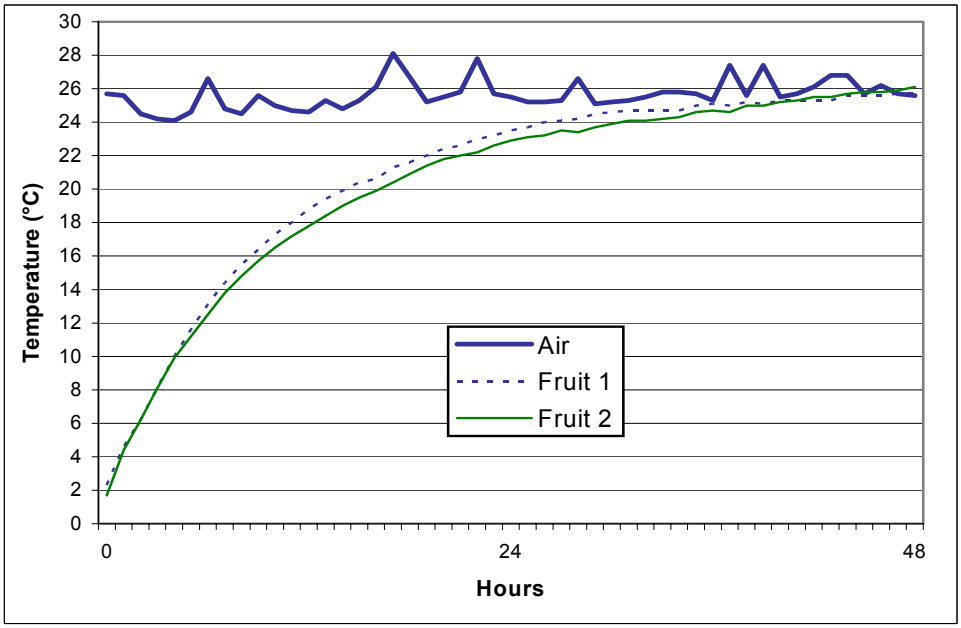

Figure 3.3.3.2. Temperatures in air and two fruit during the second incubation period of the second replicate at 26°C after fruit has been removed from the cold treatment.

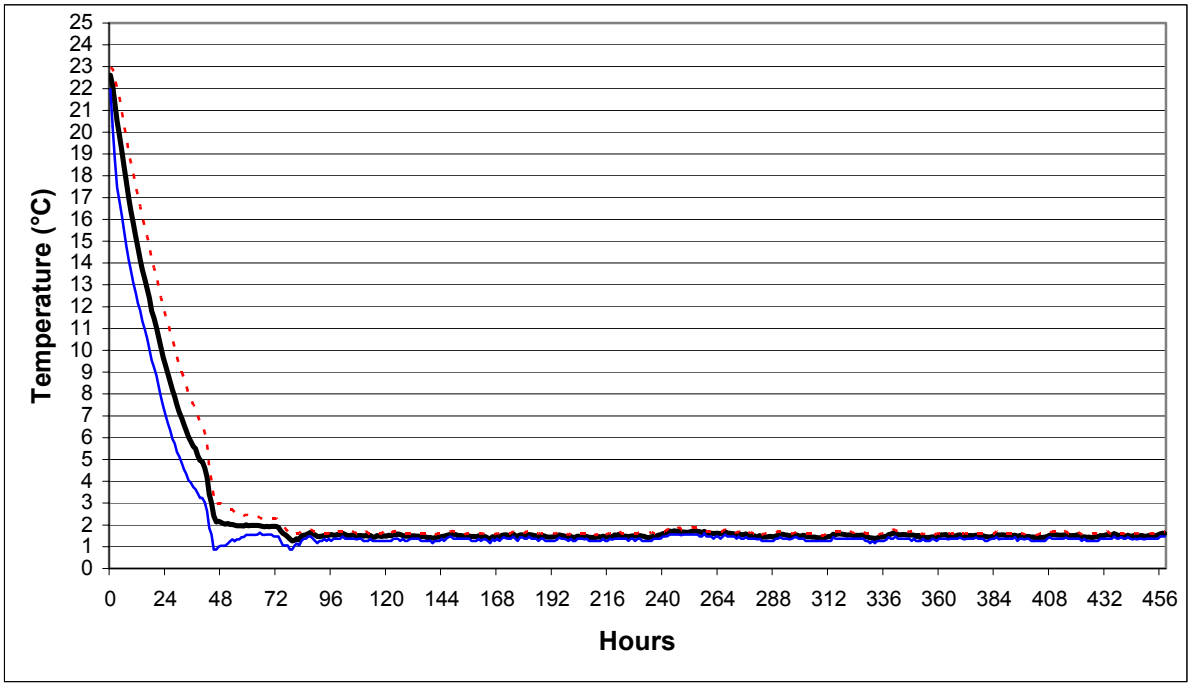

Figure 3.3.3.3. Highest (red, broken line), lowest (blue) and mean (black) temperatures of fruit in replicate 1. Long-term mean temperatures: mean 1.504, low 1.340, high 1.638.

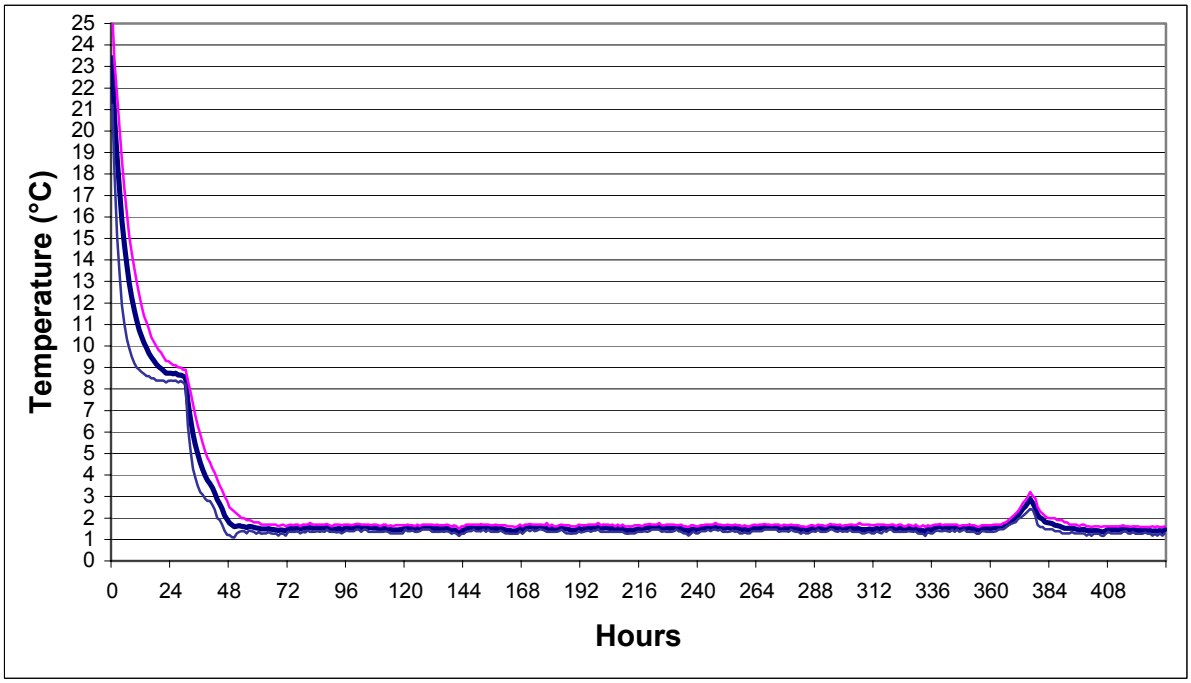

Figure 3.3.3.4. Highest (red, broken line), lowest (blue) and mean (black) temperatures of fruit in replicate 2. Long-term mean temperatures: mean 1.540, low 1.375, high 1.715.

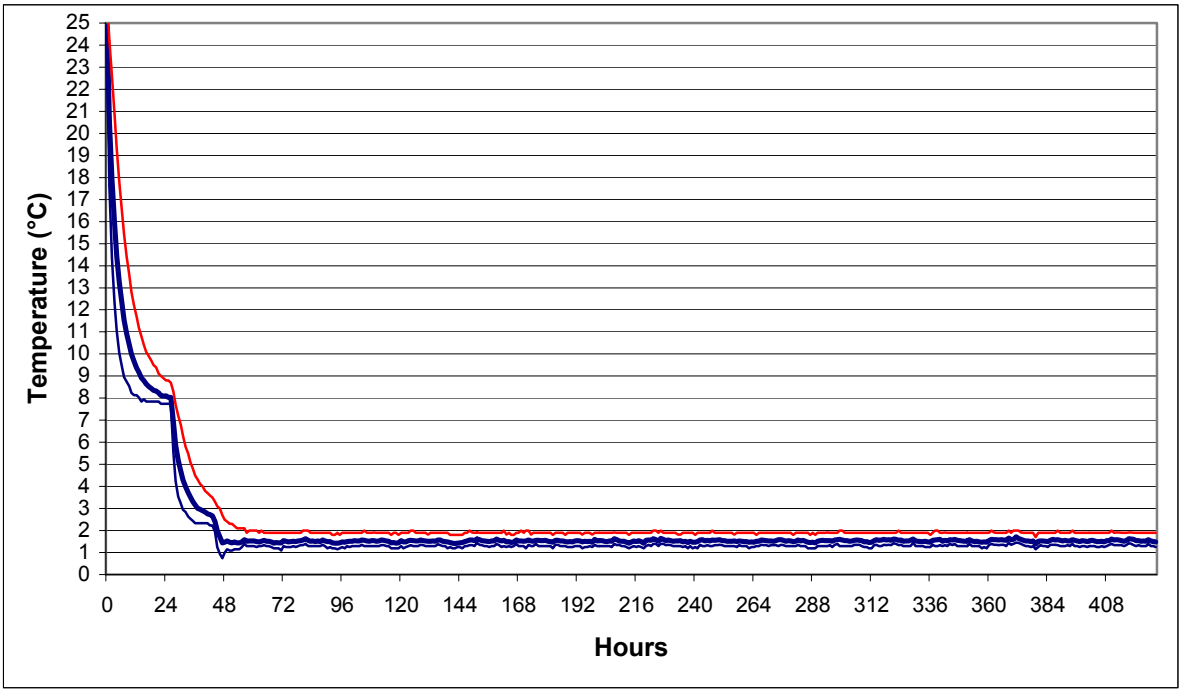

Figure 3.3.3.5. Highest (red, broken line), lowest (blue) and mean (black) temperatures of fruit in replicate 3. Long-term mean temperatures: mean 1.525, low 1.290, high 1.911.

Table 3.3.3.1. *Ceratitis capitata* mortality in controls at 26°C and after 16 days at 1.5°C

Rep	Control treatment at 26°C for 6 days				Cold treatment at 1.5°C for 16 days			
	Fruit used	Larvae found	Larvae dead	Mortality %	Fruit used	Larvae found	Larvae dead	Mortality %
1	528	4367	321	7.3506	2075	22135	22135	100
2	517	5760	355	6.1632	2080	18946	18946	100
3	508	7530	504	6.6932	2058	21411	21408	99.9860
Overall	1553	17657	1180	6.6829	6213	62492	62489	99.9952*

*Probit-9 requirement is 99.9968% mortality

3.3.4 Fruit fly bait sprays – alternatives to organophosphates

Experiment 773 by Bruce Tate and Tim Grout (CRI)

Opsomming

In 2005 het die toetse wat in hokke uitgevoer is met laboratorium vlieë daarop gedui dat imidaclopried en fiproniel belowende gifstowwe mag wees om in lokmiddels te gebruik. Fiproniel is egter 'n stadig werkende produk waarvan die effektiwiteit moontlik onderskat kon word in die veld as die tradisionele metode van dooie vlieg vangste in gemonteerde plastiekbakke onder behandelde takke gevolg sou word. Daarom is die besluit toe geneem om 'n wilde populasie toetshok te ontwikkel om die produkte te evalueer sonder om die vlieë te dood wat op die lokmiddels gevoed het. Drie prototipes met onverdunde Hymlure is geëvalueer vir lang periodes, maar geen vlieë het in die hokke ingegaan nie. Verdere toetse in hokke met laboratorium-geteelde vlieë sal in 2007 uitgevoer word met imidaclopried en fiproniel om die dosisse te bevestig. Verdere evaluasies in die veld sal uitgevoer word met materiaal wat behandel is en gehang sal word tussen bome in groot blokke.

Summary

In 2005, cage tests with laboratory flies had indicated that imidacloprid and fipronil were promising toxicants for use in baits but it was felt that the efficacy of fipronil would be underestimated in the field using the traditional method of catching dead flies in plastic basins mounted below treated branches because of this product's slow mode of action. It was therefore decided to develop a wild population testing cage for the evaluation of products without a knockdown action that would trap flies that had fed on the bait. Three prototypes were evaluated for extended periods using undiluted Hymlure but no flies entered the cages. Further cage tests using laboratory-bred flies will be resumed in 2007 with imidacloprid and fipronil in order

to confirm dosages. Then field evaluation will need to be based on treated fabric hung between trees in large blocks of trees.

3.3.5 Development of a rapid diagnostic test to distinguish Medfly larvae from other larvae

Experiment 774 by Tim Grout and John-Henry Daneel (CRI)

Opsomming

Daar is 'n behoefte om 'n vinnige metode te vind om in die pakhuis te kan bepaal of vrugte besmet is met Medvlieg larwes of ander larwes. Lateralevloei diagnostiese toetse word suksesvol in die veld gebruik vir die identifikasie van baie patogene en CRI het die Central Science Laboratorium gekontrakteer om so 'n tipe toets te ontwikkel. In 2005 is 'n LFD prototipe getoets wat nie suksesvol was nie. In September 2006 is 'n tweede LFD prototipe ontvang met die opsie van twee buffers. Positiewe identifikasies van Medvlieg papies en eiers wat fyn gemaal is in beide van die buffers was weereens nie moontlik nie. CSL sal nou 'n ander tipe van antigeen benadering moet volg wat moontlik mag beteken dat CRI nog insekte sal moet aanstuur. Dit wil egter voorkom asof die tegniek nie suksesvol gaan wees vir die larwes van insekte nie, moontlik as gevolg van die teenwoordigheid van baie komplekse proteïene.

Summary

There is a need for a means of rapidly distinguishing Medfly larvae from other larvae in fruit in a packhouse. Lateral flow diagnostics are used very successfully for the field identification of many pathogens and Central Science Laboratory was contracted to develop this type of testing system for CRI. In 2005 an LFD prototype was tested but it did not work. In September 2006 a second LFD prototype was received with the option of using two buffers. However, this prototype also failed to positively identify Medfly pupae and eggs when crushed in either buffer. CSL will now need to use a different antigen approach and will may require us to send them more insects. However, it is looking increasingly likely that this technique will not be successful for insect larvae, due perhaps to the many complex proteins present.

3.3.6 Comparative development at constant temperatures of three economically-important *Ceratitis* spp. (Diptera: Tephritidae) from southern Africa

Experiment 797 by Tim G Grout and Kim C Stoltz (CRI)

Opsomming

Drie spesies van *Ceratitis* MacLeay is van ekonomiese belang in suider Afrika. Die ontwikkelingstempo's van die Suid Afrikaanse populasies van *Ceratitis (Ceratitis) capitata* (Wiedemann), *C. (Pterandrus) rosa* Karsch en *C. (Ceratalaspis) cosyra* (Walker) is vergelyk by konstante temperature van 14, 18, 22, 26 en 30°C om te bepaal wat die invloed van temperatuur is op die ontwikkeling op die drie spesies. Die tydsduur van elke lewensstadium van elke spesie is bepaal sowel as die persentasie oorlewing van die onvolwasse stadiums. Een liniêre- en drie nie-liniêre modelle vir die tempo van ontwikkeling is gebruik om die minimum-, optimum- en maksimum ontwikkelingsdrempelwaardes te genereer addisioneel tot die termiese konstantes vir die lewenssiklus van die drie spesies. Hierdie parameter waardes was: 9.6, 28.5, 33.0 en 338 vir *C. capitata*, 9.7, 28.8, 33.2 en 376 vir *C. cosyra* en 8.6, 27.7, 33.0 en 429 vir *C. rosa* onderskeidelik. Die parameters vir *C. capitata* het ooreengestem met die waardes soos gevind deur ander navorsers vir hierdie spesie in Reunion. Die parameters vir *C. rosa* het egter beduidend verskil van die waardes soos gevind vir die populasie wat in Reunion voorkom, wat moontlik daarop dui dat dit verskillende rasse is. Die data het oor die algemeen die Briére, Lactin and Logan-6 modelle gepas. Die ooreenkomste tussen die ontwikkelings parameters vir *C. capitata* en *C. corysa* ondersteun egter nie die verskille wat bekend is oor die verspreiding van hierdie spesies nie. Ander beperkende faktore soos relatiewe humiditeit en die beskikbaarheid van gasheer spesies mag moontlik ook belangrik wees. Hierdie bevindinge waarsku teen die voorspellings van potensiële globale verspreiding van spesies wat slegs gebaseer is op lewenstabelle of klimaat parameter waardes.

Introduction

In southern Africa, three tephritids are currently of economic importance as pests; these are the Mediterranean fruit fly *Ceratitis (Ceratitis) capitata* (Wiedemann), the Natal fruit fly *Ceratitis (Pterandrus) rosa* Karsch and the Marula fruit fly *Ceratitis (Ceratalaspis) cosyra* (Walker). *Ceratitis capitata* is the most widespread of these three species and can be found throughout southern Africa, at any time of the year, on different host plants (Hancock 1989). It is a serious pest of both deciduous and subtropical fruit, including *Citrus* spp. and mango *Mangifera indica* L. and also utilizes many non-commercial fruits (White and Elson-Harris 1992). *Ceratitis rosa* is the next most economically-important fruit fly in southern Africa and has a host

range that is almost as broad as that of *C. capitata* (White and Elson-Harris 1992), but its distribution and both temporal and spatial abundance is more limited. It is most abundant during the wet summer months in the eastern and subtropical regions of South Africa and Zimbabwe (Hancock 1989) and is seldom found in the cold Cape provinces during winter. *Ceratitis cosyra* is of less economic importance than the other two species, although it does infest mango and guava *Psidium guajava* L. (White and Elson-Harris 1992, Grové 2001). It is a tropical species and its distribution in southern Africa is limited to the subtropical regions where the Marula tree *Sclerocarya birrea* (A.Rich.) Hochst. subsp. *caffra* (Sond.) is a common host (Grové 2001, Ekesi and Billah 2006).

Hancock (1984) placed the three *Ceratitis* species mentioned above into separate subgenera, but apart from the *C. cosyra* males not being attracted to trimedlure (while males of the other two species are) (Hancock 1987), the behavior of these flies is similar. However, molecular studies by Douglas and Haymer (2001) indicated that *C. rosa* from South Africa was distinct from the Kenyan *C. rosa* and they referred to them as different strains. Barr et al. (2006) also found molecular differences between specimens of *C. rosa* from different African countries. Duyck and Quilici (2002) investigated the development of *C. rosa* from Reunion at different constant temperatures and found that the larval minimum developmental threshold was 3.1°C compared with 10.2°C for *C. capitata*. This low developmental threshold for *C. rosa* is surprising considering the geographical distribution and seasonal abundance of *C. rosa* in southern Africa mentioned above. Perhaps the *C. rosa* population in Reunion originated from east Africa and is more similar to the Kenyan strain than the South African one.

Due to the economic importance of the above *Ceratitis* spp. in southern Africa, possible *C. rosa* strain differences, the lack of knowledge of *C. cosyra* and the need to know more about developmental thresholds, we conducted the following temperature-dependent developmental research on these three species using cultures originating from South Africa.

Materials and methods

Fruit Fly Cultures and Experimental Conditions

This research was conducted between 18 July 2005 and 19 April 2006. The fruit flies used were from mother cultures reared at Citrus Research International in Nelspruit that had been initiated in 1998. The *C. capitata* culture was started with flies collected in the Western Cape Province from deciduous fruit. The *C. cosyra* culture was initiated with flies reared from Marula and mango fruit collected in Mpumalanga and the *C. rosa* culture was started with flies reared from mango and guava collected in Mpumalanga. Since starting the cultures, additional wild flies from Mpumalanga have been periodically added to broaden the gene pool of each species. Adult eclosion and oviposition for *C. capitata* and *C. cosyra* in culture, take place in closed, temperature-controlled rooms at 26°C and with continuous artificial light. *C. rosa* pupae are moved to an outdoor, screened rearing room subject to ambient temperature and light due to the adult's requirement for crepuscular conditions. All three species are reared in the laboratory between egg and pupal formation at 23°C. In the mother cultures, eggs of *C. capitata* are collected in bowls of water placed below rearing cages as the adults oviposit through the gauze screens of the cages. Eggs from both *C. cosyra* and *C. rosa* are collected from perforated plastic lids on plastic honey-jars containing moist paper-towel to maintain high relative humidity. Larvae of all three species are reared on simple proprietary diets.

All developmental research was conducted in an environmental chamber that was originally a Conviron CMP3023 (Controlled Environments Limited, Manitoba, Canada), but was refurbished by Fast Flow Air-conditioning, Nelspruit, South Africa. The chamber's window was situated next to a north-facing window in the laboratory so that the chamber would receive natural light as well as artificial light. In winter, fluorescent daylight-type lights were on from 0500 to 1700 and in summer this period was shifted to 0600 until 1800. This allowed the flies to experience natural dusk conditions after the artificial lights were turned off. Temperatures were maintained at 14, 18, 22, 26 and 30ºC while relative humidity was kept above 60% by using a humidifier connected to a hygrostat and an external water container. A Grant Squirrel data logger (model SQ1025, Grant Instruments (Cambridge) Ltd, UK) was used to record temperature every hour for the duration of each trial using three probes. One probe was used to determine the mean temperature and the other two probes were used for wet and dry readings in order to calculate the relative humidity.

Development and Survival

For each life stage, three ventilated, transparent, plastic cake boxes were used to provide three replicates per species. The boxes used had a 10-litre capacity with dimensions 30 cm x 30 cm and height 14 cm. All gauze screens had 250 µm apertures in the mesh. The boxes used for *C. cosyra* and *C. rosa* had screens on each side that were 16.5 cm wide by 7.2 cm high and a screen in the lid that was 21 cm square. The

boxes used for *C. capitata* did not have side screens but had a single circular screen on the lid of 20 cm diameter. This was to facilitate egg collection. The numbers of individuals used per replicate were 300 eggs, 100 larvae, surviving pupae and whatever number of female adult flies eclosed within the first three days after eclosion started, plus an equal number of males. Individuals used for any particular life stage evaluation were taken from the production peak of the previous life stage. Where poor survival was expected, parallel cultures were used to supplement the numbers at the beginning of each life stage.

Eggs were collected from the mother cultures over a 2-h period. For *C. capitata* a bowl of clean water was placed below each fly cage for this period. For *C. cosyra*, plastic honey-jars (diameter 65.4 mm and height 128.2 mm) were used with small holes punched into the lid. The jars were rinsed internally with water just before placement to create a moist film inside the lid. For *C. rosa,* the more labor-intensive method of using apples (*Malus domestica* Borkh. cv. Granny Smith) that had been pricked six times with a row of eight pins (0.8 mm diameter and 2.5 mm apart), rather than the perforated honey-jar lid, was chosen as it yielded higher numbers of eggs per unit time. After being exposed to the flies, the apples were cut close to the holes and the eggs washed out with water. A trial run was conducted before each temperature series was started to ensure that the egg-hatching peak would occur between 0600 and 1800 on the day of hatching. For each replicate, 300 eggs were transferred onto moist filter paper in a Petri dish (6 cm diameter), using a Pasteur pipette. The filter paper had been previously sprayed with distilled water containing red food colorant in order to improve the visibility of the eggs and the filter paper was placed on a thin layer of sponge that had been saturated with distilled water. Filter papers were checked daily and moistened with distilled water if required. Three replicates were used per species. Each species was started 2 h apart to allow time for egg collection and transfer into the environmental chamber. The start time was taken as the time when the eggs were removed from the mother cultures. Eggs were observed every 2 h under a stereo-microscope to determine the development time and percentage hatch. The mean duration of each replicate's development was determined by adding the products of the duration in days and the eggs hatched at each count, then dividing this sum by the total number of eggs hatched.

Larvae that were formed during the hatching peak (usually less than 48 h) were used for the larval development studies. For each replicate, 100 larvae were transferred to a 1-cm square of filter paper that was then placed onto artificial diet in a Petri dish (9 cm diameter). The larvae readily moved to the food provided. When the larvae were in the third instar the Petri dishes were moved to smaller boxes containing a layer of fine sand on the bottom. Observations were made three times a day to determine the end of the larval stage which was defined by larvae jumping out of the medium into the sand. The start of the larval stage was taken as the time when some eggs had hatched in all three replicates of a species. The mean duration of the larval stage per replicate was determined by adding the products of the duration in days and the number of larvae jumping at each count, then dividing this sum by the total number of larvae that jumped. The percentage of larvae surviving from hatch to jump was determined for each replicate.

Pupae were sieved out of the sand three times a day and each batch of pupae kept in a small Petri dish (3.5 cm diameter). Pupae were counted under a stereo-microscope to confirm the actual numbers that had formed. The last few pupae that were formed at the tail end of production were removed and not included in further evaluations. The rest of the pupae were returned to the larger containers and observed three times a day for adult eclosion. In the cases of 14 and 30°C, some supplementary pupae were used to make numbers up to 100 per replicate due to low survival rates. The supplementary pupae were reared at 20°C for the experiment run at 14°C and were reared at 26°C for the experiment run at 30°C. The start time for pupal development was taken as the time when larvae had jumped in all three replicates of a species. The mean duration of the pupal stage per replicate was determined by adding the products of the duration in days and the number of adults eclosing at each count, then dividing this sum by the total number of adults that eclosed. The percentage surviving to adults was also determined for each replicate.

Sexing of adults of each species was done directly after the peak of fly eclosion. For *C. capitata* this was 48 h, for *C. cosyra* 72 h and for *C. rosa* 96 h, after the first fly eclosed. Sex determination of *C. capitata* was based on the spatulate orbital setae found on the males. Thick, dark setae or feathering on the mid-tibia of the male *C. rosa* were used to separate the sexes in that species. Sexes of *C. cosyra* were distinguished by the ovipositor on the females. When each replicate was sexed, all female flies were kept and an equal number of males in order to obtain a 1:1 sex ratio. Extra male flies were discarded. If male numbers were inadequate, supplementary flies were added as for the pupae. A mixture of sugar and yeast in a 7:1 ratio was used to feed the flies and this was replaced daily. Yeast is a source of protein and is important for egg production. Small specimen containers (43 mm diameter by 59 mm high) were used to provide distilled water to the flies. A hole was burnt into the lid of the container through which a dental roll was forced to serve as a wick in the water.

Eggs were collected within the rearing boxes in a similar manner to the method used to collect eggs from the mother culture. For *C. cosyra,* empty plastic honey-jars that had been rinsed with distilled water and had small holes in the lids, were placed lid-side-down on the mesh of the insect box lid. For *C. rosa,* punctured apples (Granny Smith cultivar) were provided within the rearing boxes. For *C. capitata* the box was turned on its side with the screened lid in a vertical position and a bowl of water was placed below the mesh screen to collect eggs laid through the screen. Oviposition was monitored twice daily and the time taken from the first adult eclosion in all replicates to the first egg production in all replicates, was recorded. The total number of eggs produced over a fly's lifespan was not determined due to this being extremely labor intensive.

Statistics

The mean developmental duration in days per replicate for each life stage, species and temperature was calculated as described above. The developmental durations of each species were then compared at each temperature in a two-way analysis of variance (ANOVA). The percentage survival or emergence of each life stage at each temperature was also compared between species using a two-way ANOVA, after using the arcsine-square root transformation to normalize the data. In both analyses, means were further compared using Student-Newman-Keul's test (SNK) (Steel and Torrie 1980) at $\alpha = 0.05$. All analyses were conducted with Statgraphics Plus 5.1 (2000).

Determining Thermal Requirements

The developmental rate of insects and other poikilothermic invertebrates is linearly dependent on temperature from the base temperature or lower developmental threshold (T_{min}) to the optimum temperature (T_{opt}). This is because temperature affects many physiological processes and the activity of enzymes (Trudgill et al. 2005). Higher base temperature values indicate adaptation to warmer climates and these are usually accompanied by shorter physiological development times or thermal constants (measured in degree days) compared to the same values obtained for species adapted to cooler climates (Honek 1996, Trudgill et al. 2005). In order to determine the base temperature and the thermal constant for each *Ceratitis* species the following linear regression model was used:

$$1/d = a + bT,$$

where d is the developmental time (days), T is the rearing temperature (°C), a is the developmental rate at T = 0°C and b is the slope of the regression line. The lower developmental threshold was calculated from $T_{min} = -a/b$ and the thermal constant K from $K = 1/b$ (Campbell et al. 1974). Only data from the temperatures 14 to 26°C were used in these calculations because it was clear that 30°C was above the optimal temperature in most instances (Figure 1) and the developmental rate was no longer linearly dependent on temperature (Kontodimas 2004). Simple linear regressions (using Statgraphics Plus 5.1 [2000]) were based on the mean temperature recorded in the environmental chamber and the inverse of the mean duration in days for three replicates at any particular life stage.

In order to estimate the optimum temperature T_{opt} and the maximum or lethal temperature T_{max}, three non-linear models were used. Nonlinear regressions were analyzed in Statgraphics Plus 5.1 (2000) making use of the Marquardt algorithm (Marquardt 1963). Initial estimates for parameters were obtained from other publications such as Lactin et al. (1995), Sanchez-Ramos and Castanera (2001), Kontodimas et al. (2004) and Arbab et al. (2006).

The Logan-6 model (equation 6, Logan et al. 1976) was used due to its popularity and the biological significance of its parameters. It is defined as follows:

$$1/d = \Psi(e^{(\rho T)} - e^{(\rho Tmax - (Tmax - T)/\Delta T)})$$

where Ψ is the maximum developmental rate, ρ is the biochemical reaction rate at the optimum temperature, T is the rearing temperature (°C), T_{max} is the lethal maximum temperature and ΔT is the difference between the optimum temperature and T_{max}. The optimum temperature was determined with the following equation simplified from Logan et al. (1976):

$$T_{opt} = T(1+(\Delta T/T_{max})(Log_n(\Delta T\rho))/(1-\Delta T\rho))$$

The Lactin model (modification 2, Lactin et al. 1995) is a modified version of the Logan-6 model and was chosen because it allows for a graphical estimate of T_{min}. It is defined as follows:

$$1/d = e^{(\rho T)} - e^{(\rho Tmax - (Tmax - T)/\Delta T)} + \lambda$$

where λ is a parameter that forces the curve to cross the abscissa at suboptimal temperatures and thus allows estimation of T_{min}. The other parameters are as defined for the Logan-6 model and T_{opt} can be determined in the same way as for Logan-6.

The Briére model (equation 1, Briére et al. 1999) was chosen for its simplicity and because it provided all three developmental thresholds. In the following equation, a is an empirical constant and the other parameters are as defined above:

$$1/d = aT(T-T_{min})(T_{max}-T)^{1/2}$$

The following equation from Briére et al. (1999) was used to calculate the optimum temperature:

$$T_{opt} = (4T_{max} + 3T_{min} + (16T_{max}^{2} + 9T_{min}^{2} - 16T_{min}T_{max})^{1/2})/10$$

The mean values for T_{min}, T_{opt} and T_{max} were determined for each life stage of each species using the results generated by the developmental rate models. Unrealistic values were excluded.

Results

Experimental Conditions

Actual mean temperatures recorded in the environmental chamber throughout the duration of each experiment were used in the developmental models and were: 13.96, 17.65, 21.76, 26.45 and 29.83°C. The mean relative humidities at these temperatures were 89, 84, 74, 74, 86%, respectively with the extremes being 62 and 100%.

Development and Survival

Considering the developmental durations in Table 3.3.6.1, the shortest egg-to-egg life cycle times were obtained at 26°C where *C. capitata* had a significantly shorter (P<0.05) life cycle in days than *C. cosyra,* which in turn had a significantly shorter life cycle than *C. rosa.* At this temperature, similar trends in significant differences between the species occurred in pupal development and in the time taken for adults to oviposit. However, there were no significant differences between species in larval development at this temperature. The egg and larval developmental durations varied between species at different temperatures and although there were significant differences, there were no obvious trends across temperatures. No significant differences were found between species when pupal developmental times were compared at 14 and 18°C, but at 22°C the pupal developmental time for *C. capitata* was significantly shorter than that of *C. cosyra* and at 30°C all three species were significantly different from one another in the same order as found at 26°C. The adult-to-egg developmental durations for *C. cosyra* at 14 and 18°C were only based on one replicate so were not included in the ANOVAs. But at 22 and 26°C this duration for *C. cosyra* was significantly longer than for *C. capitata.* At four of the five temperatures, the adult-to-egg developmental duration for *C. rosa* was significantly longer than for *C. capitata* and this trend was repeated when the complete life cycle durations were compared (Table 3.3.6.1).

There were few significant differences (P<0.05) in survival of the various life stages (Table 3.3.6.2). The poorest percentage of egg hatch was for *C. rosa* at 30°C. A very low percentage of *C. cosyra* and *C. rosa* larvae managed to pupate at 14°C and significantly less (P<0.05) *C. cosyra* pupated at 30°C. The percentages of adults eclosing from pupae were above 85% for all species and all temperatures except at 30°C when some adults of *C. cosyra* and *C. rosa* appeared to die from exhaustion in trying to emerge from the pupal case. *C. capitata* had the best survival rates of the three species at 30°C (Table 3.3.6.2).

Thermal Requirements

The developmental rate at the different temperatures tested was linear until 26°C for all species but the rates slowed dramatically for *C. capitata* and *C. rosa* at 30°C (Figure 3.3.6.1). The data generally fitted all of the models well with the only adjusted R-squared value of below 90% being obtained for the fit of the Logan-6 model to *C. cosyra* eggs (Table 3.3.6.3). Due to the poor performance of *C. cosyra* at the two extreme temperatures, fewer values were available for the calculation of mean parameter values than for the other two species. Overall, the values for T_{min} generated by the Lactin model were closer to those obtained from the simple regression than those generated by the Briére model, which in most cases were lower than those given by the straight line (Table 3.3.6.3). On the other hand, the T_{max} values from the Briére model tended to be slightly higher than those generated by the other two non-linear models. The T_{opt} values generated by all three non-linear models were usually within one degree of each other.

Comparing the minimum, optimum and maximum developmental thresholds for the three species, the mean egg-to-egg values for *C. capitata* and *C. cosyra* were surprisingly similar at 9.6, 28.5, 33.0 and 9.7, 28.8 and 33.2, respectively (Table 3.3.6.3). The T_{max} egg-to-egg value for *C. rosa* (33.0) was virtually the same as the other two species but T_{min} and T_{opt} for this species were approximately one degree lower than for the other two (8.6 and 27.7, respectively). In all species, the T_{min} and T_{max} values for the larvae were the least extreme of the life stages with the highest values for T_{min} and lowest values for T_{max} being obtained for the larvae of each species. However, the opposite was true for egg development which usually had the most extreme T_{min} and T_{max} values.

The egg-to-egg thermal constants for *C. capitata, C. cosyra* and *C. rosa* were 337.8, 375.9 and 429.2, respectively. The combined thermal constants for the egg and larval stages of these species were very similar at 128.3, 129.7 and 123.8, respectively. The differences between the species were therefore in the pupal development and the thermal requirement for oviposition.

Table 3.3.6.1. Mean developmental duration in days (with SD) for the three southern African *Ceratitis* species at different constant temperatures

Developmental stage	Species	14°C	18°C	22°C	26°C	30°C
Egg	C. capitata	8.2 (0.37) b	3.8 (0.02) b	3.0 (0.05) c	2.0 (0.02) a	1.9 (0.01) b
	C. cosyra	8.5 (0.47) b	3.6 (0.01) a	2.9 (0.05) b	2.2 (0.01) b	1.8 (0.00) a
	C. rosa	7.7 (0.66) a	4.0 (0.11) c	2.7 (0.02) a	2.0 (0.03) a	1.9 (0.01) b
Larval	C. capitata	31.6 (1.20) a	12.5 (0.09) a	9.4 (0.11) c	5.9 (0.06) a	6.2 (0.13) a
	C. cosyra	32.8 (1.06) a	13.9 (0.45) b	8.9 (0.22) b	5.9 (0.10) a	6.9 (0.32) a
	C. rosa	31.0 (1.21) a	12.6 (0.35) a	8.4 (0.17) a	5.8 (0.09) a	9.3 (0.54) b
Pupal	C. capitata	31.2 (1.40) a	20.0 (0.82) a	13.1 (0.29) a	8.9 (0.18) a	9.1 (0.04) a
	C. cosyra	32.7 (2.53) a	20.8 (0.76) a	14.2 (0.43) b	9.6 (0.13) b	10.4 (0.18) b
	C. rosa	28.3 (0.34) a	20.8 (0.25) a	13.9 (0.55) ab	10.0 (0.15) c	11.0 (0.34) c
Adult to egg	C. capitata	12.7 (2.89) a	6.4 (0.69) a	4.5 (0.58) a	3.4 (0.00) a	4.0 (0.00) a
	C. cosyra	21.0 (-)*	11.0 (-)	6.9 (2.01) b	5.1 (0.58) b	3.6 (0.38) a
	C. rosa	16.3 (1.15) a	11.6 (0.70) b	8.2 (0.20) b	6.6 (0.38) c	5.7 (0.58) b
Egg to egg	C. capitata	83.6 (2.91) a	42.6 (1.40) a	30.1 (0.81) a	20.2 (0.21) a	21.2 (0.17) a
	C. cosyra	91.8 (-)	48.2 (-)	32.9 (1.43) b	22.7 (0.77) b	22.7 (0.40) a
	C. rosa	83.3 (0.57) a	49.0 (1.02) b	33.2 (0.59) b	24.4 (0.61) c	27.9 (1.42) b

Means for the same developmental stage and in the same column followed by the same letter are not significantly different (P>0.05) (SNK)

* (-) denotes result of a single replicate so SD could not be calculated and data were not included in the ANOVA

Table 3.3.6.2. Mean survival percentages at each life stage event of *Ceratitis* species at different constant temperatures

Event	Species	14°C	18°C	22°C	26°C	30°C
Eggs hatching	C. capitata	88.1 a	95.9 b	67.9 a	88.1 a	70.6 b
	C. cosyra	74.2 a	88.7 b	77.7 b	66.1 a	100.0 c
	C. rosa	92.4 a	53.3 a	79.8 b	70.2 a	34.1 a
Larvae pupating	C. capitata	43.7 b	96.3 a	79.0 a	93.7 a	70.3 b
	C. cosyra	3.3 a	74.7 a	88.7 a	81.7 a	29.0 a
	C. rosa	15.3 ab	91.0 a	79.7 a	82.7 a	68.3 b
Adults eclosing	C. capitata	92.0 a	100.0 a	94.6 a	95.3 a	92.3 a
	C. cosyra	98.0 a	98.0 a	92.0 a	86.0 a	60.6 a
	C. rosa	97.7 a	94.3 a	89.3 a	93.0 a	76.0 a

Means for the same event and in the same column followed by the same letter are not significantly different (P>0.05) (SNK)

Table 3.3.6.3. Values of fitted coefficients and measurable parameters of four developmental rate models for three *Ceratitis* species

Model	Parameters	*Ceratitis capitata*					*Ceratitis cosyra*					*Ceratitis rosa*				
		Egg	**Larval**	**Pupal**	**Adult to egg**	**Egg to egg**	**Egg**	**Larval**	**Pupal**	**Adult to egg**	**Egg to egg**	**Egg**	**Larval**	**Pupal**	**Adult to egg**	**Egg to egg**
Linear[a]	Tmin	9.6	10.8	9.4	9.0	9.9	8.3	11.2	9.2	10.1	10.1	9.4	10.8	7.8	5.7	8.8
	K	33.8	94.5	155.3	58.2	337.8	38.6	91.1	169.8	82.4	375.9	33.5	90.3	190.1	135.5	429.2
	Adj R^2	97.24	97.28	98.65	99.24	99.24	93.79	99.78	98.58	99.91	99.82	99.89	99.64	98.23	99.23	99.99
Briere	Tmin	7.5	10.1	7.8	8.9	9.0	4.5*	11.2	8.0	-[b]	9.0	7.9	11.4	6.2	-	8.3
	Toptimal	30.6	28.6	29.3	26.9	28.5	33.4*	27.4	28.3	-	29.0	29.5	26.2	28.0	-	27.3
	Tmax	37.2	34.2	35.6	32.2	34.3	41.1*	32.5	34.3	-	35.0	35.7	30.9	34.1	-	32.9
	Adj R^2	96.65	95.02	97.09	98.46	97.27	94.67	97.11	95.33	-	98.89	99.77	94.56	94.27	-	97.84
Lactin	Tmin	8.6	10.5	9.1	9.2	9.9	8.9	10.9	9.0	-	10.0	9.1	10.6	7.6	6.0	8.7
	Toptimal	29.1	29.0	29.0	27.3	29.0	36.0*	28.9	28.9	-	29.2	29.0	27.6	28.9	40.2*	28.3
	Tmax	31.3	31.1	31.4	35.0	31.3	56.1*	30.8	31.3	-	31.1	37.7	32.4	31.2	67.6*	33.1
	Adj R^2	95.27	94.82	97.98	98.88	98.58	91.47	99.68	97.62	-	99.72	99.77	98.79	96.75	99.11	99.99
Logan-6	Toptimal	28.9	28.1	28.3	27.1	28.0	30.8	27.5	28.0	37.5*	28.3	28.6	26.7	27.9	31.0	27.4
	Tmax	34.7	33.1	33.0	32.5	33.3	37.6	32.2	32.2	44.4*	33.6	34.3	31.3	32.2	38.8	32.9
	Adj R^2	92.77	91.59	99.94	95.55	97.28	83.69	97.13	99.65	95.76	98.53	97.29	94.58	99.76	97.88	99.41
Means	Tmin	8.6	10.5	8.8	9.0	9.6	8.6	11.1	8.7	10.1	9.7	8.8	10.9	7.2	5.9	8.6
	SD	1.05	0.35	0.85	0.15	0.52	0.42	0.17	0.64	-	0.61	0.79	0.42	0.87	0.21	0.26
	Toptimal	29.5	28.6	28.9	27.1	28.5	30.8	27.9	28.4	-	28.8	29.0	26.8	28.3	31.0	27.7
	SD	0.93	0.45	0.51	0.2	0.5	-	0.84	0.46	-	0.47	0.45	0.71	0.55	-	0.55
	Tmax	34.4	32.8	33.3	33.2	33.0	37.6	31.8	32.6	-	33.2	35.9	31.5	32.5	38.8	33.0
	SD	2.96	1.57	2.12	1.54	1.53	-	0.91	1.54	-	1.98	1.71	0.78	1.47	-	0.12

[a] The last or highest temperature value was omitted from the linear regression due to deviation from a straight line;
[b] - Parameters could not be estimated with the model
* Unrealistic values not used for calculation of means and standard deviation (SD)

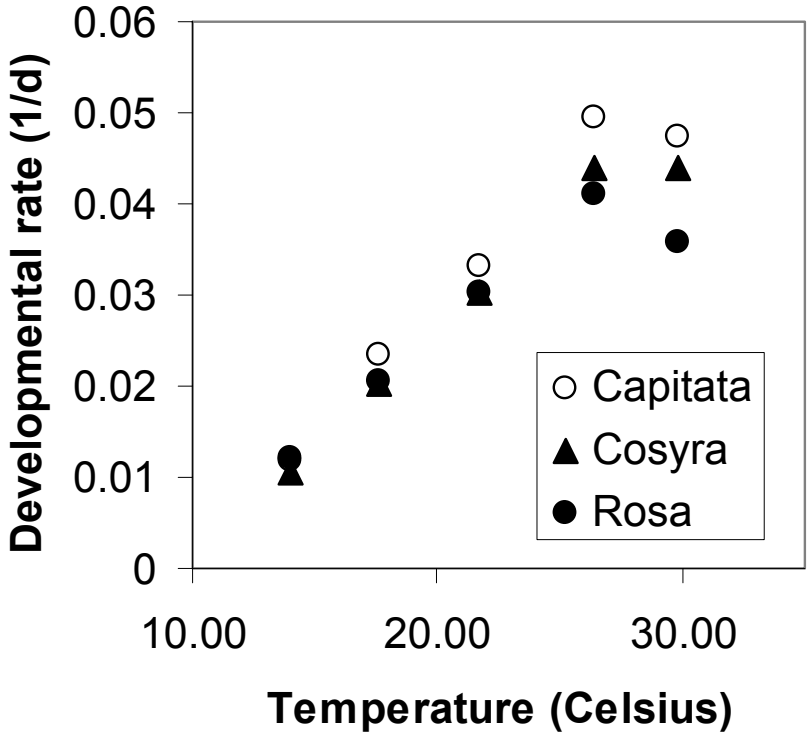

Fig. 3.3.6.1. Egg to egg developmental rates of three *Ceratitis* species at five constant temperatures.

Discussion

The developmental rate models used all appeared to fit the data reasonably well with the exception of some *C. cosyra* parameters due to a poorer dataset. Several researchers besides those cited for the regression parameters have recommended combinations of the linear regression model for the lower developmental minimum and thermal constant and one or more non-linear models for the other parameters, e.g., Chen et al. (2006) (linear and Lactin), Chong and Oetting (2006) (linear and Logan-6), Roy et al. (2002) (linear, Briére and Lactin). The Logan-6 model fitted the data the most often of the three non-linear models, although it does not provide a lower developmental threshold. The other two models were very similar in their ability to produce realistic parameters (Table 3.3.6.3).

The statistical analyses of the developmental durations (Table 3.3.6.1) and the parameters generated by the developmental rate models (Table 3.3.6.3) support one another in showing that *C. capitata* is more similar to *C. cosyra* than *C. rosa*. The egg-to-egg thermal constant for *C. cosyra* was slightly greater than that for *C. capitata* (376 versus 338) but close enough to support a tropical origin for both these species (Trudgill et al. 2005). The thermal constant obtained for the total development of the immature stages of *C. capitata* was 284 which was similar to the 260 obtained by Duyck and Quilici (2002) for a Reunion population of this species. However, our thermal constant value for the total development of the immature stages of *C. rosa* was 314 which differed markedly from Duyck and Quilici's (2002) value of 405 for *C. rosa* in Reunion. Our egg-to-egg thermal constant for *C. rosa* was 429 and the egg-to-egg T_{min} value was 8.6°C, which was one degree lower than the T_{min} value we obtained for *C. capitata*. This supports a more subtropical or coastal origin for *C. rosa* so the name of Natal fruit fly may be very appropriate as this refers to a coastal region of South Africa. Our T_{min} value for larval development of *C. rosa* was 10.9°C compared with Duyck and Quilici's (2002) value of 3.1°C for the same life stage, whereas our T_{min} value for larval development of *C. capitata* was 10.5°C and Duyck and Quilici's (2002) was 10.2°C. It therefore seems that the populations of *C. capitata* in South Africa and Reunion can be considered to belong to the same strain but that the *C. rosa* populations represent different strains. This is further supported by Tate and Ware (2003) who showed that South African *C. rosa* larvae experience 100% mortality when held in oranges at 3°C for 7 days. As mentioned above, Douglas and Haymer (2001) indicated that *C. rosa* from South Africa was molecularly distinct from the Kenyan *C. rosa* so perhaps the Reunion strain originated from east Africa and a genetic bottleneck has resulted in further diversification with time and adaptation to colder temperatures. De Meyer

et al. (2006) have made some geographical distribution predictions for *C. rosa* and suspect it to be more tolerant to colder conditions than *C. capitata*. Furthermore, they suggest that *C. rosa* could probably survive in areas at higher northern latitudes that are unsuitable for *C. capitata*. However, De Meyer et al. (2006) used Duyck and Quilici's (2002) developmental data for *C. rosa* from Reunion in their models and collection records for both strains. Their predictions for the possible distribution of *C. rosa* may be partly applicable to *C. rosa* from Reunion if other non-climatic parameter values are not important. However, their predictions contrast markedly with the known distribution of *C. rosa* in South Africa because it is seldom found in the colder, southern-most provinces where *C. capitata* is more abundant. *C. capitata* is also found throughout South Africa while *C. rosa* is most abundant in the wetter, coastal or low altitude regions and the subtropics.

Considering the developmental thresholds obtained for the immature life stages of the three species in South Africa, the ability of the eggs of all species to withstand more extreme temperatures than the larvae is understandable because the eggs will normally be close to the skin of the fruit and subjected to more extreme temperatures. Larvae have the ability to move further into the fruit where they are not subjected to such extreme temperatures.

These studies could not investigate the influence of relative humidity or vapor pressure deficit on development and this may be important in determining the differences in distribution of *C. capitata* and *C. cosyra* in South Africa, because the developmental thresholds of these species are very similar. The availability of favored host plant species may also influence distribution. This finding and the experience with *C. rosa* discussed above, therefore caution against basing predictions of potential global distributions of species on methods that assign high weighting to specific life table or climatic parameter values without information on a species' colonization abilities or competitive fitness. For example, the short time required between adult eclosion and oviposition in *C. capitata* (3.4 d at 26°C) relative to *C. cosyra* (5.1 d at 26°C) and *C. rosa* (6.6 d at 26°C) would be advantageous in conditions detrimental to the adult such as low relative humidity, wind, or exposure to toxic baits or predators. This may help to explain why *C. capitata* is the most widespread of the three *Ceratitis* species in southern Africa.

Conclusion

Developmental rate models were used to generate the minimum, optimum and maximum developmental thresholds in addition to the life cycle thermal constants for the three species of *Ceratitis*. These parameter values were: 9.6, 28.5, 33.0 and 338 for *C. capitata*, 9.7, 28.8, 33.2 and 376 for *C. cosyra* and 8.6, 27.7, 33.0 and 429 for *C. rosa,* respectively. The parameters for *C. capitata* are similar to those found by other researchers for this species in Reunion but the parameters for *C. rosa* differ substantially from a Reunion population, suggesting that these are different strains. The similarities between the developmental parameters for *C. capitata* and *C. cosyra* do not support known differences in the distribution of these species so other limiting factors such as relative humidity and the availability of host species may be important.

Future research

No further research is planned. Ms M de Villiers may use these data in Climex models based on her collection records. The manuscript has been submitted to Environmental Entomology.

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3.3.7 **Fruit Fly – Field Control other than OP substitutes**

Experiment 801 by John-Henry Daneel, Tim Grout and Rooikie Beck (CRI)

Opsomming

Vrugtevlieë is belangrike fitosanitêre plae vir al ons markte maar die EU skenk toenemend al hoe meer aandag aan ander vrugtevlieë as Medvlieg en daarom moet ons beheermaareëls vir die Natalse vrugtevlieg verbeter word. Alternatiewe gifstowwe vir die gebruik in vrugtevlieg lokmiddels mag moontlik ook in die toekoms langer voor-oes weerhoudingsperiodes vereis wat dus ondersoeke na wysigings in die toedieningsmetodes noodsaak. Volgens laboratorium ondersoeke word slegs ’n lae persentasie vrugtevlieë gelok as lokmiddels op die grond geplaas word. Die bevindinge is bevestig in drie kommersiële boordproewe waar die getalle van vrugtevlieë nie noemenswaardig afgeneem het toe die lokmiddels op die grond of grondbedekking geplaas is nie. Die metode kan dus nie as ’n alternatief in die toedieningsstrategie gebruik word nie. Die vangste van losgelate vlieë wat in die laboratorium geteel is in Sensus lokvalle het getoon dat Capilure en Ceratitislure meer effektief was vir geslags volwasse vlieë terwyl ouderdom nie

belangrik was in die geval van Questlure nie. Geen wysigings gaan aangebring word aan die aanbevelings vir vrugtevlieg lokmiddels nie tensy afkeurings as gevolg van 'n spesifieke spesie daarop dui dat monitering en beheerpraktyke onvoldoende is. 'n Evaluasie van moontlike vrugtevlieg lokmiddels het getoon dat een van die produkte effektief was teenoor alle *Ceratitis* wyfies en sal dus verdere aandag geniet. Navorsing op die aanwending van lokmiddels op materiaal wat hang sal uitgevoer word indien dit goedkoper sal wees as lokvalstasies.

Introduction

Although the Mediterranean fruit fly *Ceratitis capitata* is common in parts of the Mediterranean, the Natal fruit fly *C. rosa* is not and there are concerns that the phytosanitary status of this pest may increase in the future. The same control measures have successfully been used for both these *Ceratitis* species in the past but it is possible that the currently-used organophosphate (OP) toxicants may not be available in the future and alternative toxicants may not have acceptable residues. It is therefore necessary to evaluate the application of baits on the ground or ground-cover to see whether fruit residues can be avoided in this way. Further research was conducted to compare the attractiveness of various lures in Sensus traps to known numbers of released laboratory fruit flies. Alternative attractants to the only registered protein hydrolysate on the market in South Africa, were also evaluated.

Materials and methods

Will fruit fly bait applications be effective if applied to the ground to avoid fruit residues?

Little is known about the flight behaviour of the southern African *Ceratitis* spp. and whether they will readily feed on bait applied to the ground or ground-cover. Recent work by Nascimento et al. (2006) suggests that ground baits in vineyards in Brazil could not be an effective control method for Medfly because numbers of these flies caught in traps with protein hydrolysate were much lower at a low height than when traps were positioned higher. We conducted some laboratory experiments to determine whether our *Ceratitis* spp. have a preference for attractants at certain heights above the ground and to confirm the attractiveness of some fruit fly attractants that could be used in baits. Three field trials were also conducted to evaluate the registered Hym-lure plus OP bait applied to trees and applied to the ground.

Laboratory trials

At the CRI premises a trial was conducted indoors in a passage (27.05 m x 1.98 m x 2.65 m) with light green walls, white ceiling and light grey floor. Both ends of the passage received natural daylight. Three thousand flies (1000 of each *Ceratitis* species) were released in the middle of the passage. At both ends of the passage a "pool noodle" (closed-cell foam rod with length 140 cm, diameter 5 cm) was placed vertically, 10 m from the release point, which was at ground level. Normal Sensus capsules (filled with 2 ml undiluted Hymlure, Ready to Use) were placed in the noodle, 25 cm apart, with the lowest capsule 11 cm above the ground. Five A4-sized Gladwrap sheets were placed around the foam tube above each other and painted with Flytac adhesive. The sheet closest to the ground was marked number 1 and the highest 5.

The experiment in the passage was repeated using sticky transparencies only. Eight transparencies (four on each side of the release point) were painted with Flytac and placed on the wall 10 cm above the ground. In the middle of each transparency a capsule was fixed with glue. Four of the capsules were filled with water and four with Hymlure (800 ml/100 ℓ). The transparencies were placed at the following distances from the release point: 2.5 m (Hymlure), 5 m (water), 7.5 m (Hymlure) and 10 m (water). Again, 3000 fruit flies were released in the middle of the passage and left for 72 hours. This method failed to attract flies to the ground although three thousand flies were released in a closed environment. In both these passage trials, most flies were dead at the end of the trial even with water being supplied during the second trial. In both cases, flies were only fed sugar before the releases.

Due to the low numbers of flies being caught in the passage, it was decided to do more indoor trials with different attractants at different heights. Four more trials were conducted in the test room at CRI that is naturally lit through large windows. The testroom is a rectangular room 11.76 m x 4.98 m x 2.83 m. The wall with three outside windows is dark green, the opposite wall is painted light green and the two shorter walls are both painted dark blue.

The aim of the first trial was to compare four different attractants with each other. Four transparencies were placed on the green wall, 2 m apart, near the ceiling and opposite the windows. A capsule was glued in the middle of each transparency, filled with an attractant and the transparency painted with Flytac. The four

attractants were Hymlure (Ready to Use) and water (1:1), Molasses and water (1:1), GF 120 (0.3 ml/10 ml) and water only.

At the same time trial 2 was also started. Three transparencies were placed on the opposite dark green wall containing the three windows. The capsules on the transparencies were loaded with Hymlure (Ready to Use) (1:1). The first transparency was placed below the ceiling (270 cm above the ground), the second in the middle (158 cm high) and the third near the ground (20 cm above the ground). Marula and Natal fruit flies (one thousand of each and sugar-fed only), were released in the middle of the room 95 cm above the ground.

Trials 3 and 4 commenced in the same room 72 hours later. In trial 3, four transparencies were placed on one of the blue walls below the ceiling and the same attractants and concentrations were used as in trial 1. At the same time the positions of the transparencies in trial 1 were randomly rotated. Trial 4 was similar to trial 2 but this time the transparencies were placed on a string 1 m away from the wall at exactly the same height. Again the same amount of flies were released into the room exposing trial 1 and 2 to two thousand flies of each of the two species, and 3 and 4 to one thousand flies of each species. All the transparencies were counted 72 hours after the second release.

Orchard trials

From 9 May to 12 September 2006 a trial was conducted in a guava orchard at BronPro, 30 km from Nelspruit on the Sabie road. The orchard was divided into three regions and each of these subdivided into three blocks. Each block was at least five rows wide and comprised at least 150 trees. Two Sensus traps were hung in each block, one with Capilure and one with Ceratitislure. The traps were usually 12 to 15 trees apart depending on the shape of the block. Three treatments were allocated randomly to the blocks within each region. These were: untreated control, bait applied to the trees in the conventional manner and the same volume of bait applied on the ground or ground cover near the trees. Traps were emptied weekly and all flies sexed and counted. Due to the low numbers of fruit flies caught at this site it was decided that this trial should be repeated elsewhere. A block of mango trees at the Lowveld Agricultural College was therefore used and another mango orchard at Oewersig, Alkmar.

Traps were placed at the Agricultural College on 23 November 2006 in a mango and adjacent litchi orchard to determine if there were any flies present. In each orchard only one trap containing Questlure, Ceratitislure, Capilure, Hymlure (800 ml/100 ℓ) and molasses (2 g/10 ml) was placed. In the litchi orchard another two traps containing undiluted Hymlure and molasses were also placed. Over a period of two weeks it was established that Marula and Natal fruit flies were present in the two orchards but only two Medfly females were caught. Only the mango orchard was used at the Agricultural College as the litchi orchard was one week away from harvesting by this time. The mango orchard was divided into three blocks, a control, a ground application and a normal spray onto the trees. Two Sensus traps per treatment were hung out, one with Ceratitislure and one with Capilure. These traps were put out a week before spraying and were examined weekly to determine treatment efficacy. Spraying started on the 2 January with the last spray applied on the 25 January.

A mango orchard of 6 ha was identified at Alkmaar on the farm Oewersig where no commercial fruit fly treatments were planned. Three treatments were again used, an application to the ground, the normal application to the trees and an untreated control. The orchard was naturally divided by a road into four blocks, but only three blocks were used. Each block was divided into three to provide three replicates of the three treatments. In each replicate two traps, one with Capilure and one with Ceratitislure were hung to compare the effectiveness of the bait application methods. Traps were put out a week before the first application due on 2 January. The last spray was on the 25 January.

At all three sites, baits were sprayed with a hand-operated Guarany knapsack (16 ℓ) using a 56-whirler and D5 nozzle at a constant pressure of 1 bar. Sprays were applied weekly to one side of the tree only, alternating between the sides each week. At BronPro every tree received 50 ml with the correlated amount applied onto the ground for the same distance. Oewersig and the Agricultural College received 100 ml/ tree with the mango trees being bigger than the guava trees at BronPro. Results were analyzed using a multifactor ANOVA after applying a square root (x+0.1) transformation to normalise the data. Where F tests were significant for treatments at P=0.05, means were compared further using Student-Newman-Keul's test at P=0.05.

Alternative baits for fruit fly

Green Trading requested the assistance of CRI to test the effectiveness of 13 different fruit fly attractants for *C. capitata, C. cosyra* and *C. rosa* in March 2006. Each attractant was placed in a Sensus trap (3 ml) and compared with Questlure and Capilure placed in both Sensus and Biagro traps (yellow McPhail type). Two samples of each attractant were hung in the biocontrol orchard at the Lowveld Agricultural College and all traps were within 30 m of each other. Traps were monitored every 7 days and after being emptied were moved four places forward to ensure rotation in the orchard. The trial started on 7 March and was terminated on 30 March.

Five different attractants were received from Green Trading to test on 12 October. The attractants, Prolure (100%), Prolure (70%), Buminal, Hymlure and Cresol were each placed in a Sensus trap (3 ml in a capsule). Three traps with each attractant were placed in a mixed citrus orchard (Empress, pomelos, Ellendale, Clementine and Satsuma) 30 m apart and were rotated weekly until 24 October.

The competitive efficiency of Questlure, Ceratitislure and Capilure in Sensus traps for a known number of released flies

The work conducted in 2005 with 3-day-old flies by Daneel et al. (2005) was repeated at the same mango orchard (Neos Estates in the Onderberg area of Mpumalanga Province) but using 12-day-old flies. All techniques were the same as before apart from the age of the flies. Protein was withheld, although the adults were allowed water and granulated white sugar *ad lib*. The flies were released (9000 per species) on 29 May, 21 June and 23 September 2006.

Traps were emptied before each release of the flies. Sensus traps were placed in trees that were two rows east of the release row. A Sensus trap containing Questlure was placed in the first tree. Three trees further down the row a Sensus trap with Ceratitislure was hung and a further three trees down the row a Sensus-Capilure trap was used. The sequence was repeated until six traps of each Sensus-lure combination were positioned. The arrangement of Sensus traps with lures was repeated two rows west of the release row. All lures were replaced every six weeks. Two larger, yellow, Probodelt traps (Fig. 3.3.7.1) (McPhail-type but with 4 additional holes near the lid) with Biolure (3-component) were placed on either side of the rows of Sensus traps, two rows away and diagonally opposite one another. They were each 4 rows away from the release row and flies had to first pass the Sensus traps before reaching the Biolures.

The traps were monitored and emptied daily for the first six days after a release of flies and weekly thereafter. However, by the fifth day after release, numbers of released flies being caught were almost negligible so the first four days after release were used for comparison purposes. Flies with traces of dye were identified to species and sexed in the laboratory.

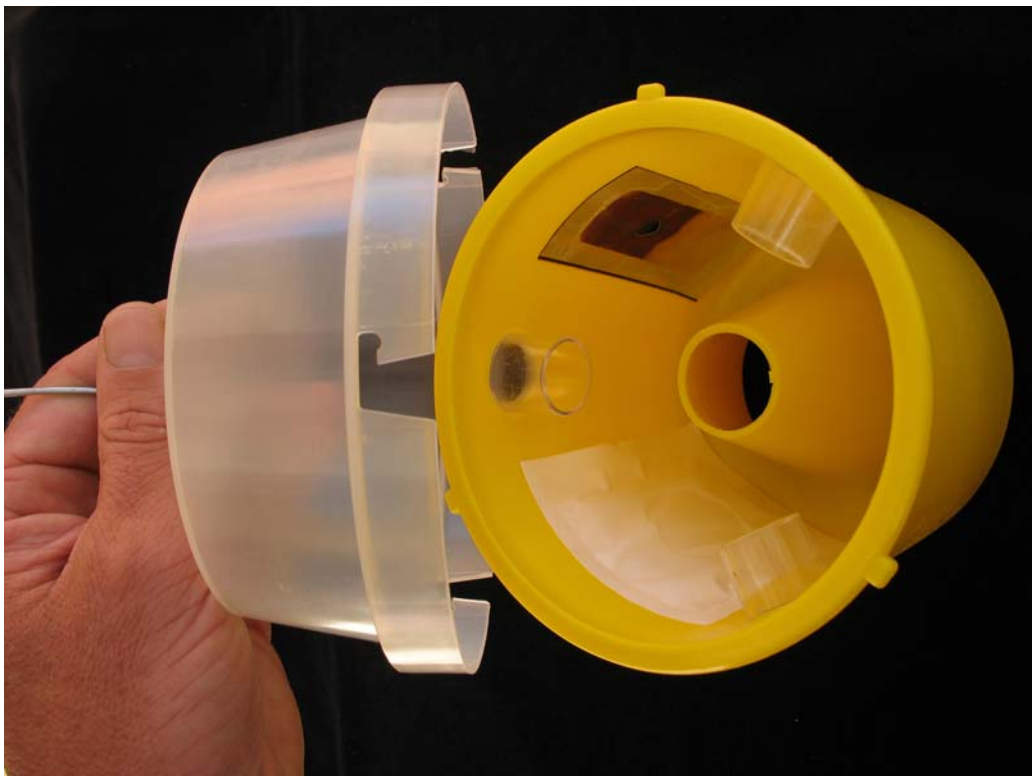

Figure 3.3.7.1. Probodelt trap with 3-component sachets attached to inner walls.

Results and discussion

Will fruit fly bait applications be effective if applied to the ground to avoid fruit residues?

Laboratory trials

Immediately after the release in the passage it was noticed that most flies gathered at the ceiling or just below on the walls. It was decided that the flies would be left for 72 hours before counting. However, such low numbers were trapped on the noodles that no conclusion could be drawn on what height the flies flew at or if there were any differences between the species (Table 3.3.7.1). The two noodles were then moved to an outside location where they were protected from rain but over a period of two weeks no fruit flies were caught. It was later found that the Flytac sold by Insect Science was not effective in catching flies so many may have escaped from the sticky surface.

Table 3.3.7.1. Released flies caught on pool noodles in the passage.

HEIGHT	POOL NOODLE A						POOL NOODLE B					
	MEDFLY		MARULA		NATAL		MEDFLY		MARULA		NATAL	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
1	1	0	0	1	0	1	0	0	0	0	0	1
2	0	0	0	1	0	0	0	0	0	0	0	0
3	0	0	0	0	0	1	0	0	0	0	0	0
4	0	0	0	0	0	0	1	0	0	0	0	0
5	0	0	0	1	0	0	0	0	0	0	0	0

Very few flies were caught on the low sticky transparencies after the second release in the passage (Table 3.3.7.2) and no trends were evident.

Table 3.3.7.2. Flies caught on sticky transparencies near the ground in the passage

DISTANCE FROM RELEASE	HYMLURE (800 ml/100 ℓ)						DISTANCE FROM RELEASE	WATER					
	MEDFLY		MARULA		NATAL			MEDFLY		MARULA		NATAL	
	♂	♀	♂	♀	♂	♀		♂	♀	♂	♀	♂	♀
2.5 m	2	0	1	2	0	0	5 m	0	0	0	0	0	0

DISTANCE FROM RELEASE	HYMLURE (800 ml/100 ℓ)						DISTANCE FROM RELEASE	WATER					
	MEDFLY		MARULA		NATAL			MEDFLY		MARULA		NATAL	
	♂	♀	♂	♀	♂	♀		♂	♀	♂	♀	♂	♀
2.5 m	2	0	1	0	0	0	5 m	1	0	0	0	0	0
7.5 m	2	0	0	2	0	0	10 m	0	1	0	0	0	0
7.5 m	1	0	0	0	0	0	10 m	0	1	1	1	2	0

Table 3.3.7.3. Trial 1 in the test room, transparencies below the ceiling on the green wall exposed to two thousand flies of each species. Two releases over a total period of 144 hours.

ATTRACTANT	MARULA FRUITFLY		NATAL FRUITFLY	
	♂	♀	♂	♀
HYMLURE, READY TO USE (1:1)	10	6	3	7
MOLASSES (1:1)	5	4	3	3
GF120 (0.3 ml/10 ml)	6	2	4	6
WATER	5	1	1	2

Again, very low numbers of the released fruit flies were caught in trial 1 in the test room (Table 3.3.7.3); 2% of Marula and 1.5% of Natal fruit fly. Hymlure caught more Marula fruit flies with minor differences between Hymlure and the control. In Natal fruit fly these differences were even smaller, with no attractant resulting in higher numbers caught. In the second trial in the test room there was a visible trend with the height of the lures (Table 3.3.7.4), with the least flies being caught at the lowest height.

Table 3.3.7.4. Trial 2, three transparencies placed at different heights on the wall. Two releases over a total period of 144 hours.

HEIGHT ABOVE GROUND	MARULA FRUITFLY		NATAL FRUITFLY	
	♂	♀	♂	♀
BELOW THE CEILING	21	15	22	25
MIDDLE	13	9	16	17
GROUND	1	2	1	0

In the third trial in the test room (Table 3.3.7.5), catches of fruit flies attracted to the different lures were again very low and little conclusion could be drawn other than the fact that water was not attractive. In this case the low number of flies may have been due to the dark blue background of the wall. The fourth trial (Table 3.3.7.6) again showed a trend of low numbers of flies trapped near the ground and higher numbers near the ceiling.

Table 3.3.7.5. Trial 3, transparencies below the ceiling on the blue wall exposed to one thousand flies of each species. One release over a period of 72 hours.

ATTRACTANT	MARULA FRUITFLY		NATAL FRUITFLY	
	♂	♀	♂	♀
HYMLURE, READY TO USE (1:1)	0	2	2	1
MOLASSES (1:1)	1	4	1	1
GF120 (0.3 ml/10 ml)	1	1	0	0
WATER	0	0	0	0

Table 3.3.7.6. Trial 4, three transparencies placed at different heights on a string 1 m away from the wall. One release over a period of 72 hours.

HEIGHT ABOVE GROUND	MARULA FRUITFLY		NATAL FRUITFLY	
	♂	♀	♂	♀
BELOW THE CEILING	5	8	3	2
MIDDLE	2	3	1	1
GROUND	3	1	0	0

In all of these small trials, the numbers of flies trapped were extremely low considering the numbers released. Apart from the poor quality of the adhesive used, the dark wall colour may have had some influence but there may have also been a dispersal response after being confined in a box. Most flies usually sat on the ceiling or the upper parts of the walls. The attraction of the lures may have also been reduced due to the confined space becoming saturated with the odour and making it difficult for the flies to find the source. Trials 2 and 4 are the only two that show a reliable trend and that is that few flies were caught at the lowest height. This does suggest that the spraying of baits on the ground cover or ground will be less effective than spraying higher up in the tree.

Orchard trials

Numbers of fruit flies at BronPro remained surprisingly low for a long period, even when baits were only applied every second week. After 16 weeks the results based on Capilure traps showed that the conventional bait application in the trees reduced numbers of Medfly males significantly ($P<0.05$) compared with the untreated blocks and where bait had been applied to the ground (Table 3.3.7.7). Differences between numbers of Natal fruit fly attracted to Capilure were not significant between treatments, but showed the same trend with the baits on the tree being the lowest. However, numbers of Natal fruit fly in traps with Ceratitislure were significantly different between the blocks with bait on the trees versus the untreated areas. Marula fruit flies attracted to Ceratitislure showed no significant differences between treatments and no trend in numbers (Table 3.3.7.7).

Table 3.3.7.7. Means of weekly total catch in three traps per treatment over 16 weeks of bait applications in a guava orchard at BronPro near Nelspruit

Treatments	Capilure in Sensus trap		Ceratitislure in Sensus trap		
	Medfly males	Natal fly males	Natal fly males	Natal fly females	Marula fly males
No bait	0.7 b	1.3 a	1.8 b	1.1 a	2.6 a
Bait on ground	1.1 b	1.1 a	1.2 ab	1.2 a	3.0 a
Bait on tree	0.4 a	0.4 a	0.8 a	0.6 a	2.1 a

Means in the same column followed by the same letter are not significantly different at P=0.05 (SNK)

Although the treatments used at the Agricultural College were not replicated and the numbers of fruit flies were low, the trends are similar to those found at BronPro (Table 3.3.7.8). The only significant difference was found in the numbers of Natal fruit fly males attracted to Ceratitislure where the standard bait on the tree resulted in significantly fewer flies than the bait on the ground or the untreated control (Table 3.3.7.8). There were no Medflies in this orchard and there were no significant differences in the numbers of Marula fruit flies trapped in each treatment using Ceratitislure.

Table 3.3.7.8. Means of weekly catch per trap per treatment over four weeks of bait applications in a mango orchard at the Lowveld Agricultural College near Nelspruit

Treatments	Capilure in Sensus trap	Ceratitislure in Sensus trap		
	Natal fly males	Natal fly males	Natal fly females	Marula fly males
No bait	1.3 a	12.3 b	0.8 a	196.3 a
Bait on ground	4.0 a	10.3 b	1.3 a	155.8 a
Bait on tree	1.5 a	4.8 a	1.0 a	250.0 a

Means in the same column followed by the same letter are not significantly different at P=0.05 (SNK)

In the mangoes at Oewersig there were also no Medflies present but numbers of Natal fruit fly and Marula fruit fly were monitored in traps with Capilure and Ceratitislure. At this site the numbers of flies were higher than at BronPro or the Agricultural College and good results were obtained after four weeks of baiting and monitoring (Table 3.3.7.9). Numbers of Natal fruit fly males in traps with Capilure were significantly lower in blocks with bait applied to the trees ($P<0.05$) than either blocks with bait on the ground or untreated blocks. The differences in Natal fruit fly numbers (male and female) in traps with Ceratitislure showed the same trends as for Capilure but the differences were only significant for bait in the trees versus no bait, with the ground baits showing a slight suppressive effect. Once again there were no significant differences between any of the treatments regarding Marula fruit fly numbers (Table 3.3.7.9), although in this case there appeared to be a trend towards lower numbers in the baited treatments.

Table 3.3.7.9. Means of weekly catch per trap per treatment over four weeks of bait applications in a mango orchard at Oewersig near Nelspruit

Treatments	Capilure in Sensus trap	Ceratitislure in Sensus trap		
	Natal fly males	Natal fly males	Natal fly females	Marula fly males
No bait	24.1 b	36.6 b	3.2 b	96.8 a
Bait on ground	26.7 b	23.5 ab	1.9 ab	31.3 a
Bait on tree	4.3 a	11.7 a	0.4 a	27.3 a

Means in the same column followed by the same letter are not significantly different at P=0.05 (SNK)

Alternative baits for fruit fly

The mean numbers of the three fruit fly species caught per trap per week were compared for the 13 prospective attractants and four standards (Figures 3.3.7.2-3.3.7.4). The Biagro trap caught higher numbers of all species than Sensus traps with the same lures, but it is not known whether this was due to the different colour of the trap or just the large holes through which the flies can enter. The attractant number 8 was promising for females of all *Ceratitis* species and will be investigated further.

In the second comparison of attractants, no flies were caught during the exposure period so the trial will have to be repeated at another time.

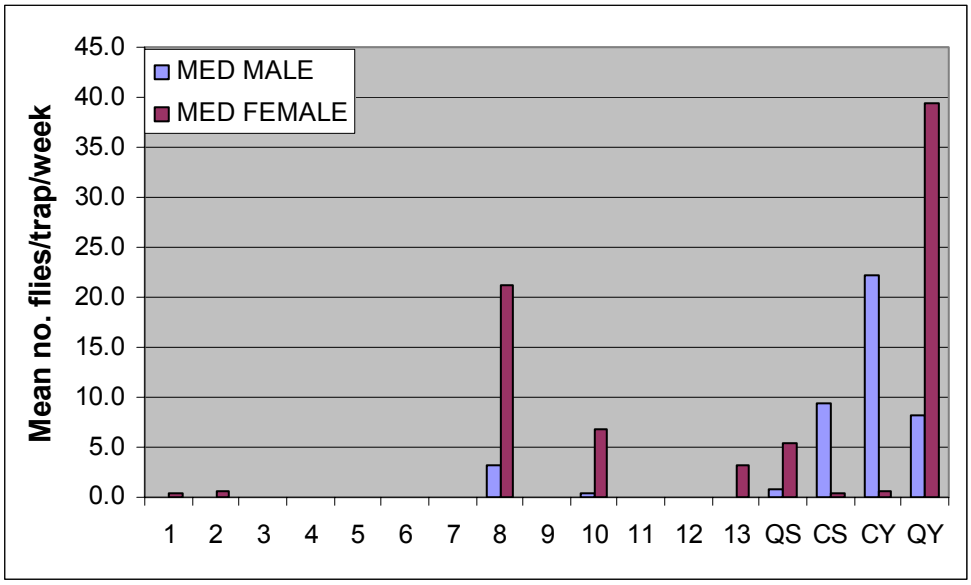

Figure 3.3.7.2. Evaluation of prospective fruit fly attractants in Sensus traps against Medfly where QS=Questlure in Sensus, CS=Capilure in Sensus, CY=Capilure in yellow Biagro McPhail and QY= Questlure in Biagro McPhail

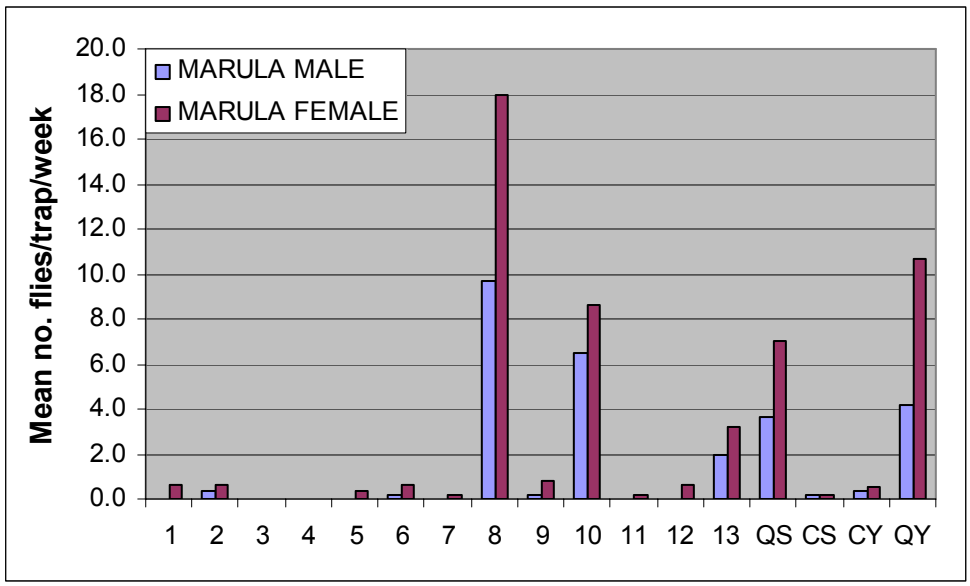

Figure 3.3.7.3. Evaluation of prospective fruit fly attractants in Sensus traps against Marula fruit fly where QS=Questlure in Sensus, CS=Capilure in Sensus, CY=Capilure in yellow Biagro McPhail and QY= Questlure in Biagro McPhail

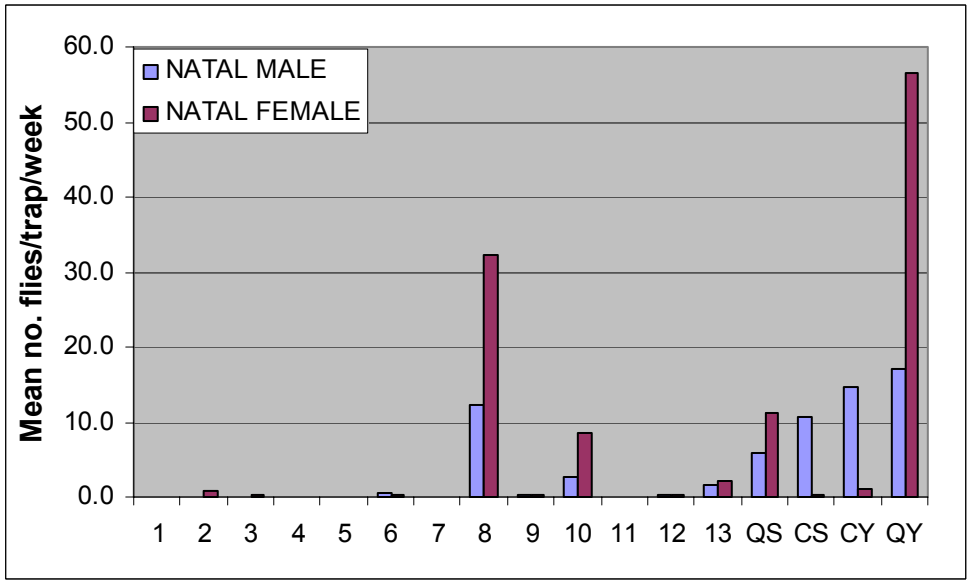

Figure 3.3.7.4. Evaluation of prospective fruit fly attractants in Sensus traps against Natal fruit fly where QS=Questlure in Sensus, CS=Capilure in Sensus, CY=Capilure in yellow Biagro McPhail and QY= Questlure in Biagro McPhail

<u>The competitive efficiency of Questlure, Ceratitis lure and Capilure for a known number of released flies</u>

The numbers of flies trapped during the first four days after releases in 2005 were determined from the raw data of that year and are expressed in Table 3.3.7.9. These results were then used in various comparisons with the data from 2006 (Table 3.3.7.10). Unfortunately, there was a lot of variability in the data between releases of the same year so very few significant differences were found. After the work was conducted in 2005 (Daneel et al. 2005), concern was expressed that Natal fruit flies were immature when released and this may have affected their interest in certain lures. This concern appears to have been justified because the recovery of Natal fruit fly in traps with Capilure and Ceratitislure was higher with 12-day-old flies than with 3-day-old flies (Figure 3.3.7.5). Capilure is a parapheromone or type of sex attractant and Ceratitislure contains caryophyllene which is a sesquiterpene found in citrus rind and presumably is more attractive when the flies are looking for oviposition sites. From the work conducted by Grout and Stoltz (section 3.3.6) it is known that Medfly reared at 26°C is ready to oviposit 3.4 d after eclosion, whereas Marula fruit fly requires 5.1 d and Natal fruit fly 6.6 d. With the releases of young flies in 2005, many of the Natal fruit flies would have died before seeking oviposition sites or mates and to a lesser extent this would have applied to some of the Marula fruit flies. The highest total number of flies trapped with 3-day-old flies was for Medfly males

(Table 3.3.7.9) but the highest number trapped of the 12-day-old flies was Marula fruit fly males (Table 3.3.7.10). Questlure showed the fewest differences between the ages of the flies because it is primarily a food-type of lure and its attractiveness is not affected by sexual maturity. However, the recovery percentages for Questlure were the lowest of all the lures (Figure 3.3.7.5). Although the recovery percentages in Figure 3.3.7.5 appear low, they are similar to those found for *Bactrocera tryoni* in a recent study in Queensland, Australia where the mean recovery rate for released males attracted to traps with cue-lure was 10% (Weldon and Meats 2007).

Table 3.3.7.9. Flies trapped in 12 traps per lure type during the 4 days following each release of 9000 flies (3 days old) per species in 2005.

Lure	Species	Sex	Release 1	Release 2	Release 3	Total trapped
Questlure	Medfly	M	1	24	5	30 ab
		F	20	120	106	246 ab
	Marula	M	2	0	2	4 ab
		F	2	5	1	8 ab
	Natal	M	121	67	24	212 ab
		F	106	79	19	204 ab
Capilure	Medfly	M	550	440	1033	2023 d
		F	71	39	170	280 ab
	Marula	M	0	0	0	0 a
		F	0	0	0	0 a
	Natal	M	16	4	12	32 ab
		F	1	1	3	5 ab
Ceratitislure	Medfly	M	262	278	727	1267 c
		F	28	72	208	308 b
	Marula	M	192	446	291	929 c
		F	1	3	2	6 ab
	Natal	M	26	20	10	56 ab
		F	13	9	8	30 ab

Means in the last column followed by the same letter are not significantly different at P=0.05 (SNK)

Table 3.3.7.10. Flies trapped in 12 traps per lure type during the 4 days following each release of 9000 flies (12 days old) per species in 2006.

Lure	Species	Sex	Release 1	Release 2	Release 3	Total trapped
Questlure	Medfly	M	6	16	7	29
		F	213	194	55	462 bc
	Marula	M	3	1	2	6 a
		F	28	5	4	37 ab
	Natal	M	26	39	24	89 abc
		F	34	46	29	109 abc
Capilure	Medfly	M	440	557	794	1791 d
		F	5	9	30	44 ab
	Marula	M	0	0	0	0 a
		F	0	0	0	0 a
	Natal	M	13	102	406	521abc
		F	0	1	5	6 a
Ceratitislure	Medfly	M	776	609	330	1715 d
		F	72	51	69	192 abc
	Marula	M	2036	861	1096	3993 e
		F	7	23	5	35 ab
	Natal	M	72	282	328	682 c
		F	8	9	12	29 ab

Means in the last column followed by the same letter are not significantly different at P=0.05 (SNK)

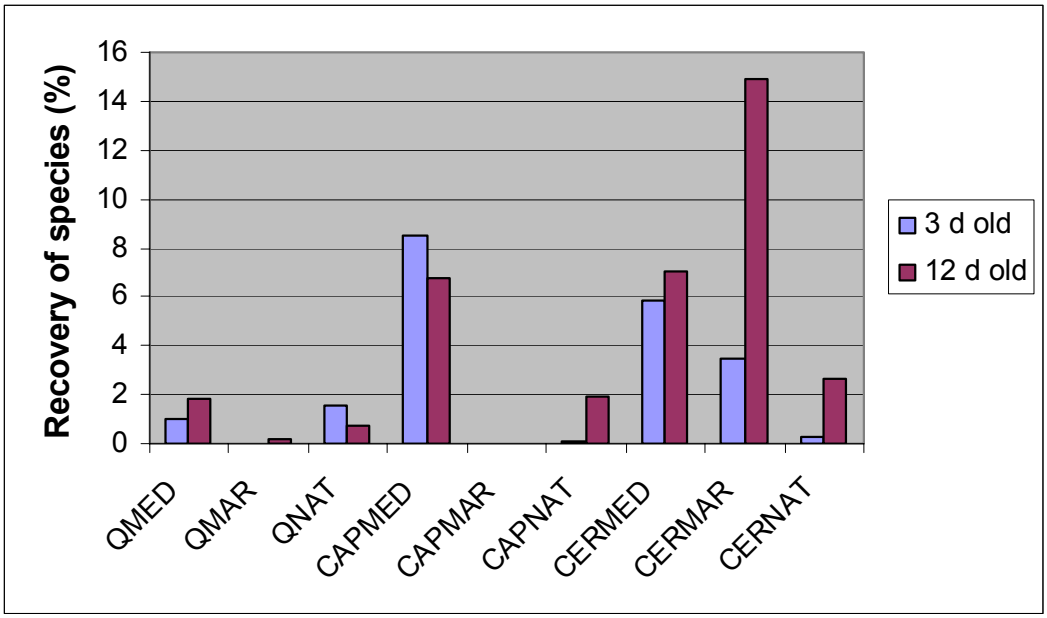

Figure 3.3.7.5. Percentage recovered of each species released at different ages where Q, Cap and Cer refer to Questlure, Capilure and Ceratitislure, and Med, Mar and Nat refer to Medfly, Marula fruit fly and Natal fruit fly, respectively. None of the differences due to age at any particular lure and species combination were significant at P=0.05.

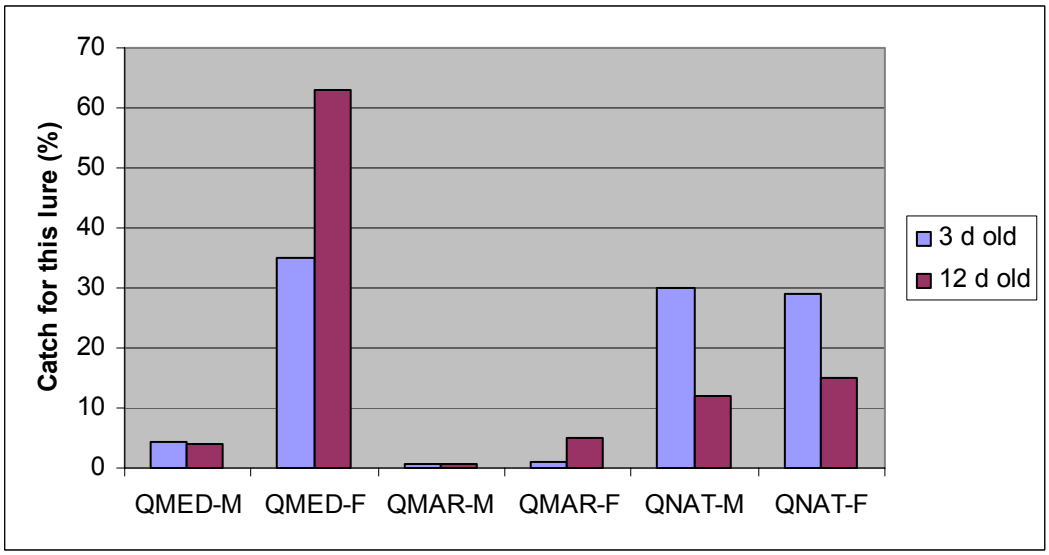

Figure 3.3.7.6. Percentage catch for Questlure for different sexes and species released at two different ages, where Med, Mar and Nat refer to Medfly, Marula fruit fly and Natal fruit fly, respectively, and M and F refer to the sex. None of the differences due to age at any particular sex and species combination were significant at P=0.05.

Considering the catches per sex and species as a percentage of the total catches per lure, provided a slightly different perspective. For 3-day-old fruit flies, catches with Questlure were fairly evenly distributed between female Medflies, and both male and female Natal fruit flies (Figure 3.3.7.6). Capilure is known to be ineffective for Marula fruit fly (Hancock 1987) and this was confirmed (Figure 3.3.7.7). This lure mostly caught male Medfly with an increase in male Natal fruit fly when 12-day-old flies were used.

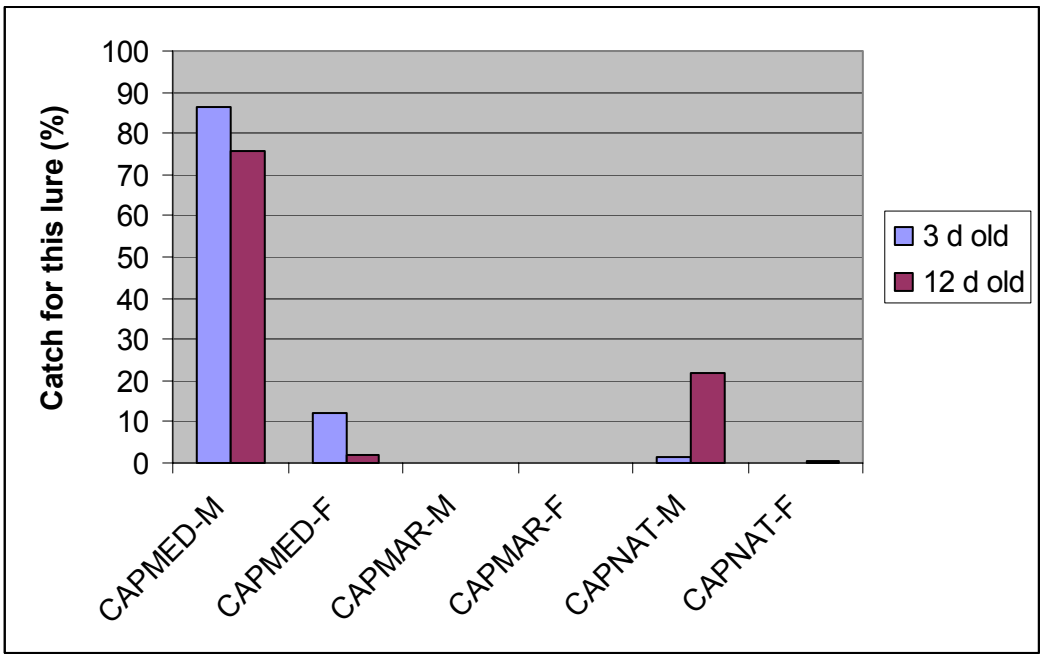

Figure 3.3.7.7. Percentage catch for Capilure for different sexes and species released at two different ages, where Med, Mar and Nat refer to Medfly, Marula fruit fly and Natal fruit fly, respectively, and M and F refer to the sex (Capilure is known to have no attraction for Marula fruit fly). The difference due to age for Medfly females attracted to this lure was significant ($P<0.05$) but there were no other significant differences.

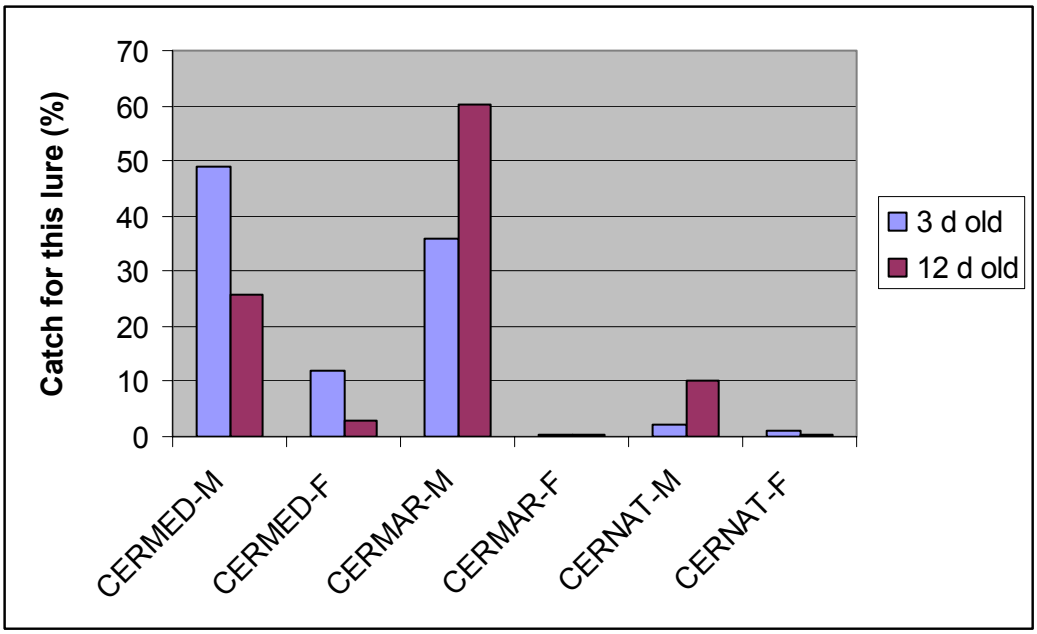

Figure 3.3.7.8. Percentage catch for Ceratitislure for different sexes and species released at two different ages, where Med, Mar and Nat refer to Medfly, Marula fruit fly and Natal fruit fly, respectively, and M and F refer to the sex. None of the differences due to age at any particular sex and species combination were significant at $P=0.05$.

Ceratitislure was most effective for Medfly males and Marula fruit fly males, the latter showing an increased attraction with fly age (Figure 3.3.7.8). Although not part of the main comparison, the two McPhail traps with Biolure showed that the fly age had no apparent effect on this trap-lure combination (Table 3.3.7.11). Biolure was not very effective for either sex of Marula fruit fly and caught primarily female Medfly. Numbers of caught female Natal fruit flies only amounted to approximately one-third of the numbers of female Medfly.

In crops such as mangoes where Marula fruit fly is an important pest, Biolure would not be a good choice for monitoring purposes and Ceratitislure would be the best option. Ceratitislure provided the best recovery of all three *Ceratitis* species but is more effective for mature flies. It must be remembered that these results were obtained where the flies had the option to go to the different lures. Where one particular type of lure is

used in an orchard as a monitoring system without competition with other lures, the recovery level may be higher. Further monitoring of rejections for infestation of citrus by fruit fly will be conducted to determine whether rejections are more often due to one species than another. This will then provide an indication of whether monitoring of a particular species such as Natal fruit fly is inadequate when using either Capilure or Questlure, and whether a change in lure is required. At this stage, no change is recommended.

Table 3.3.7.11. Mean totals of flies caught in two yellow plastic Probodelt traps with Biolure over the 4 days after each release.

Species	Sex	3-day-old flies	Catch (%) 3-d	12-day-old flies	Catch (%) 12-d
Medfly	M	35. 7	8.7	8.5	2.2
	F	215.0	52.2	255.0	65.1
Marula	M	3.3	0.8	3.0	0.8
	F	6.0	1.4	6.0	1.5
Natal	M	70. 7	17.2	47.0	12.0
	F	81.0	19.7	72.0	18.4

Conclusions

Two laboratory trials suggested that Natal fruit fly and Marula fruit fly do not forage just above the ground. Three orchard trials confirmed that numbers of Medflies and Natal fruit flies were reduced significantly by bait applications to the trees but not to the ground. This means that fruit residues cannot be avoided by spraying baits on the ground, but application of baits to hanging fabrics will be investigated. Marula fruit flies were not effectively controlled with the use of protein baits. However, one of 13 prospective fruit fly attractants gave promising results for females of all *Ceratitis* spp. and will be investigated further.

Differences were found in the attractiveness of Capilure and Ceratitislure with change in fruit fly maturity. This was not the case for Questlure and Biolure that are primarily food-type lures. The percentage recovery of released flies in a competitive situation was lowest for Questlure. However, in the absence of other lures, the use of this lure appears to be practical. Fruit fly rejections will be monitored to determine whether any changes to the recommendations for monitoring should be made.

Future research

Bait application research will now turn to the application of baits to hanging fabric, if it is substantially cheaper than using M3s. Further research on monitoring systems and lures will only be required if rejections for a particular species of fruit fly increase. Some research will be conducted on Marula fruit fly control methods in case the EU becomes concerned about this fly in the future.

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3.3.8 **Global distribution of Natal Fruit Fly**
Experiment 805 by M de Villiers (SU)

Summary

Natal fruit fly is a pest of international phytosanitary concern. Therefore, certain phytosanitary restrictions are placed on international citrus trade. If the current distribution of the pest is determined, it will be possible to model its potential to invade other parts of the world, thereby providing a scientific basis to evaluate the relevance of both present and future phytosanitary restrictions. The goal of the latter is to reduce the risk of introducing Natal fruit fly into importing countries. Only through obtaining reliable, scientific information, can

phytosanitary restrictions on citrus trade be contested. It is therefore considered critical to obtain such information in order to gain and maintain market access.

In order to model the potential global distribution of the pest, detailed distribution data need to be obtained. Information on the biology of the pest is also important. A literature search was therefore undertaken. Museums and other researchers were also contacted to obtain further information regarding its distribution and biology. To get the best results from modelling, more detailed distribution and abundance data is needed. Since such information is not currently available, surveys are required. The objective of the surveys is to determine the relative abundance of the pest in southern Africa, as well as to determine the geographical limits in the distribution of Natal fruit fly within southern Africa. To meet these objectives, traps for fruit flies were placed in Stellenbosch, Citrusdal, Swellendam, Knysna, Onseepkans, Keimoes, Britstown, Jan Kempdorp, Addo, King William's Town, Bloemfontein, Pietermaritzburg, Nkwalini, Groblersdal/Marble Hall, Nelspruit, Komatipoort, Tshipise, Tom Burke, Tzaneen, Rustenburg, as well as Harare (Zimbabwe). Fruit fly traps will probably also be placed in Alexander Bay during January 2007. To determine the geographical limits of Natal fruit fly, traps were also placed in Onrus/Vermont, Somerset West, Paarl, Riebeeck Kasteel, Piketberg, Porterville, Clanwilliam, Vanrhynsdorp, Beaufort West, Garies, Springbok, Olifantshoek, Gariepdam and Vryburg. Three Chempac bucket traps baited with Biolure were used per locality. Monitoring started during August, September or October. Traps were serviced on a monthly basis.

No insect samples or data were obtained from Tom Burke. Fruit fly samples or data were obtained from all other areas. For Harare, samples for December have not been received. For Gariepdam both November and December samples are still lacking. Natal fruit fly was absent from Clanwilliam, Swellendam, Beaufort West, Garies, Springbok, Onseepkans, Keimoes, Britstown, Olifantshoek and Gariepdam. Counts were very low, with counts of less than 10 Natal flies per trap per month, in Porterville, Citrusdal, Vanrhynsdorp, Jan Kempdorp, Tshipise and Vryburg. Counts varied between very low and low (10 to 29.9 flies per trap per month) in Onrus/Vermont, Riebeeck Kasteel, Knysna, Bloemfontein, Groblersdal/Marble Hall, Tzaneen and Rustenburg. Counts were higher in Nelspruit and Komatipoort, mostly with monthly averages of 30 to 99.9 flies per trap. High counts (100 to 499.9 flies per trap) were observed during certain months in Piketberg, Addo and Pietermaritzburg and very high counts (500 to 1920 flies per trap) during certain months in Somerset West, Stellenbosch, Paarl, King William's Town and Harare. A full report will be given upon completion of the study.

Opsomming

Natalse vrugtevlieg is 'n plaag van internasionale fitosanitêre belang, met die gevolg dat sekere fitosanitêre beperkings op internasionale sitrushandel geplaas word. Indien die huidige verspreiding van hierdie plaag bepaal word, sal dit moontlik wees om die potensiaal daarvan om ander wêrelddele binne te dring, te modelleer. Sodoende word 'n wetenskaplike basis verskaf om die relevansie van beide huidige en toekomstige fitosanitêre beperkings te evalueer. Die doel van laasgenoemde is om die risiko van binnedringing van Natalse vrugtevlieg in invoerende lande te verminder. Slegs deur betroubare, wetenskaplike inligting te verkry, kan fitosanitêre beperkings op sitrushandel beveg word. Dit word dus as krities beskou dat sulke inligting bekom word ten einde marktoegang te verkry en te handhaaf.

Ten einde die potensiële globale verspreiding van die plaag te modelleer, moet volledige verspreidingsdata verkry word. Inligting oor die biologie van die plaag is ook noodsaaklik. 'n Literatuurstudie is derhalwe uitgevoer. Museums en ander navorsers is ook gekontak om verdere verspreidings- en biologiese data te verkry. Om die beste resultate vanuit modellering te verkry, is vollediger data rakende die verspreiding en volopheid van die plaag nodig. Aangesien sulke inligting nie tans beskikbaar is nie, moet opnames gedoen word. Die doel van die opnames is om die relatiewe volopheid van hierdie plaag in suidelike Afrika te bepaal, asook om die geografiese afsnypunte in die verspreiding van Natalse vrugtevlieg in suidelike Afrika te bepaal. Om hierdie doelwitte te bereik, is vrugtevliegvalle in Stellenbosch, Citrusdal, Swellendam, Knysna, Onseepkans, Keimoes, Britstown, Jan-Kempdorp, Addo, King William's Town, Bloemfontein, Pietermaritzburg, Nkwalini, Groblersdal/Marble Hall, Nelspruit, Komatipoort, Tshipise, Tom Burke, Tzaneen, Rustenburg, asook Harare (Zimbabwe) uitgeplaas. Vrugtevliegvalle behoort gedurende Januarie 2007 ook in Alexanderbaai gehang te word. Ten einde die geografiese afsnypunte van Natalse vrugtevlieg te bepaal, is lokvalle ook in Onrus/Vermont, Somerset-Wes, Paarl, Riebeeck-Kasteel, Piketberg, Porterville, Clanwilliam, Vanrhynsdorp, Beaufort-Wes, Garies, Springbok, Olifantshoek, Gariepdam en Vryburg uitgeplaas. Drie "Chempac bucket" valle met Biolure-lokaas is per lokaliteit gebruik. Monitering het gedurende Augustus, September of Oktober begin. Valle is maandeliks nagegaan.

Geen insekmonsters of data is vanaf Tom Burke verkry nie. Vrugtevliegmonsters of data is vanaf alle ander areas verkry. Vir Harare is monsters vir Desember nog uitstaande. Vir Gariepdam is beide November en Desember-monsters nog uitstaande. Natalse vrugtevlieg was afwesig in Clanwilliam, Swellendam, Beaufort-

Wes, Garies, Springbok, Onseepkans, Keimoes, Britstown, Olifantshoek en Gariepdam. Vangste was baie laag, met getalle van minder as 10 vlieë per val per maand in Porterville, Citrusdal, Vanrhynsdorp, Jan-Kempdorp, Tshipise en Vryburg. Vangste het gewissel tussen baie laag en laag (10 tot 29.9 vlieë per val per maand) in Onrus/Vermont, Riebeeck-Kasteel, Knysna, Bloemfontein, Groblersdal/Marble Hall, Tzaneen en Rustenburg. Vangste was hoër in Nelspruit en Komatipoort, meestal met maandelikse gemiddeldes van 30 tot 99.9 vlieë per val. Hoë vangste (100 tot 499.9 vlieë per val) is gedurende sekere maande in Piketberg, Addo en Pietermaritzburg waargeneem en baie hoë getalle (500 tot 1920 vlieë per val) gedurende sekere maande in Somerset-Wes, Stellenbosch, Paarl, King William's Town en Harare. 'n Volledige verslag sal met voltooing van die studie gegee word.

3.4 PROJECT: COSMETIC PESTS

Project coordinator: Tim G. Grout (CRI)

3.4.1 Project summary

The control of cosmetic pests sometimes requires long-residual chemicals or frequent applications of short-residual chemicals and both approaches can result in the disruption of natural enemies of key pests such as red scale and mealybug. Recently, the usage of some of the plant protection products commonly sprayed against these pests was restricted due to changes in the maximum residue limits accepted by our markets. There is therefore an increasing need for new, less disruptive treatments for cosmetic pests and more efficient use of existing IPM-compatible treatments. Further promising results were obtained with the use of a virus for the control of bollworm and the product Helicovir should be registered soon (3.4.2). The development of a rearing technique for a parasitoid of citrus thrips has been delayed while the rearing method for the citrus thrips themselves is being improved (3.4.3). Growers requested a comparison between available formulations of abamectin to determine whether they were equally efficacious. This did prove to be the case, although trial conditions were not ideal (3.4.4). The use of the adjuvant BreakThru at 5 ml/hl with abamectin appeared to be equivalent to the use of horticultural mineral oil (3.4.5). No suitable infestations of grey mite could be found for research to be conducted on this pest but previously-infested sites are being monitored (3.4.6).

Projekopsomming

Chemikalieë met langer residuele nawerkings sowel as gereelde bespuitings met chemikalieë met 'n korter residuele nawerking word gebruik in die beheer van kosmetiese plae. Beide benaderings kan egter lei tot die ontwrigting van natuurlike vyande van sleutelplae soos rooi dopluis en witluis. Die gebruik van sommige van die algemene plantbeskermingsprodukte teen die plae is egter onlangs beperk weens die veranderinge in die maksimum toelaatbare residuvlakke wat deur ons markte aanvaar is. 'n Toenemende behoefte bestaan dus vir nuwe, minder ontwrigtende behandelings teen hierdie plae asook vir die meer effektiewe gebruik van bestaande GPV-behandelings. Verdere belowende resultate is verkry met die gebruik van 'n virus vir die beheer van bolwurm en die produk Helicovir behoort binnekort geregistreer te word (3.4.2). Die ontwikkeling van 'n tegniek om sitrus blaaspootjie parasitoïede te teel is uitgestel maar die tegniek om die blaaspootjies self te teel, is verbeter (3.4.3). Produsente het versoek dat die verskillende beskikbare formulasies van abamectin vergelyk moet word om te bepaal of die doeltreffendheid dieselfde is. Die proewe het aangetoon dat daar nie verskille is nie, alhoewel die omstandighede waaronder die proewe uitgevoer is, nie ideaal was nie (3.4.4). Die gebruik van die hulpmiddel BreakThru, teen 5 ml/hl met abamectin, skyn om ekwavalent te wees aan die gebruik van minerale olies (3.4.5). Bestaande bronne van grysmyt besmettings is gemonitor terwyl geen ander bronne wat geskik was vir navorsing, gevind kon word nie (3.4.6).

3.4.2 Evaluation of the *Helicoverpa armigera* nuclearpolyhedrovirus (HearNPV) for control of bollworm on citrus

Experiment 782 by Sean D. Moore, Wayne Kirkman and Peter Stephen (CRI)

Opsomming

Gedurende 1996-1998 is 'n Suid-Afrikaanse isolaat van *Helicoverpa armigera* nukliêre polihedrovirus (HearNPV) teen bolwurm (*Helicoverpa armigera*) met uitstekende resultate op sitrus getoets. Desondanks is navorsing daarop nie voortgesit nie omdat die virus nie kommersieel beskikbaar was nie. 'n Australiese maatskappy het onlangs 'n HearNPV-produk begin vervaardig. Die produk is oorspronklik as Vivus en Vivus Gold bekend maar word nou deur River Bioscience as Helicovir geregistreer. Geduurende die 2005/06 seisoen is Helicovir in twee boordproewe met Dursban, Dipel en Beta-Bak vergelyk. Beta-Bak is 'n plaaslik vervaardigde Bt isolaat. In al twee proewe het die Helicovir behandelings bolwurm beskadigde vrugte beteknisvol verminder. In een proef is vrugverlies ook betekenisvol verminder tot dieselfde mate as wat met

Dursban bereik is. Beta-Bak het swak gewerk. In een van die proewe was daar 'n positiewe tendens tussen bolwurm skade en uitstop nawels. Geduurende die 2006/07 seisoen, is drie proewe in nawel lemoen boorde uitgevoer en een proef in Midknight Valencias. Helicovir is vergelyk met mevinphos of Lannate, Dipel, Beta-Bak en 'n mengsel van virus en Bt. Bolwurm besmetting in een van die proewe het 'n baie hoë vlak bereik (77.5%). Al die produkte het gesukkel om goeie en vinnige beheer uit te oefen. Al het Helicovir die beste gewerk, sal skade evaluasies wys om sy werking voldoende genoeg was. Voor finale gevolgtrekkings gemaak kan word moet vrugskade- en oesevaluasies uitgevoer word.

Introduction

From 1996-1998 a South African isolate of *Helicoverpa armigera* nuclearpolyhedrovirus (HearNPV) was tested against bollworm (*Helicoverpa armigera*) on citrus, with excellent results (Moore *et al*., 2004c). Despite this, work on this experiment was terminated, as the virus was not commercially available. Recently, an Australian company, Ag-Biotech Australia, began commercially producing HearNPV. The product was previously known as Vivus and Vivus-Gold, but will be commercialised in South Africa by River Bioscience as Helicovir. In the trials recounted in this report, a suspension concentration formulation of 2×10^9 viral occlusion bodies (OBs) per ml, was used. However, the formulation which will be registered will have a concentration of 5×10^9 OBs/ml. During the 2005/06 season, two trials were conducted with Helicovir. Bollworm control in these trials was described in the last annual report (Moore *et al*., 2005). Results of these trials, regarding reduction in fruit damage and crop loss, are reported here. Further trials, with Helicovir, were conducted during the 2005/06 season. If similarly good results are achieved with this commercial product in a second season, its registration for the control of bollworm on citrus in South Africa will be considered. If Helicovir could replace the organophosphate, carbamate or pyrethroid usually used for bollworm control in spring, growers will be greatly assisted towards a reduction in the use of chemicals and the implementation of a bio-intensive IPM programme.

Materials and methods

2005/06 season

Two navel orange orchards in the Eastern Cape were selected for conducting field trials during the 2005/06 season. A trial site was also sought in Mpumalanga. However, bollworm infestation in this region was too low. The first trial was conducted in an orchard of eight-year-old Palmer Navel orange trees on rough lemon rootstock on Scheepersvlakte Farm in the Sundays River Valley. These trees were planted at a spacing of 5.8 x 2.5 m (rows x trees) giving 690 trees per hectare. The second trial was applied in an orchard of 53 year-old Washington navel orange trees interplanted with 24 year-old trees of the same variety, on Tierhok Farm in the Gamtoos River Valley. These trees were planted at a spacing of 7 x 3.5 m (rows x trees) giving 408 trees per hectare. Ten trees, randomly selected in each orchard, were inspected for bollworm infestation, weekly from early in September 2005. Treatments were applied as soon as the first bollworm larvae were observed. The trials were laid out in single-tree random design, with each treatment replicated 12 times.

The same six treatments were applied at both sites: Dursban (100 ml/100 L); Dipel (12.5 g/100 L) and Kynobuff (100 ml/100 L); Helicovir (15 ml/100 L); Helicovir (30 ml/100 L); Beta-Bak (20 ml/100 L); Beta-Bak (40 ml/100 L). Untreated control trees were also used. Beta-Bak is an experimental, locally produced Bt.

In the Scheepersvlakte trial, an average of 12.5 L of spray mix was applied per tree. Therefore the rates of virus per hectare were approximately 2.6×10^{12} OBs and 5.2×10^{12} OBs. This trial was applied on 29-30 September 2005.

In the Tierhok trial, an average of 16.3 L of spray mix was applied per tree. Therefore the rates of virus per hectare were approximately 2.0×10^{12} OBs and 4.0×10^{12} OBs. This trial was applied on 6 October 2005.

Techniques and results of evaluations of bollworm infestation, after applications, are given in the last annual report (Moore et al. 2005). During March and April 2006, fruit damage caused by bollworm was evaluated. Twenty randomly-selected fruit from each tree were inspected and categorised as clean, blemished, or culled (non-exportable) according to stipulated standards (Anonymous, 1995). The crop load was then estimated by placing a 0.125 m^3 frame at uniform height (1 m above the ground) into both the northern and southern sides of each tree, and counting the number of fruit within the frame. Fruit counts from both aspects of the tree were added together and the mean counts calculated for each treatment. Furthermore, evaluations were conducted to measure navel-end malformation. Twenty randomly-selected fruit on each tree were inspected. Navel-ends were categorised as normal, enlarged (without protruding) or protruding.

In all trials, data were analysed using ANOVA and means compared using the Bonferroni LSD multiple range test at the 95% significance level. Proportions (percentages/100) were subjected to an arc sine transformation, where necessary, in order to normalise the data. A zero percentage was counted as 1/(4n) and 100% as (n - 0.25)/n before transformation (Bartlett, 1947; Snedecor & Cochran, 1980).

2006/07 season

A spray trial was conducted in an orchard of 8-year old Midknight Valencia orange trees at Blydevallei Canyon Pakkers in Mpumalanga. Treatments were applied on 18 September 2006, at which time 20.0% of blossom clusters were infested with one or more bollworm larvae of varying life-stages. The average larval size was greater than what was considered ideal for optimum efficacy of a virus or a bacterium. Treatments were applied as medium cover sprays (10.4 L per tree) in a single-tree randomized block format, replicated 10 times. The following treatments were applied (at concentrations per 100 L water): Helicovir (20 ml), Helicovir (25 ml), Lannate (20 g), Dipel (12.5 g) plus Kynobuff (100 ml), Beta-Bak (40 g) plus Kynobuff (100 ml), Beta-Bak (80 g) plus Kynobuff (100 ml), and Beta-Bak (40 g) plus Helicovir (20 ml) plus Kynobuff (100 ml).

Nine days after spraying, on 27 September, bollworm infestation was evaluated. This was done by inspecting 10 blossom or fruitlet clusters per tree and recording the clusters as infested or clean. Statistical analyses were conducted as explained for the 2005/06 trials.

A spray trial was conducted in a navel orange orchard, on Scheepersvlakte Farm in the Sundays River Valley. This was the same navel orange orchard which was used for the trial conducted during the 2005/06 season. Treatments were applied as soon as hatching of bollworm eggs was observed – on 28 and 29 September. At this time, 57.5% of all blossom clusters inspected, were infested with one or more life-stages of bollworm. Treatments were applied as medium cover sprays in a single-tree randomized block format, replicated 10 times. The following treatments were applied (at concentrations per 100 L water): Helicovir (25 ml), Helicovir (30 ml), Mevinphos (100 ml), Dipel (12.5 g) plus Comodobuff (50 ml), Beta-Bak (40 g) plus Comodobuff (50 ml), Beta-Bak (80 g) plus Comodobuff (50 ml), Beta-Bak (40 g) plus Helicovir (20 ml) plus Comodobuff (50 ml), Dipel (12.5 g) plus Comodobuff (50 ml) plus Helicovir (20 ml), an experimental HearNPV (5 g). The 5 g per 100 L water concentration of the experimental HearNPV had an equivalent number of viral occlusion bodies to the 30 ml per 100 L water concentration of Helicovir.

Two field trials were applied in the Western Cape: in the Citrusdal and Swellendam regions. The trial in Citrusdal, in which the same treatments were applied as in the Scheepersvlakte trial, was inadvertently sprayed out by the grower, approximately 10 days after treatments were applied. This was before any evaluations could be conducted.

The trial at Swellendam was applied to an orchard of six year old Washington navel oranges on Thornlands farm. The trees were spaced at 6 m x 3 m. The following treatments were applied (at concentrations per 100 L water): Helicovir (25 ml) plus Comodobuff (50 ml), Helicovir (25 ml) plus Comodobuff plus Agral 90 (18 ml), Helicovir (25 ml) plus Comodobuff plus Agral 90 (18 ml) plus molasses (250 ml). Shortly after treatments were applied (on 9 October 2006), light rain was experienced. Consequently, treatments were reapplied the following day (10 October) on different trees adjacent to the original trial block. Bollworm infestation was evaluated in both trial blocks two weeks after application.

Results and discussion

2005/06 season

All treatments, apart from the Helicovir at 30 ml, significantly reduced bollworm damage (Table 3.4.2.1). More importantly, all treatments significantly reduced fruit culled due to bollworm. Although not significantly, the most effective treatments in reducing bollworm cull were Dursban and Helicovir (30 ml). Bollworm infestation had obviously not been high enough to cause any reduction in yield (Table 3.4.2.1). Infestation in the untreated control had peaked at 42.5 % (clusters infested) during early October 2005 (Moore et al. 2005).

Table 3.4.2.1. Fruit (navel orange) damage and yield for various bollworm treatments at Scheepersvlakte Farm in Sundays River Valley. Treatments were applied on 19 and 30 September 2005. Fruit damage and yield were evaluated on 9 March 2006

Treatment	Concentration per 100 L water	Fruit damage		Yield index
		Scar	Cull	
Untreated control	-	27a	26a	181a
Dursban	100 ml	10b	6c	165a
Dipel Kynobuff	12.5 g 100 ml	15b	7bc	151a
Helicovir	15 ml	13b	9bc	174a
Helicovir	30 ml	17ab	6c	160a
Beta-Bak Kynobuff	20 g 100 ml	12b	9bc	182a
Beta-Bak Kynobuff	40 g 100 ml	14b	14b	193a

*Values in the same column followed by the same letter are not significantly different (P>0.05; LSD multiple range test).

At Scheepersvlakte, it was also determined that 7.95% of fruit with bollworm induced scars (i.e. not cullable damage), appeared on the sides of fruit, whereas 5.00% of fruit had bollworm-scarred navel ends. This difference was statistically significant. Conversely, a marginally higher percentage of fruit culled for bollworm damage – 4.88% - bore the damage on the navel end, rather than on the side of the fruit – 4.29%. This difference was not statistically significant. These results did not support earlier findings, demonstrating that the vast majority of damage was inflicted on the navel (stylar) end of the fruit (Moore et al. 2004b).

The same study established a relationship between bollworm damage and malformed (enlarged and/or protruding) navels (Moore et al. 2004b). Navel end malformation was measured in the Scheepersvlakte trial in order to again examine this relationship. No regression analyses were conducted. However, a higher percentage of protruding navels and all types of malformed navels, was recorded for untreated control fruit than for any of the treatments (Table 3.4.2.2). This was not highly significant.

Table 3.4.2.2. Navel-end malformation of navel oranges for various bollworm treatments at Scheepersvlakte Farm in Sundays River Valley. Treatments were applied on 19 and 30 September 2005. Navel-ends were evaluated on 25 April 2006

Treatment	Concentration per 100 L water	Navel-end malformation		
		Enlarged	Protruding	Total
Untreated	-	8.75ab	7.08a	15.83a
Dursban	100 ml	8.75ab	3.75ab	12.50a
Dipel Kynobuff	12.5 g 100 ml	10.83a	3.75ab	14.58a
Helicovir	15 ml	5.83ab	3.75ab	9.58a
Helicovir	30 ml	9.17ab	2.08b	11.25a
Beta-Bak Kynobuff	20 g 100 ml	4.17b	5.83ab	10.00a
Beta-Bak Kynobuff	40 g 100 ml	5.83ab	3.75ab	9.58a

*Values in the same column followed by the same letter are not significantly different (P>0.05; LSD multiple range test).

In the Tierhok trial, only Dursban significantly reduced bollworm damage (Table 3.4.3). However, Helicovir (both concentrations) and Dursban significantly reduced bollworm induced fruit cull. Damage was less for the higher concentration of Helicovir. However, there was no difference in fruit cull for the two concentrations. Bollworm infestation peaked at 52.5% in October (Moore et al. 2005). Consequently, yield was significantly lower in the untreated control and the Bt treatments (Table 3.4.2.3). Results with Dipel were disappointing. It was clear at this site that a second Dipel application should have been made.

Table 3.4.2.3. Fruit (navel orange) damage and yield for various bollworm treatments at Tierhok Farm in Gamtoos River Valley. Treatments were applied on 6 October 2005. Fruit damage and yield were evaluated on 6 April 2006

Treatment	Concentration per 100 L water	Fruit damage		Yield index
		Scar	Cull	
		21ab	13a	76a
Dursban	100 ml	3c	2b	164c
Dipel Kynobuff	12.5 g 100 ml	20ab	11a	108ab
Helicovir	15 ml	20ab	1b	147bc
Helicovir	30 ml	11bc	1b	151bc
Beta-Bak Kynobuff	20 g 100 ml	24a	12a	92a
Beta-Bak Kynobuff	40 g 100 ml	20ab	11a	73a

*Values in the same column followed by the same letter are not significantly different (P>0.05; LSD multiple range test).

A higher percentage of enlarged navels were recorded for the untreated control and the two Beta-Bak treatments, which worked very poorly (Table 3.4.2.4). However, there did not appear to be a logical trend for protruding navels.

Table 3.4.2.4. Navel-end malformation of navel oranges for various bollworm treatments at Tierhok Farm in Gamtoos River Valley. Treatments were applied on 6 October 2005. Navel-ends were evaluated on 26 April 2006

Treatment	Concentration per 100 L water	Navel-end malformation		
		Enlarged	Protruding	Total
Untreated	-	5.83abc	2.08a	7.92abc
Dursban	100 ml	2.92bc	1.25a	4.17c
Dipel Kynobuff	12.5 g 100 ml	2.08c	5.83b	7.92abc
Helicovir	15 ml	3.33bc	1.25a	4.58bc
Helicovir	30 ml	3.75abc	3.33ab	7.08abc
Beta-Bak Kynobuff	20 g 100 ml	7.08a	2.92ab	10.00ab
Beta-Bak Kynobuff	40 g 100 ml	5.83ab	5.42b	11.25a

*Values in the same column followed by the same letter are not significantly different (P>0.05; LSD multiple range test).

2006/07 season

At the time of applying the treatments at Blydevallei Canyon Pakkers, 20.0% of blossom or fruitlet clusters were infested with bollworm. At this time, only 22.7% of larvae were smaller than 12 mm in length; 45.4% of larvae were between 12 mm and 19 mm long; and 31.8% of larvae were larger than 19 mm long. It is generally accepted that both Bt and baculoviruses are far less effective against larger larvae. Despite this, it was surprising that only nine days after application, five of the treatments were free of bollworm larvae (Table 3.4.2.5). Bollworm was recorded in the untreated control, the Lannate treatment and the combined Beta-Bak and Helicovir treatment. It is interesting that the latter appeared to be less efficacious than the two treatments on their own – although not significantly so. This might be because Bt caused a rapid reduction in feeding activity, thus potentially reducing the amount of virus ingested.

Table 3.4.2.5. Efficacy of various treatments in controlling bollworm on Midknight Valencia orange trees at Blydevallei Canyon Pakkers in Mpumalanga, evaluated on 27 September 2006, nine days after treatment

Treatment	Blossom/fruitlet clusters infested with bollworm (%)	
	Total infestation	Infestation excluding large larvae
Untreated control	7.3a	5.4a
Lannate (20 g)	0.9b	0.9b
Dipel (12.5 g) + Kynobuff (100 ml)	0b	0b
Helicovir (20 ml)	0b	0b
Helicovir (25 ml)	0b	0b
Beta-Bak (40 g) + Kynobuff (100 ml)	0b	0b
Beta-Bak (80 g) + Kynobuff (100 ml)	0b	0b
Beta-Bak (40 g) + Helicovir (20 ml) + Kynobuff (100 ml)	0.9b	0b

*Values in the same column followed by the same letter are not significantly different (P>0.05; LSD multiple range test).

At the Scheepersvlakte trial site used during the 2006/07 season, a higher level of bollworm infestation was recorded than had ever previously been recorded during the course of these virus trials, dating back to 1996 (Moore et al. 2004b). Consequently, treatments seemed to be less effective than in other trials. Also, the higher concentration of Helicovir (30 ml/100 L water), which was the most effective treatment at three weeks after application, was notably (but not significantly) more effective than the lower concentration (25 ml/100 L water). It was this result, which swayed River Bioscience to register the product at a rate of no less than 30 ml per 100 L water. Despite HearNPV having worked well in all trials to date, its slow knock-down will not make it easily acceptable to growers. In this trial conducted at Scheepersvlakte, it will be interesting to see if Helicovir was able to significantly and acceptably reduce bollworm damage.

Table 3.4.2.6. Efficacy of various treatments in controlling bollworm on navel orange trees at Scheepersvlakte farm in the Sundays River Valley

Treatment	Blossom/fruitlet clusters infested with bollworm (%)			
	1 WAT*	2 WAT	3 WAT	4 WAT
Untreated control	77.50a	69.17a	40.83a	10.00abcd
Mevinphos (100 ml)	24.17f	34.17c	23.33c	10.83abc
Dipel (12.5 g) + Comodobuff (50 ml)	35.00ef	45.00bc	28.33bc	15.83a
Helicovir (25 ml)	60.83bc	39.17bc	18.33cd	5.00cde
Helicovir (30 ml)	67.50bc	32.50c	9.17d	1.67e
Beta-Bak (40 g) + Comodobuff (50 ml)	69.17b	66.67a	36.67ab	7.5bcde
Beta-Bak (80 g) + Comodobuff (50 ml)	67.5bc	61.67a	38.33ab	6.67bcde
Beta-Bak (40 g) + Helicovir (20 ml) + Comodobuff	55.83cd	48.33b	25.00c	5.00cde
Dipel (12.5 g) + Comodobuff (50 ml) + Helicovir (20 ml)	40.00e	41.67bc	27.50bc	12.50ab
Experimental virus (5 g)	45.83de	40.00bc	22.50c	4.17de

*weeks after treatment; *Values in the same column followed by the same letter are not significantly different (P>0.05; LSD multiple range test).

The experimental virus (Table 3.4.2.6), although used at a lower mass per volume, was used at a similar concentration of occlusion bodies as was Helicovir. This virus appeared to be marginally inferior to Helicovir. Beta-Bak was once again inadequately effective and probably does not have the potential to be registered for use on citrus. Combining Helicovir with Bt did not improve efficacy. However, this was with a lower concentration of Helicovir than was used on its own.

The rainfall experienced at the Swellendam trial site did not reduce the efficacy of Helicovir (Table 3.4.2.7). The rainfall could have been too light, or the virus might be rainfast in a similar manner to that recorded for Cryptogran (Moore et al. 2004a). The addition of molasses also did not improve the efficacy of the virus. Most of the larvae found during the evaluations were first or second instar larvae, which would indicate that a "second" infestation took place after the treatments were applied.

Table 3.4.2.7. Efficacy of various treatments in controlling bollworm on navel orange trees at Thornlands farm near Swellendam

Treatment	Blossom/fruitlet clusters infested with bollworm (%) after 2 weeks	
	Trial 1 (rainfall)	Trial 2 (no rainfall)
Untreated control	15.00a	15.00a
Helicovir (25 ml) + Comodobuff (50 ml)	7.50b	8.33b
Helicovir (25 ml) + Comodobuff (50 ml) + Agral 90 (18 ml)	7.50b	9.17b
Helicovir (25 ml) + Comodobuff (50 ml) + Agral 90 (18 ml) + molasses (250ml)	9.17ab	7.50b

*Values in the same column followed by the same letter are not significantly different (P>0.05; LSD multiple range test).

Conclusion

In the two trials conducted during the 2005/06 season, Helicovir was very effective in significantly reducing the percentage of fruit culled for bollworm damage. Beta-Bak was not very effective. During the 2006/07 season Helicovir significantly reduced bollworm infestation. However, it will shortly be determined whether this was adequate to acceptably reduce bollworm damage where infestation was extremely high. An application for the registration (according to Act 36 of 1947) of Helicovir for control of bollworm on citrus has been submitted.

Future research

During March 2007, bollworm damage to fruit and crop load will be evaluated.

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3.4.3 Develop a rearing technique for the citrus thrips parasitoid *Goetheana incerta*

Experiment 809 by Tim G Grout and Kim Stoltz (CRI)

Summary

South African citrus thrips *Scirtothrips aurantii* is being maintained in an environmental chamber on *Bryophyllum pinnatum* phyllodes in small disposable plastic containers without water or soil. However, it has not yet been possible to increase numbers sufficiently to allow for rearing of the parasitoid. A limiting factor that is preventing high numbers of thrips from being reared in this manner has not yet been resolved. The size of phyllodes being used has been reduced as it was thought that those produced during high temperatures in summer may be too tough for the first instars to feed on. This culture will also be required for microbial control research so the rearing technique is currently receiving further attention. As soon as

sufficient numbers of citrus thrips are available, a culture of *Goetheana incerta* will be started in another environmental chamber.

Opsomming

Scirtothrips aurantii, die Suid Afrikaanse sitrus blaaspootjie, is vir 'n jaar in 'n broeikamer, op blaarstele van *Bryophyllum pinnatum* aangehou in klein, weggooibare plastiekhouers, sonder water of sand. Dit was egter nog nie moontlik om die getalle genoegsaam te vermeerder vir die teling van die parasitoïed nie aangesien verskeie faktore wat die proses belemmer nog nie opgelos kon word nie. Die grootte van die blaarstele wat gebruik is, is verklein omdat daar vermoed is dat die blaarstele wat tydens die hoë somer temperature gevorm is moontlik te taai kan wees vir die eerste instars. Om ontsnapping te voorkom is die ontwerp van die kaste ook aangepas. Sodra genoegsame getalle van die blaaspootjies beskibaar is, sal 'n kultuur van *Goetheana incerta* in 'n ander broeikamer gevestig word. 'n Volledige verslag sal beskikbaar gestel word sodra die telingstegniek vir *Goetheana incerta* ontwikkel is.

3.4.4 Efficacy evaluation of abamectin formulations against citrus thrips

Experiment 853 by Tim Grout, Peter Stephen and Bruce Tate (CRI)

Opsomming

Op versoek van sitrus produsente het CRI die verskillende beskikbare formulasies van abamectin vergelyk om te bepaal om daar enige verskille is in effektiwiteit en ook of dit fitotoksisiteit kan veroorsaak. Al die formulasies, in vergelyking met die kontrole, was effektief en het die sitrus blaaspootjie populasies betekenisvol verminder. Daar was egter geen noemenswaardige verskille ($P>0.05$) tussen die formulasies nie en ook geen indikasie van fitotoksisiteit nie. Die proef is herhaal op 'n tweede perseel, maar geen evaluasies was moontlik nie omdat die sitrus blaaspootjie besmetting tot niet gegaan het. Geen verdere vergelykings tussen die abamectin formulasies word beplan nie.

Introduction

There are currently at least five formulations of abamectin available to citrus farmers for the control of citrus thrips (Morse 1994, Grout & Stephen 2006). When the first generic abamectin formulations became available they were closely scrutinised by Syngenta to determine whether they were equivalent to Agrimec. However, the currently available formulations have not had this scrutiny and variable results in the field have led to a request from growers for a comparison of efficacy. Furthermore, some of the recent problems with ring burn on fruit may be connected to abamectin plus oil sprays so treatments can be evaluated for fruit burn at the same time. For this reason the following research was conducted.

Materials and methods

A trial was laid out in a Valencia orchard at Johan Beukes' farm near Burgersfort because in the last three years, this area has consistently had the highest population levels of citrus thrips in the region. The orchard was divided into two sections and each section further divided into treatment blocks that were at least three rows wide and seven trees long. Spray applications were made with the use of high pressure hand-guns on 7 October 2006 after 100% petal fall. The temperature ranged from 26.5 to 33.5°C and relative humidity from 20 to 40%. All abamectin formulations (abamectin 18 g/ℓ EC) were applied at 20 ml per 100 ℓ water plus 300 ml BP medium oil. Three weeks later it was discovered that a severe fruit drop had occurred shortly after spraying, particularly on one side of the orchard. This drop has been ascribed to the dry weather conditions and a marginally effective drip irrigation system. Numbers of citrus thrips were also low so an assessment of thrips infestation levels was delayed until 8 November when the proportion of 20 fruit per tree that were infested with either citrus thrips larvae or adults was determined. Only one evaluation was conducted. Results were analysed by two-way ANOVA and means compared further using Student-Newman-Keul's test at $\alpha=0.05$.

In November, all treatments were repeated at a site at the Lowveld Agricultural College, Nelspruit. Prior to spraying, fruit counts showed a 14% infestation of thrips larvae. However, two weeks after spraying the controls showed only 1.3% infestation and no evaluation was possible.

Results and discussion

With the scarcity of fruit on some trees, the incidence of larval thrips infestation varied widely, presumably because it was difficult for the adult thrips to find solitary fruit. This resulted in considerable differences

between replicates of the same treatment. Although all the abamectin formulations had significantly fewer ($P<0.05$) thrips larvae than the untreated control, there were no significant differences between formulations (Table 3.4.3.1). The adult infestations of fruit were even more variable due to the time elapsed since spraying and only one treatment was significantly different from the control. None of the fruit showed signs of ring burn or phytotoxic reactions.

Table 3.4.4.1. Percentage infestation of fruit by citrus thrips on 8 November 2006

Treatments	% Larvae	%Adults
Control	26.7 a	12.1 a
Mecti	7.3 b	4.0 ab
Biomectin	5.8 b	2.1 b
Sanamectin	7.1 b	5.4 ab
Agromectin	8.8 b	5.8 ab
Agrimec	4.2 b	2.9 ab
Abamec plus	14.6 b	9.6 ab
Abamec Insecticide	10.0 b	5.8 ab

Means in the same column followed by the same letter were not significantly different ($P>0.05$) (SNK)

Conclusions

No differences were found between the abamectin formulations with regard to efficacy and no fruit burn was found.

Future research

No further comparisons of abamectin formulations are planned.

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3.4.5 Evaluation of Solitaire and BreakThru in combination with abamectin against citrus thrips

Experiment 897 by T G Grout, P R Stephen and B A Tate (CRI)

Opsomming

Op versoek van Degussa Africa is die twee hulpmiddels, BreakThru en Solitaire tesame met BP Medium Olie, deur CRI, as byvoegsels tot abamectin vergelyk vir die beheer van sitrus blaaspootjie. Alhoewel die vlakke van besmetting en die volopheid van vrugte nie ideaal was nie, het die resultate getoon dat BreakThru 0.005% effektief was as 'n alternatief tot medium graad minrerale oile teen 0.3%. Verdere proewe word egter benodig om die dosis waarde te bevestig en ook die effektiwiteit van abamectin met Solitaire te bepaal.

Introduction

Degussa Africa (Pty) Ltd approached CRI to evaluate two adjuvants as possible alternatives to oil for combination with abamectin for control of citrus thrips. The research was therefore conducted on a contract basis.

Materials and methods

The trial was conducted in the same Valencia orchard at Johan Beukes' farm near Burgersfort as experiment 853 (section 3.4.4). The orchard was divided into two sections and treatments were randomly assigned to blocks within each section. Each treatment block was at least four rows wide and seven trees long. Trees were sprayed by hand using approximately 11.9 to 13.4 L spray mix per tree and while temperatures ranged from 25.2 to 27.0°C and relative humidity from 32 to 36%. Treatments (Table 3.4.6.1) were applied on 11 October 2006 and evaluated on 15 November by determining the proportion of 20 fruit per tree infested by

either larvae or adult citrus thrips. Results were analysed by two-way ANOVA and means compared further using Student-Newman-Keul's test at α=0.05.

Results and discussion

Unfortunately, the trees in this trial also suffered from water stress and lost many of their fruit. Only a single evaluation was conducted and an attempt to repeat the trial was aborted when thrips numbers in the second site collapsed. Due to the movement of adults over time, results based on larval infestation are usually less variable. In this case, all treatments had significantly lower larval infestation levels than the control and the treatment with BreakThru at 5 ml/hl had the lowest infestation level (Table 3.4.5.1). The higher dosages of BreakThru were less effective, perhaps due to more run-off taking place and there being less absorption of abamectin in the plant tissue to provide a residual effect. Although there were few significant differences between treatments it appeared that the rates of Solitaire and BreakThru used could serve as alternatives to medium grade horticultural oil at 0.3%, although the results with Solitaire were less convincing than with BreakThru.

Table 3.4.5.1. Percentage infestation of fruit by citrus thrips on 15 November 2006

Treatments on 11 Oct 2006	% Larvae	% Adults
Biomectin 20 ml plus BP Medium oil 300 ml/hl	7.5 bc	11.3 ab
Biomectin 20 ml plus Solitaire 0.025%	14.6 bc	15.8 ab
Biomectin 20 ml plus Solitaire 0.075%	6.9 bc	5.9 bc
Biomectin 20 ml plus BreakThru 0.005% (5 ml/hl)	1.6 c	2.8 c
Biomectin 20 ml plus BreakThru 0.01%	14.7 b	14.7 ab
Biomectin 20 ml plus BreakThru 0.025%	8.3 bc	7.6 abc
Control	23.8 a	15.3 a

Means in the same column followed by the same letter were not significantly different (P>0.05) (SNK)

Conclusion

Although the infestation level in the trial was low and fruit were sparse the results indicated that BreakThru at 0.005% was effective in a combination with abamectin for the control of citrus thrips.

Future research

This research should be repeated to obtain more information on suitable dosage rates for the adjuvants.

3.4.6 Improving management of citrus grey mite, *Calacarus citrifolii*

Experiment 856 by T G Grout (CRI)

Summary

No suitable sites for research could be found near Ohrigstad as the pest was under control. With Dassie Smith's help a site NW of Mookgopong was eventually found. Unusual feeding symptoms seen on navel orange leaves without the typical concentric ring blotch or necrotic spot were observed. This may be feeding damage without the presence of the pathogen. However, no live mites could be found at the time. Sticky traps and Vaseline-coated slides were set up in transects near indigenous bush to see whether grey mite is blowing into the orchard from the bush as claimed by more than one citrus grower. At the time of writing the report, no citrus grey mite had been found. Research will continue at this or another site.

Opsomming

Geen geskikte proefpersele kon gevind word vir navorsing naby Ohrigstad nie omdat die plaag daar onder beheer was. 'n Perseel is egter gevind NW van Mookgopong met die hulp van Dassie Smith. Ongewone voedingsimptome sonder die tipiese konsentriese ring vlekke of nekrotiese vlekke is gevind op blare van nawel lemoene. Dit mag moontlik voedingskade wees in die afwesigheid van die patogeen. Geen lewende myte kon egter op daardie stadium gevind word nie. Taai valle en Vaseline bedekte glasplaatjies is geplaas in "transects" naby inheemse bosveld om so vas te stel of grysmyte deur die wind gewaai kan word vanaf die bosveld na die boorde, soos beweer word deur meer as een produsent. Geen grysmyte kon egter gevind word teen die tyd dat die verslag saamgestel is nie. Navorsing sal egter voortgesit word by hierdie perseel of ander persele wat gemonitor word.

3.5 PROJECT: BIOCONTROL DISRUPTION

Project coordinator: Tim G. Grout (CRI)

3.5.1 Project summary

Biological control remains the mainstay of IPM and is the easiest method of reducing residues on fruit. The use of long-residual pesticides for the control of false codling moth or citrus thrips disrupts the biocontrol of mealybug and other pests by killing their natural enemies. Ants can cause similar disruption of biocontrol when present in the trees, even though they may be beneficial predators when on the orchard floor. The ideal tool for ant management would be a repellent that keeps them out of trees but does not eliminate the ants themselves. However, the best prospective repellents evaluated only worked against the pugnacious ant for five days which is not long enough for commercial purposes (3.5.2). Parallel research on the development of ant baits got as far as demonstrating that the pugnacious ant is attracted to both a sugar-based bait and one containing protein, whereas the brown house ant is mostly attracted to protein (3.5.4). Research will continue with a protein, sugar, plant oil combination. Although non-target effect bioassays are regularly conducted on a contract basis when new products are being registered, none were required during 2006. However, the non-target effects of several key pesticides were tested against green lacewing larvae as little is known about these predators of soft-bodied arthropods. Some unusual results were obtained with Mesurol being very harmful, as in the case of predatory mites, and Regent and Hunter being classified as slightly harmful. Dursban was also categorised as very harmful whereas some other organophosphates were found to be harmless (3.5.3). Future research in this project will concentrate on ant control.

Projekopsomming

Biologiese beheer is steeds die belangrikste onderbou van IPB en ook die maklikste manier om residue op vrugte te verminder. Die gebruik van chemikalieë met ’n lang residuele nawerking in die beheer van valskodlingmot of sitrus blaaspootjie ontwrig egter die biologiese beheer van witluise en ander plae omdat hul natuurlike vyande gedood word. Miere kan ook soortgelyke ontwrigting van biologiese beheer veroorsaak as dit in bome teenwoordig is alhoewel hul voordelig kan wees as predatore op die vloer van die boord. Die ideale manier vir mierbeheer sal ’n afweermiddel wees wat die miere uit die bome hou, maar hul nie dood nie. Die beste moontlike afweermiddels wat geëvalueer is, was slegs vir 5 dae effektief teen die malmier. Dit is egter nie lonend vir kommersiële gebruik nie (3.5.2) Parallele navorsing op die ontwikkeling van ’n lokmiddel vir miere het so ver gevorder as ’n demonstrasie dat die malmier gelok word deur ’n middel met ’n suikerbasis sowel as ’n middel wat proteïene bevat, terwyl die bruin huismier meestal deur die proteïene aangelok word (3.5.4). Navorsing sal verder gedoen word met ’n middel wat ’n kombinasie sal wees van ’n proteien, suiker en ’n plantolie. Alhoewel nie-teiken effek biologiese ondersoeke op ’n gereelde kontrak basis uitgevoer word wanneer nuwe produkte geregistreer word, is geen van die ondersoeke in 2006 uitgevoer nie. Die nie-teiken effekte van verskeie sleutel chemikalieë is egter getoets teen die larwes van gaasvlerkies omdat daar min bekend is oor die predatore van sagte dop arthropode. Ongewone resultate is verkry met Mesurol wat baie skadelik was teenoor predatoriese myte, terwyl Regent en Hunter slegs geklassifiseer is as effens skadelik. Dursban is ook geklassifiseer as baie skadelik terwyl ander organofsfate as nie-skadelik gevind is (3.5.3). Verdere navorsing in hierdie projek sal fokus op die beheer van miere.

3.5.2 To develop an ant repellent that will keep ants out of citrus trees without destroying their nest

Experiment 798 by Tim G Grout and Kim Stoltz (CRI)

Opsomming

Om afweermiddels te gebruik vir die beheer van miere is baie voordelig want sodoende kan hul predatoriese voordele op die vloer van die boorde behou word sonder ontwrigting van hul natuurlike vyande in die bome. Verskeie produkte is getoets as mierafweermiddels teen die malmier, *Anoplolepis custodiens*, omdat dit die moeilikste mier is om te beheer met ander metodes. Verskeie van die produkte is gevind om effektief te wees maar ’n effek kon egter net vir ’n periode van 5 dae verkry word. Dit is egter te kort om van enige kommersiële waarde te wees vir die produsent en daarom word geen verdere navorsing met die produkte beplan nie.

Introduction

Ants are attracted to honeydew-producing homopteran insects in citrus trees and often protect these pests from their natural enemies. In addition, ant activity in trees can disrupt the natural enemies of non-honeydew-producing pests such as red scale *Aonidiella aurantii* and result in an increase in population density of this pest. However, ants on the orchard floor can play a beneficial role in preying on late larval instars or pupae of various citrus pests including fruit flies, false codling moth and other lepidoptera, and

citrus thrips. Rather than destroying the nests on the orchard floor it would therefore be beneficial to stop the ants from gaining access to the tree. This has been done in the past with sticky ant bands (Samways & Tate 1985) and chemical-mechanical barriers such as The Protector but these are labour-intensive to maintain and the re-treating of The Protector sometimes results in pyrethroid insecticide being sprayed up into the tree. If natural products could be found that could be applied to tree trunks to repel ants without killing them they would be more cost effective than physical barriers. Some promising work was done in California with the use of farnesol as a barrier (Shorey et al. 1992, Shorey et al. 1996) but this product was never commercialised as the control period was too short (P.A. Phillips, pers. comm.). The emphasis of the work below was on the pugnacious ant *Anoplolepis custodiens* as this ant is the most difficult to control.

Materials and methods

Research in 2005 (Grout et al. 2005) had led to the elimination of many products and those that looked promising as short-term repellents were mostly botanical products. The focus of the research then changed to the persistence of the repellents as it was reasoned that any repellent that would not last at least a month would be of no commercial interest. Initially, prospective repellents were used without any dilution but in the later persistence tests the products were diluted to make them more cost effective.

Trial 1 was initiated on 30 January 2006. Wooden dowels, 50 cm long and 18 mm diameter were used to each support a Petri dish containing a food attractant. The repellents were applied to the rods to attempt to prevent access to the attractant. The dowels were cut at a 45 degree angle at one end to enable easy penetration of the soil. Fifteen centimetres above the lower end of each rod, a single layer of brown packaging tape (48 mm wide) was wrapped around the rod as a foundation for a single wrap of Elastoplast Fab roll (25 mm wide). Approximately 1 cm^3 cotton wool was used in the centre of the plaster to absorb 0.5 ml of each repellent. The repellent was applied to the cotton wool before the plaster was attached to the rod. At the top end of the dowel, a 6.5 cm diameter Petri dish was attached by means of a thumb tack pushed through a 1 mm diameter hole that had been melted into the centre of the dish. Each Petri dish also had two holes of approximately 5 mm diameter provide drainage during and after rain. A smaller, 3.8 cm diameter Petri dish was used to provide a food attractant in the form of fish paste (Pecks Anchovette) and Golden syrup. The attractant dishes could be prepared in the laboratory and placed on the larger Petri dishes in the orchard in a few minutes. Eight replicates were used per repellent and all treatments for each replicate were placed in the vicinity of an active nest opening. Counts of the number of ants visiting the food in 30 s were made one hour after placing the food in the Petri dishes. Evaluations were made on the day of setting up the trial and repeated after 24 hours and 48 hours. Repellents used were all plant products.

Trial 2 was initiated on 1 February 2006. A different type of plaster was used (Elastoplast Sport Rigid Strapping tape 50 mm wide) due to the repellents causing the previous plasters to peel off. Otherwise, the method was the same with the second plaster being applied higher up the rod from where the previous one was. However, the repellents were diluted to a rate of 10%. Only one evaluation was made after 24 hrs. Repellents used were the same as for Trial 1.

Trial 3 was initiated on 5 February 2006. Plasters were no longer used to absorb the repellent but the repellent was applied with a paintbrush directly to the wood. Repellent mixtures were made up at 20% and 0.1 ml of this mixture was painted in a band around each rod. A separate brush was used for each repellent. Evaluations were made after 24 and 72 hours and food was not replenished at the time of evaluation. Repellents used were the same as for Trials 1 and 2.

Trial 4 was initiated on 6 December 2006. Food was replaced daily due to high ant activity. Only five replicates were used per treatment and ant activity was not quantified, only noted as present or absent. The application of the repellents was changed as follows. Blue dishwashing cloths (Pick 'n Pay No Name Heavy Duty Wipes 60 x 33 cm) were cut into strips 6 cm wide and 30 cm long. The end of each strip was cut in the middle to create pieces (6 cm long) that could be used to tie the material into position securely around the rods. The material was positioned approximately 15 cm above the ground. A Sigma Spray Kit was used to spray each product onto the dishcloth bands after the rod had been inserted in the ground near the nest opening. The repellents were diluted to 20%. The Sigma Spray Kit uses an aerosol can to spray a liquid from a vial using the venturi principle. Each rod was sprayed with 2 ml of the repellent mixture. Water was sprayed between each treatment to remove any residues and new pipes and vials were used for each treatment, to prevent contamination. Evaluations were conducted after the repellents were exposed for one, two and five days.

Trial 5 was initiated on 11 December 2006 using the same technique as for Trial 4. Evaluations were made after one, two, three, seven and eight days.

Results and discussion

With the undiluted repellents, all treatments were successfully repelling ants soon after setting up the trial but the products affected the plasters so that they lifted in places and allowed ants through in the later evaluations (Table 3.5.2.1). This trial was therefore stopped after 48 hours. In Trials 2 and 3, none of the diluted treatments were successful in repelling ants for 24 hours or longer and the different type of plaster in Trial 2 was still negatively affected by the oils.

Table 3.5.2.1. Results of undiluted repellents under Elastoplast band (Trial 1)

Treatments	Percentage of replicates with ants obviously repelled or no food removed		
	1 h	24 h	48 h
Control	0	0	0
Product A	100	62.5	50.0
Product B	100	62.5	87.5
Product C	100	87.5	50.0
Product D	100	87.5	100
Product E	100	100	87.5
Product F	100	100	100

In Trial 4, all the repellents were effective in all replicates for up to two days but by five days none of them were effective (Table 3.5.2.2). In Trial 5 the repellents were breaking down by about the seventh day after application and were ineffective after eight days (Table 3.5.2.3).

The longest control that can be expected with a spray onto an absorbent band is for approximately five days for the pugnacious ant. Direct application to bark without an absorbent band may not even be effective for this long. Labour would be required to apply the band or barrier and frequent application costs would probably be prohibitive. It therefore does not look as if this approach would be cost effective for a commercial grower, although it may have some value for small scale organic production or home gardens. It is possible that some type of extender would provide control for a longer period and this will be investigated against both pugnacious ant and the brown house ant. However, the Californian product (Shorey et al. 1996) was effective for a much longer period than those evaluated here and it could not be commercialised, so these results are not very promising.

Table 3.5.2.2. Results of diluted repellents on cloth band (Trial 4)

Treatments	Percentage of replicates with ants obviously repelled or no food removed		
	1 d	2 d	5 d
Control	0	0	0
Product A	100	100	0
Product G	100	100	0
Product H	100	100	0

Table 3.5.2.3. Results of diluted repellents on cloth band (Trial 5)

Treatments	Percentage of replicates with ants obviously repelled or no food removed				
	1 d	2 d	3 d	7 d	8 d
Control	0	0	0	0	0
Product B	100	100	100	40	0
Product D	100	100	100	20	0
Product F	100	100	100	60	0

Conclusion

The longest period of control obtained against pugnacious ants with repellents was approximately five days. With some type of absorbent trunk barrier this period may be extended slightly but the additional cost and labour involved would probably exceed any savings on spray. A possible extender will be evaluated but if this is not effective, no further research on repellents is planned.

Future research

Apart from some small-scale evaluations with repellents on ant caps and with possible extenders, no further research is planned.

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3.5.3 Rearing of the lacewing predator *Chrysoperla pudica* and its susceptibility to key pesticides used on citrus

Experiment 810 by P. R. Stephen and T. G. Grout (CRI)

Opsomming

Verskillende spesies van goudogies kom in sitrusboorde voor, en almal is predatore van plantluise, witluise, sagtedopluise, sitrusblaaspootjies en myte. Om die voordeel van hierdie algemene predatore ten volle te benut, is kennis ten opsigte van hulle sensitiwiteit teenoor die mees algemeen gebruikte plaagdoders nodig. Hierdie inligting sal ook nodig wees as insektariums goudogies wil teel vir vrylatings in sitrusboorde. Volwasse goudogie spesies van *Chrysoperla pudica,* is versamel en in die laboratorium geteel. Nadat die populasiegetalle hoog genoeg was is die evaluasie proses begin. Die larfstadium is met vrugtevliegeiers (Ceratitis sp.) en moteiers (*Ephestia sp.)* gevoed, terwyl die volwassenes gevoed het op ’n mengsel van heuning en gis. Daaglikse eierproduksie is in afsonderlike houers geteel. Gesnipperde papier in die houers dien as skuiling vir die larwes. Die lewenssiklus van *C. Carnea* beloop ’n minimum van 30 dae by 23°C. Vir die evaluasieproses is 50, twee dag oue, larwes blootgestel aan blare met 24-uur oue residue van elke plaagdoder. Elke behandeling het bestaan uit 10 herhalings met 5 larwes per blaar. Tellings om mortalitiet te bepaal is na 24 en 48 uur gedoen. Resultate het getoon dat die larwes van *C. pudica* redelik bestand is teenoor die meeste plaagdoders wat getoets is. Daarinteen was drie van die middels, naamlik, Dursban WG teen 64 g/hl, Meothrin EC teen 50 ml/hl en Mesurol WP teen 10 g/hl met 200 g/hl suiker, wel toksies en het ’n 80-100 % mortaliteit getoon.

Introduction

Lacewings are generalist predators and so can be beneficial in citrus orchards by preying on many different pests. Very little is known about our local lacewing species, except that they are sporadically active in orchards and sometimes seem to have a major effect on certain pests. Some basic knowledge of their susceptibility to pesticides regularly used in citrus orchards could provide insights into their occurrence. For this reason a rearing technique was developed for one of the most common green lacewings, and its susceptibility to several commonly used pesticides was determined.

Materials and methods

A colony of *Chrysoperla pudica* was started from wild adult lacewings that were collected in Nelspruit, Mpumalanga in April 2005. Adults were fed a 40:60 mixture of yeast hydrolysate powder and honey. A water container with a dental roll wick provided adequate water per box of approximately 100 adults. Initially moth eggs (*Ephestia* sp.) were used as food for the larvae. Later it was discovered that fruit fly eggs also provided good nutrition and these were provided at least every second day while *Ephestia* eggs were given once a week.

Plastic boxes 270 x 270 x 150 mm were used for rearing both adults and larvae. In the side of each box was a circular, gauze door, 100 mm diameter. Adults were moved to a fresh box each day for egg laying by connecting the new and old boxes via a 100 mm diameter PVC tube. The old box was covered with a black cloth and the adults moved from the darkened box into the new box. The old box containing freshly laid eggs

was then provided with larval food and shredded paper as a substrate. About 10 days after emergence from eggs most of the larvae in a box had formed pupae, which were moved to an adult emergence box with a glass lid. Once the adults had emerged they were fed in this box for several days while they matured and were then transferred to an adult box. The purpose of this rearing method was the production of larvae of uniform age and size for testing. The two-day-old larvae used were 2 to 2.5 mm long, active and less inclined to cannibalism than larger larvae. They were therefore considered the most suitable life stage for use in bioassays.

Test Procedure: Each product tested (Table 3.5.3.1) was applied to three rough lemon seedlings in pots using a hand held sprayer. Treatments normally used as baits were applied as diffuse droplets; all other treatments were applied to the point of runoff. Suitable leaves were picked 24 h after spraying and control leaves were picked from untreated seedlings. Leaves were placed in test cells (Fig. 3.5.3.1) and 5 two-day-old larvae were placed into each cell. Small drops of fruit fly eggs were provided as food. Cells were placed on a plenum that provided fresh air to each cell. Assessments were made after 24 and 48 hours of exposure. Percentage mortality for each material tested was corrected for natural mortality using Abbott's (1925) formula.

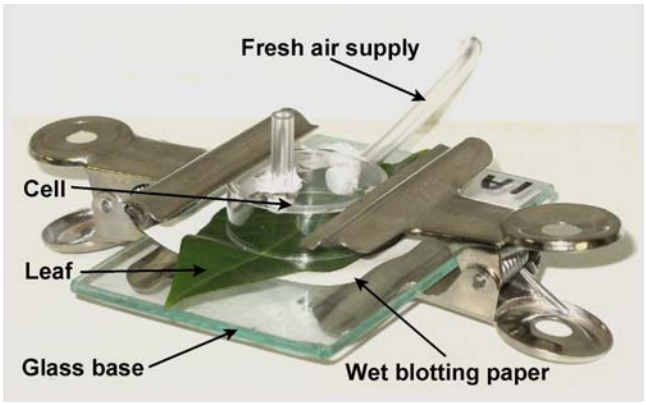

Fig. 3.5.3.1. Test cell with 34 mm diameter Petri-dish enclosure.

Results and discussion

The adults of *C. pudica* were found to live at least 3 months (90 days). At 23ºC the eggs took 4 days to hatch and the larvae started forming pupae 9 days later. The pupal stage lasted about 12 days and once emerged, adult females took about 7 days to start laying eggs. This gives an "egg to egg" lifecycle time of 32 days at 23ºC.

The bioassay results (Table 3.5.3.1) show *C. pudica* larvae to be relatively tolerant to most materials commonly used on citrus. Only three of the materials tested were very harmful. The result with Mesurol was similar to that found with predatory mites but very different to its impact on *Chilocorus* and *Aphytis.* The toxicity of organophosphates varied from no effect (mevinphos) to 100% mortality (Dursban). The toxicity of Meothrin was similar to that found previously with parasitoids but Regent and Hunter were less toxic than expected. These results suggest that most of the products used for thrips control should have little or no effect on lacewing populations.

Table 3.5.3.1. Products used, dosage rates and mortality after 24 and 28 hours exposure

Product (common name)	Dosage rate/hl	Corrected mortality (%)		Hazard category
		24 hr	48 hr	
Abamectin + Orchex	20 ml + 300 ml	0.0	1.7	1
Dursban (chlorpyrifos) WG	64 g	100	100	4
Endosulfan	113 g	3.6	3.5	1
Erador (pyrethrum & neem)	75 ml	0.0	0.0	1

Product (common name)	Dosage rate/hl	Corrected mortality (%)		Hazard category
		24 hr	48 hr	
Hunter (chlorfenapyr)	30 ml	20.4	41.2	2
Lannate (methomyl)	20 g	6.0	10.2	1
Mancozeb	200 g	0.0	0.0	1
Meothrin (fenpropathrin)	50 ml	94.1	100	4
Mesurol + Sugar (methiocarb) BAIT	10 g + 200 g	76.8	84.1	4
Mevinphos SL	30 ml	0.0	0.0	1
Orchex	1.25%	15.1	13.4	1
Regent (fipronil)	10 ml	15.2	25.7	2
Tartar emetic + Sugar BAIT	200 g + 200 g	3.9	5.9	1
Torque (fenbutatin-oxide)	55 ml	1.9	5.9	1
Tracer (spinosad) + Orchex	15 ml + 300 ml	1.8	5.3	1
Ultracide (methidathion)	100 ml	11.1	23.0	1

Hazard (Impact) category: 1 = harmless. 2 = Slightly harmful. 3 = harmful. 4 = very harmful. Based on 48 hour count.

Conclusion

A successful rearing technique for *C. pudica* was developed which could be adapted and used in the future for other lacewing species. Bioassays showed that the residual toxicities of pesticides to this green lacewing were very different to those known for parasitoids. When there is a desire to conserve lacewings populations that are active in orchards, only the use of materials categorised as harmless should be considered.

Future research

No further research is planned on this natural enemy.

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3.5.4 Development of ant baits and the use of bait stations

Experiment 857 by Tim Grout and Bruce Tate (CRI)

Opsomming

Sitrus produsente sal in die toekoms meer moet vertrou op die natuurlike beheer van witluise. Dit sal beteken dat miere baie goed beheer sal moet word. Min produkte is tans beskikbaar vir die beheer van miere en die aanbring van stamversperrings is ook baie arbeidsintensief. Navorsing word dus gedoen op lokmiddels vir miere. Voedselsoorte wat proteïene bevat was baie meer aanloklik vir die bruin huismier as lokmiddels met 'n suikerbasis. Beide tipes was egter geskik vir die malmier. Verdere navorsing word beplan met 'n lokmiddel wat sal bestaan uit 'n mengsel van proteïene, suiker en plantolies.

Introduction

Mealybugs are largely controlled by organophosphate sprays but the reduced number of available products in the future may compel growers to depend more on biocontrol for mealybug. Cost effective ant control forms the basis for any biocontrol programme. The disruptive effects of ants in citrus orchards and the outbreak of other secondary pests such as red scale are well known, resulting in the use of costly corrective treatments. The proper use of trunk barriers and repellents requires considerable management of aspects such as skirt-pruning, weed control and frequent re-application and rejuvenation of these barriers.

Materials and methods

Two baits with potential for the most common ant pests in citrus orchards, the pugnacious ant, *Anoplolepis custodiens*, and the brown house ant, *Pheidole megacephala*, were tested. These baits are the sugar based gel "TG" formulated by Tim Grout as a bait for pugnacious ants and "DB", a bait comprised of equal

quantities of syrup, fish paste and peanut butter, formulated by Deon Begemann, which is used for both ant species.

For both ants, the baits were presented in 40 mm diameter plastic Petri dish lids but to make counting the smaller *Pheidole* ants easier, the Petri dishes were glued onto 15 cm long 7 mm diameter wooden dowels, these were stuck into the ground allowing about 10 cm to protrude. Ant movement up the dowel was easily followed. For pugnacious ants, the Petri dish lids were left on the ground as they seemed reluctant to climb the dowels to investigate the bait.

Seven replicates of each treatment were used and the number of ants moving up the dowel or visiting the Petri dishes for 60 s was counted after the bait had been placed and sufficient time (30-60 min) had lapsed to allow the ants to discover the baits. In some cases more favoured baits were completely consumed before the counts were completed. Some components of the diets were changed to see if cheaper alternatives to syrup and fish paste could be used in the DB diet. To this end the syrup was substituted with the TG sugar gel, and the fish paste with a cheap sardine-based cat food. Various combinations were tested both with and without the addition of peanut butter, fish paste or syrup. No toxicants were added to the baits at this stage.

Two sites were used between November 2006 and January 2007. An Empress mandarin orchard at Hilltop near Nelspruit, with all trees having at least one *Pheidole* nest at the base of the trunk, and a Valencia orchard at Burgersfort, with a large number of active pugnacious ant nests. A few pugnacious ant nests were located later at Hilltop.

Baits and their ingredients were given abbreviated names, i.e.

Deon Begemann bait mix	-	DB
Tim Grout sugar gel	-	TG
Golden Syrup	-	SY
Peanut Butter	-	PNB
Fish Paste	-	FP
Sardine-based cat food	-	SCF

Results and discussion

Pheidole megacephala results (Tables 3.5.4.1 and 3.5.4.3) indicate that these ants prefer baits with a protein component of either FP or SCF and a product high in vegetable oil content such as PNB, the sugar-based sweeteners such as SY or TG are not essential ingredients for these baits. The PNB, SCF and TG bait combination remains attractive up to 36 days for *Pheidole megacephala*. After first exposure, these baits were not exposed to the weather but were kept indoors and were hard and dry.

With the pugnacious ants, the baits consisting of protein only (SCF and FP) were attractive (Tables 3.5.4.2 and 3.5.4.4) but impractical for use alone due to them being perishable and possibly being eaten by wildlife. Peanut butter combinations have the added benefit of long shelf-life and are attractive to *Pheidole megacephala.* The sugar based baits are suitable for pugnacious ants only but the addition of sugar would also enhance the shelf-life of other baits. The TG gel is a cheaper alternative to syrup.

Conclusion

During the summer months the brown house ants were not attracted to sugar-based baits and baits containing fish protein were the most effective. Proteinaceous baits were also attractive to the pugnacious ants but sugar baits were sometimes equally attractive. The PNB, SCF, TG bait could be a suitable bait for both of these pest ants.

Future research

Further research will be conducted with these baits and the use of bait stations for these and commercial baits will be evaluated.

Table 3.5.4.1. Tests with *Pheidole megacephala* at Hilltop

BAIT STATION NO.	NUMBER OF ANTS CLIMBING UP DOWEL TO VISIT BAITS IN 1 MINUTE																		
	Trial 1 30/11/06		Trial 2 01/12/06		Trial 3 08/12/06		Trial 4 21/12/06 7 day-old		Trial 5 03/01/2007							Trial 6 18/01/07	Trial 7 19/01/07		
									21 day-old	21 day-old								36 day-old	
	TG	DB	TG	DB	TG	DB	TG	DB	DB	TG	TG PNB	TG FP	PNB SY	PNB FP	FP SY	SCF PNB SY	SCF PNB TG	TG	DB
1	1	23	1	4	0	0	0	7	0	0	0	5	0	10	1	39	14	0	46
2	5	22	0	3	0	0	1	7	9	0	0	0	0	0	5	130	42	0	25
3	0	18	0	33	1	24	0	13	41	0	2	0	3	26	0	68	50	0	0
4	2	20	0	8	0	4	0	26	0	0	3	2	0	14	7	72	21	0	0
5	1	9	0	20	0	0	0	11	0	0	1	10	8	0	0	79	49	0	30
6	1	11	0	1	0	0	0	14	2	0	0	2	1	11	3	49	53		
7	0	13	0	0	0	0	0	5			0	4	0	15	2	68	69		
TOTALS	**10**	**116**	**1**	**69**	**1**	**28**	**1**	**83**	**52**	**0**	**6**	**23**	**12**	**76**	**18**	**505**	**298**	**0**	**101**

Table 3.5.4.2. Tests with pugnacious ants at Burgersfort and Hilltop

BAIT STATION NO.	NUMBER OF ANTS VISITING BAITS IN 1 MINUTE																	
	Trial 1						Trial 2					Trial 3			Trial 4		
	BURGERSFORT						BURGERSFORT					HILLTOP			HILLTOP		
	14/12/06		14/12/06 On stick		14/12/06 In shade		04/01/07					18/01/07			19/01/07		
	TG	DB	TG	DB	TG	DB	TG	FP	FP SY	FP TG	PNB FP	PNB SY SCF	SCF PNB	SCF	SCF PNB TG	SCF PNB	TG
1	1	5	0	0	0	0	2	9	2	1	3	6	6	12	0	0	0
2	8	1	0	0	0	0	0	9	1	0	3	4	11	21	9	5	8
3	4	8	0	1	0	0	0	5	2	0	1	0	12	16	1	1	0
4	2	5	0	0	0	0	0	0	0	2	0	7	5	17	10	5	6
5	4	0	0	0	0	0	0	0	0	0	0	9	6	14	0	0	13
6	4	0	0	0	0	0	0	0	0	0	0	2	15	15	0	0	12
7							0	3	0	0	0	4	4	16	0	0	0
8							0	0	0	2	0						
TOTALS	**23**	**19**	**0**	**1**	**0**	**0**	**2**	**26**	**5**	**5**	**7**	**32**	**59**	**111**	**20**	**11**	**39**

Table 3.5.4.3. Numbers of *Pheidole megacephala* visiting baits per minute at Hilltop.

TRIAL NO.	DATE	BAITS								
		TG	DB	PNB SY	PNB TG	PNB FP	FP SY	FP TG	SCF PNB SY	SCF PNB TG
1	30/11/06	10	116							
2	01/12/06	1	69							
3	08/12/06	1	28							
4	21/12/06 (7 day old 7 reps)	1	83							
5	03/01/07 (21 day old 6 reps)	0	52	2	6	76	18	23		
6	18/01/07								505	
7	19/01/07 (36 day old 5 reps)	0	101							298
TOTALS		**13**	**449**	**2**	**6**	**76**	**18**	**23**	**505**	**298**

Table 3.5.4.4. Numbers of pugnacious ants visiting baits per minute: Trial 1 and 2 Burgersfort; 3 and 4 Hilltop.

TRIAL NO.	DATE	BAITS									
		TG	DB	PNB FP	FP TG	FP SY	FP	PNB SCF SY	SCF PNB	PNB	SCF
1A	14/12/06 (on ground)	23	19								
1B	14/12/06 (raised)	0	1								
1C	14/12/06 (shade)	0	0								
2	04/01/07 (8 reps)	2		7	5	5	26				
3	18/01/07							32	59		111
4	19/01/07	39							11	20	
TOTALS		**64**	**20**	**7**	**5**	**5**	**26**	**32**	**70**	**20**	**111**

3.6 PROJECT: MEALYBUG AND OTHER PHYTOSANITARY PESTS

Project coordinator: Sean Moore (CRI)

3.6.1 Project summary

Five experiments were conducted within this project during 2006. In the first, live trapping was conducted for parasitoids of oleander mealybug (3.6.2). The following species were identified as parasitoids of oleander mealybug: *Leptomastix* sp., *Coccidoxenoides perminutus*, *Leptomastix thukumiensis*, *Anagyrus* sp. and *Allotropa* sp. *Leptomastix* sp. is being reared. In the second experiment, mealybug surveys were conducted in citrus orchards in different regions of the country (3.6.3). It was shown that citrus mealybug was dominant in most of the orchards surveyed throughout the country. In the third experiment, five different trap types were tested with *Planococcus citri* pheromones in mealybug-infested orchards (3.6.4). Small yellow delta traps caught higher numbers of mealybug males than did any of the other trap types. In the fourth experiment, post-harvest dip treatments were conducted for control of the grain chinch bug (GCB) (3.6.5). A natural pyrethrum treatment, Xterminator®, caused 100% mortality of GCB and is now registered as a postharvest treatment on citrus. In the fifth and final experiment, Break-Thru was compared with Agral 90 as a wetter with corrective mealybug treatments (3.6.6). Treatments were equally effective with both wetters.

Projekopsomming

Vyf eksperimente is onder hierdie projek in 2006 uitgevoer. In die eerste een is parasiete van oleanderwitluis gelok en lewendig gevang (3.6.2). Die volgende spesies is as parasiete van oleanderwitluis gekry: *Leptomastix* sp., *Coccidoxenoides perminutus*, *Leptomastix thukumiensis*, *Anagyrus* sp. en *Allotropa* sp. *Leptomastix* sp. word geteel. In die tweede eksperiment is witluis opnames in sitrus boorde in verskillende streke van die land uitgevoer (3.6.3). Dit is gewys dat sitruswitluis die dominante spesie in meeste boorde deur die land is. In die derde eksperiment is vyf verskillende lokval tiepes getoets met *Planococcus citri* feromone in witluis besmette boorde (3.6.4). Klein geel delta lokvalle het hoër getalle witluis mannetjies as die ander lokval tiepes gevang. In die vierde eksperiment is na-oes doop behandelings uitgevoer vir die beheer van die graanstinkbesie (GSB) (3.6.5). 'n Natuurlike piretrum behandeling,

Xterminator®, het 100% mortaliteit van GSB veroorsaak en is nou as 'n na-oes behandeling op sitrus geregistreer. In die vyfde en finale eksperiment is Break-Thru met Agral 90 vergelyk as 'n benatter met korrektiewe witluis behandelings (3.6.6). Behandelings met albei benatters is ewer doeltreffend.

3.6.2 **Investigating biocontrol agents of mealybug species other than citrus mealybug**
Experiment 692 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

Die plaagstatus en derhalwe die belangrikheid van *Paracoccus burnerae* op sitrus, het in Suid-Afrika verhoog, veral in die Oos-Kaap. Belangrike en doeltreffende parasitoïede van 'n paar witluisspesies wat op sitrus voorkom, is geïdentifiseer. Soortgelyke inligting word egter nog vir *P. burnerae* benodig. 'n Proef is uitgevoer om sulke inligting te versamel.

Deur om oleander witluis besmette sitrus saailinge in witluis besmette boorde te plaas vir twee weke op 'n slag is parasiete gelok om die witluis op die saailinge aan te val. In uitbroeingskaste in die laboratorium is die volgende spesies as parasiete van oleander witluis geïdentifiseer: *Leptomastix* sp., *Coccidoxenoides perminutus*, *Leptomastix thukumiensis*, *Anagyrus* sp. en *Allotropa* sp. Die laaste drie spesies is nie voorheen in hierdie studie as parasiete van oleander witluis aangetoon nie. *Leptomastix* sp. parasiete wat van witluis op die saailinge uitgebroei het is gevang en aan nog oleander witluis op aardappel saailinge blootgestel. Dit is gedoen met die doel om 'n laboratorium kultuur van *Leptomastix* sp. te stig.

Hierdie eksperiment word voortgesit. Teel tegnieke vir *Leptomastix* spp. sal ontwikkel word met die doel om aanvullende loslatings vir oleander witluis beheer uit te voer. As gevolg van onverwagte en oorheersende verpligtinge op ander projekte is vordering hiermee vertraag.

Introduction

Citrus mealybug, *Planococcus citri*, is known to be effectively controlled by natural enemies. This control has been substantially enhanced with the development of the augmentation technique for the parasitoid *Coccidoxenoides perminutus* (*peregrinus*). However, it has been shown that the oleander mealybug, *Paracoccus burnerae*, is approximately 100 times less suitable a host for *C. perminutus* than is *P. citri* (Hattingh & Tate, 1997). What makes this a serious situation justifying further investigation is that *P. burnerae* is regarded by certain important markets e.g. USA and Korea (and potentially many others) as being a phytosanitary pest; and that *P. burnerae* has increased in dominance in citrus orchards, at least in the Eastern Cape. Important and effective natural enemies of *P. burnerae*, and other important mealybug species, should be identified. Ultimately, the objective should be to establish an augmentation technique with natural enemies effective against these "other" species of mealybug. Recently, Wakgari & Gilliomee (2002) qualified and quantified the species of parasitoids in the natural enemy complexes attacking citrophilous mealybug, *Pseudococcus calceolariae*, longtailed mealybug, *Pseudococcus longispinus*, and *P. citri*. However, this has not been done for *P. burnerae.*

Materials and methods

An oleander mealybug culture was initially maintained on potted citrus seedlings in a temperature controlled glass house. Due to various problems, the culture collapsed. Subsequently, a replacement culture of oleander mealybug was obtained from Welma Pieterse, of the National Department of Agriculture in Stellenbosch and reared on sprouting potatoes. The culture was then transferred onto young citrus seedlings and kept in the CRI laboratories in Port Elizabeth, under Growlux lights and at a constant temperature of 27°C. Periodically, new seedlings were introduced and mealybug was transferred onto these.

In November and December mealybug infested trees were placed into each of two orchards in which it was known there were meaningful levels of mealybug infestation. These were a Delta Valencia orchard on the farm of Steve Nichols in Sundays River Valley, and a Palmer navel orange orchard on Tierhok Farm in the Gamtoos River Valley. Trees were collected after two weeks and returned to the laboratory. A twig, infested with approximately 100 mealybug of different life stages, was removed from each tree and placed into a emergence box, after recording the mealybug life stages. The remainder of each tree was placed in a large emergence box.

Parasitoids emerging from the enumerated mealybug, infesting the twigs, were stored in 70% alcohol, counted and sent to the Biosystematics Unit of the Plant Protection Research Institute (Agricultural Research Council) for identification.

Leptomastix sp. parasitoids, which emerged from mealybug infesting the trees in the large emergence boxes, were caught and exposed to more oleander mealybug on potato seedlings. This was done in an attempt to establish a laboratory culture of the *Leptomastix* sp.

Results and discussion

Numerous problems were experienced with the oleander mealybug culture, which was maintained on potted citrus seedlings in the temperature controlled glass house. These included a faulty temperature controller, a faulty irrigation timer, and contamination of seedlings with citrus mealybug (*P. citri*). Consequently, it was not possible to place mealybug infested seedlings (for baiting parasitoids) into orchards during the first half of 2006.

During July 2006, a new oleander mealybug culture was established under Growlux lights. Parasitoids were recovered from mealybug placed into both orchards, during both November and December. Samples of parasitoids recovered during November were identified (Table 3.6.2.1). Identifications of parasitoids collected during December are still pending.

Table 3.6.2.1. Parasitoids emerging from oleander mealybug individuals placed in orchards for two weeks during each of November and December 2006

Farm (area)	Dates trap tree was in orchard	Mealybug life stages recorded on twig placed into emergence box				Parasitoids emerged*					
		Egg sacs	Crawl-ers	Pre-adults	Adults	Total	*Leptomastix* sp.	*Leptomastix thukumiensis*	*Coccido-xenoides perminutus*	*Anagyrus* sp.	*Allotropa* sp.
Steve Nicholls (SRV)	8-23/11	3	62	219	21	97	4		X	-	X
	13-27/12	2	27	56	8	?	-	-	-	-	-
Tierhok (GRV)	7-22/11	12	98	42	9	11	-	-	-	11	-
	26/12-9/1	1	81	23	2	?	-	-	-	-	-

*X indicates that individuals of these species were identified but that total numbers were not established.

Five species of parasitoids were identified from oleander mealybug from the November surveys (Table 3.6.2.1). Three species were identified for the first time during the course of this study, namely *Leptomastix thukumiensis*, *Anagyrus* sp. and *Allotropa* sp. Previous work reported by Prinsloo (1984) and Hattingh *et al*. (1998) lists the occurrence of various species of parasitoids and the relative dominance of *Anagyrus* sp. (probably *pseudococi*) and *Coccidoxenoides perminutus* in the complex. However, it is acknowledged that there is insufficient information on which parasitoids species attack which mealybug species, of which there are seven occurring on citrus (Hattingh & Moore, 2003). Of the five species of parasitoids recorded in this study, *C. perminutus* and *Anagyrus* sp. have been previously reported (Prinsloo, 1984). *Allotropa* sp. could be *Allotropa kamburovi* (Gerhard Prinsloo, personal communication). Both *Leptomastix* sp. (which is not *Leptomastix dactylopii*) and L. *thukumiensis* appear to be new recordings.

By the end of this period of the study (January to December 2006), most of the parasitoids baited during December had emerged and been collected. However, parasitoids counts and species identifications had not yet been conducted.

Leptomastix sp. parasitoids, which emerged from mealybug infesting the trees in the large emergence boxes, were caught and exposed to more oleander mealybug on potato seedlings. This was done in an attempt to establish a laboratory culture of the *Leptomastix* sp., as this was shown to be one of the important parasitoid of Oleander mealybug in previous seasons (Moore & Kirkman, 2003; 2005). Subsequent to initiating this culture, it was discovered that there are at least two *Leptomastix* species, namely *L. thukumiensis* and an unidentified *Leptomastix* species. It is therefore highly likely that individuals from both species have been placed into the parasitoids rearing jars. Parasitoid samples will be submitted to the PPRI Biosystematics Unit for identification to species. To date, several second generation parasitoids have been produced and attempts are being made to develop suitable rearing techniques.

Conclusion

Five species were identified as parasitoids of oleander mealybug: *Leptomastix* sp., *Coccidoxenoides perminutus*, *Leptomastix thukumiensis*, *Anagyrus* sp. and *Allotropa* sp. The last three species had not previously been recorded as parasitoids of oleander mealybug in this study. *Leptomastix* sp. parasitoids, which emerged from mealybug infesting the trees in the large emergence boxes, were caught and exposed to more oleander mealybug on potato seedlings in an attempt to establish a laboratory culture of *Leptomastix* sp.

Future research

This experiment is ongoing. Rearing techniques for *Leptomastix* spp. will be developed with the objective of conducting augmentative releases for oleander mealybug control. Due to excessive and unscheduled commitments on other projects, progress on this objective has been delayed.

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3.6.3 A survey of the mealybug species complex on citrus throughout South Africa

Experiment 792 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

Geduurende die 1990s, is die sitruswitluis, *Planococcus citri*, ongetwyfeld die dominante witluis spesie op sitrus in Suid Afrika. In 2003 en 2004 is dit gewys dat oleander witluis, *Paracoccus burnerae*, die dominante witluis spesie op sitrus in die Oos-Kaap geword het. Hierdie studie is aan die gang gesit om te bepaal of hierdie tendens ook elders in die land nou voorkom en om te bepaal of díe tendens in die Oos-Kaap voortegduur het. Van Januarie tot Maart 2006 is witluis opnames in nege sitrus boorde buite die Oos-Kaap uitgevoer. In teenstelling met resultate van die 2005 opname is sitruswitluis nou as die dominante witluis spesie in boorde in die Wes-Kaap aangetoon. Sitruswitluis is as die dominante witluis spesie op sitrus in die Malelane streek van Mpumalanga gekry. ’n Mengsel van spesies is opgemerk in die Loskop Vallei van Mpumalanga vir twee agtereenvolgende jare. Geduurende Desember 2006 is ’n opname van boorde in die Oos-Kaap wat voorheen ondersoek is beplan. Dit was om te bepaal of oleanderwitluis nog die dominante spesie is. Witluis besmetting kon net in twee van die 17 boorde gekry word. Al is oleanderwitluis in albei van die boorde nog teenwoordig, is sitruswiltuis weereens as die dominante spesie opgemerk. Geen verdere werk word op hierdie eksperiment beplan nie.

Introduction

During the 1990s, when research was being conducted on augmentation of *Coccidoxenoides perminutus* parasitoids, the citrus mealybug, *Planococcus citri*, was undeniably the dominant mealybug species on citrus throughout South Africa (Hattingh *et al*., 1998). It has recently been demonstrated that oleander mealybug, *Paracoccus burnerae*, has become the dominant mealybug species on citrus in the Eastern Cape (Moore & Kirkman, 2003). There are two factors which make this a serious situation:

1. *P. burnerae* is regarded by certain important markets e.g. USA and Korea (and potentially many others) as being a phytosanitary pest (Hattingh & Moore, 2003).
2. *P. burnerae*, is approximately 100 times less suitable a host for *C. perminutus* than is *P. citri* (Hattingh & Tate, 1997), making augmentation of *C. perminutus* unsuitable for *P. burnerae* control.

Consequently, a study has been initiated to investigate the parasitoid complex of *P. burnerae* (experiment 692; Moore & Kirkman, 2003 & 2004). What is also important is to determine whether the trend recorded in the Eastern Cape, of *P. burnerae* becoming the dominant mealybug species, has occurred elsewhere in the country too. This experiment proposes to determine this. It is also endeavoured to determine whether the change in mealybug species dominance in the Eastern Cape remains or reverts.

Materials and methods

Results of a mealybug survey conducted in six Malelane orchards during 2005 was previously reported (Moore & Kirkman, 2005). However, final identifications from the Biosystematics Unit of the Plant Protection Research Institute (PPRI) (of the Agricultural Research Council (ARC)) in Pretoria, had not yet been received. The final results of these identifications are reported here.

Samples of mealybug-infested fruit (approximately 20 fruit per orchard) were collected from nine orchards in three different production regions of South Africa, from January to March 2006. The three areas were the Citrusdal region of the Western Cape, the Swellendam region of the Western Cape and the Marble Hall (Loskop Valley) of Mpumalanga. Samples were microscopically inspected to identify as many specimens within each sample, up to species level. In some instances, where uncertainty existed, individuals from samples were preserved in glycerol and glacial acetic acid and sent either to the Biosystematics Unit of the Plant Protection Research Institute (PPRI) (of the Agricultural Research Council (ARC)) in Pretoria or to Welma Pieterse of the Department of Agriculture in Stellenbosch, for identification.

A survey of mealybug species was conducted during January 2003 in 14 orchards in the Gamtoos River Valley (Moore & Kirkman, 2003). Another survey of mealybug species was conducted during February 2004 in three orchards in Sunday River Valley, which had been surveyed about eight years previously (Hattingh *et al*., 1997; 1998). These orchards were again surveyed in December 2006, when mealybug was expected to be approaching a peak in level of infestation. This was done in order to determine whether there was any change in the mealybug species complex over time.

Results and discussion

Citrus mealybug was the dominant species in five out of the six orchards monitored in Mpumalanga in 2005 (Table 3.6.3.1).

Table 3.6.3.1. Mealybug species occurring on citrus fruit collected during December 2005 from orchards in Mpumalanga

Area	Farmer (or Farm)	Cultivar	Individuals of each species out of total (%)*		
			Oleander mealybug	**Citrus mealybug**	**Karoo thorn mealybug**
Loskop Valley	Schoeman	Navel oranges	**100**	0	0
	Oudestad	Navel oranges	25.9	**74.1**	0
Malelane	Vergenoeg (TSB)	Star Ruby grapefruit	0	**100**	0
	Vergenoeg (TSB)	Marsh grapefruit	0	**100**	0
	Vergenoeg (TSB)	Rosé grapefruit	0	**100**	0
	Vergenoeg (TSB)	Marsh grapefruit	0	**100**	0

*Percentage for dominant species in each orchard is in bold.

The mealybug survey conducted in January and February 2006 in the Western Cape revealed that citrus mealybug was the dominant species in all orchards in which it was possible to identify the species (Table 3.6.3.2). This is good news, considering the high phytosanitary status of oleander mealybug for the USA market, which is supplied mainly from the Western Cape. This also contradicted findings from a survey conducted during 2005, in which it was shown that oleander mealybug was the dominant mealybug species in Western Cape orchards (Moore & Kirkman, 2005). Only two orchards were surveyed in the Loskop Valley of Mpumalanga: one was infested with citrus mealybug and the other with oleander mealybug (Table

3.6.3.2). The previous survey conducted also showed a mixture of species in the Loskop Valley (Table 3.6.3.1). However, in the Malelane region of Mpumalanga, citrus mealybug was the dominant species.

Table 3.6.3.2. Mealybug species occurring on citrus fruit collected from January to March 2006 from orchards in different parts of South Africa.

Area	Farm	Orch. no.	Cultivar	Mealybug species (% of total)		
				Citrus	Oleander	Unidentified
W. Cape: Citrusdal	Groenkloof	3	Washington navels	**90**	10	0
		5	Washington navels	0	0	100
		9	Washington navels	**20**	0	80
		11	Washington navels	0	0	100
		15	Midknights	**80**	20	0
		16	Washington navels	**100**	0	0
W. Cape: Swellendam	Neethling	?	Various	**100**	0	0
Mpumalanga: Marble Hall	Schoeman Boerdery	?	Navels	0	**100**	0
		?	Navels	**100**	0	0

? Orchard numbers are not available for the last three orchards listed.

A survey of mealybug species which was conducted during January 2003 in 14 orchards in the Gamtoos River Valley revealed that oleander mealybug was the dominant species in 78.6% of the orchards and that 68.6% of individuals, which were identifiable, were recorded as oleander mealybug (Moore & Kirkman, 2003). Citrus mealybug was the dominant species in only 7.1% of orchards and long-tailed mealybug was dominant in 14.3% of orchards.

Another survey of mealybug species, which was conducted during February 2004 in three orchards in Sunday River Valley showed that oleander mealybug was the dominant species in all three of the orchards inspected (Table 3.6.4.3). Surveys conducted in the same orchards eight years previously, revealed that citrus mealybug, *Planococcus citri*, was the dominant species in all orchards (Hattingh *et al*., 1997; 1998).

Table 3.6.3.3. Mealybug species occurring on citrus fruit collected during February 2004 from orchards in Sundays River Valley.

Farm	Orchard no.	Cultivar	Mealybug species (% of total identified)		
			Citrus	Oleander	Long-tailed
Mfuleni	54	Lemons	6.2	**93.8**	0
	8	Robyn navel oranges	33.3	**67.7**	0
Sun Orange	C11	Robyn navel oranges	33.3	**58.3**	8.4

The orchards surveyed in the Gamtoos River Valley during 2003 and in the Sundays River Valley during 2004, were again inspected in December 2006, when mealybug was expected to be approaching a peak in level of infestation. Unfortunately, mealybug infestation could only be found in two of these orchards. These were two orchards of Palmer navel oranges on Tierhok Farm: orchards 1 and 8. It was found that oleander mealybug was no longer dominant. Although oleander mealybug was conspicuously present, citrus mealybug had again become the dominant species (Table 3.6.4.4).

Table 3.6.3.4. Mealybug species occurring on Palmer navel oranges on Tierhok Farm, Gamtoos River Valley, in 2003 (surveyed 22-29 January) and 2006 (surveyed 16 January).

Orch. no.	Year	Mealybug species (% of total identified)		
		Citrus	**Oleander**	**Longtailed**
1	2004	0	**100**	0
	2006	**93.2**	6.8	0
8	2004	28.6	**57.1**	14.3
	2006	**92.1**	1.4	6.5

Conclusion

Citrus mealybug was found to be the dominant mealybug species in citrus orchards in the Western Cape and Malelane (Mpumlanaga). A mixture of species was recorded for two consecutive years in the Loskop Valley of Mpumalanga. Mealybug surveys conducted in the Eastern Cape during 1997 and 1998 revealed that citrus mealybug was the dominant species. In 2003 and 2004, oleander mealybug had become the dominant species. A survey conducted in 2006 in two of these orchards showed that citrus mealybug was again dominant.

Future research

No further work is planned on this experiment.

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3.6.4 Evaluation of *Planococcus citri* pheromone traps for monitoring infestation levels

Experiment 845 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

Vyf verskillende tiepes lokvalle met *Planococcus citri* feromone is in witluis besmette boorde getoets. Die doel van die studie was om te bepaal of enige lokval tiepe geneig was om hoër getalle witluis mannetjies te vang as die ander lokvalle. Klein geel delta lokvalle het hoër getalle witluis mannetjies as die ander lokvalle gevang, alhoewel die verskil nie statisties betekenisvol was nie. Geduurende die 2006/07 seisoen sal proewe uitgevoer word om te kyk of witluis feromoon lokvalle gebruik kan word om visuele verkenning of te vervang of by te voeg. Dit sal bepaal word deur 'n ondersoek van die verhouding tussen lokval vangstes en vrugbesmetting.

Introduction

Citrus Research International was contracted by Insect Science to compare the sensitivity of five different trap types for monitoring of citrus mealybug, *Planococcus citri*, males with the Insect Science PheroLure product in citrus orchards. PheroLure is a dispenser impregnated with an analogue of the *P. citri* female

pheromone. The objective of the study was to determine if any one of the trap types facilitates the capture of higher numbers of mealybug males than the other traps. Further work may then be conducted with such a trap. This work will entail investigation of the usefulness of pheromone impregnated traps for accurate monitoring of citrus mealybug and hence decision making on the need for and timing of intervention (chemical or biological).

Materials and methods

Site

Two adjacent orchards on Sun Orange Farm in Sundays River Valley, Eastern Cape Province, were selected for the trial. Each orchard was approximately 1 ha in size. One was Valencia orange trees and the other was navel orange trees. Together they formed a single trial site.

Infestation and trap catches did not reach expectations and the trial was therefore terminated after two weeks. A similar trial was initiated at a new trial site on Tierhok Farm, Gamtoos River Vally, Eastern Cape Province. This was a large orchard of Midknight Valencia orange trees, with a conspicuous infestation of mealybug. Approximately 2 ha of the orchard were used for the trial.

Materials

The following trap types were compared:
1. Standard yellow delta trap
2. Small yellow delta trap
3. Small red delta trap
4. Small white delta trap
5. Plastic white scale card

Traps were furnished with PheroLure dispensers. Sticky floors were placed into delta traps. White card traps were smeared with Antbar polyurethane glue.

Layout

Six of each trap type were used. These were spaced regularly, but in random order, throughout each orchard. Traps were hung approximately 20 cm within tree canopies, approximately 1.8 m above the ground and on the northern side of trees.

Procedure

Each week, the card traps and the sticky floors in the delta traps were replaced and numbers of mealybug males on each were microscopically counted. Traps were rotated one position. Simultaneously, mealybug infestation of fruit was evaluated by inspecting 10 fruit on each of 10 trees positioned diagonally through the trial area.

Data analysis

Due to the low level of mealybug at the first trial site, none of the data was statistically analysed.

From the second trial site, mean numbers of mealybug males per trap per date were compared between trap types. This was done by using an ANOVA and the LSD multiple range test. The same procedure was used for comparing mean numbers per trap for each trap type, over the full trial period and for the first three weeks of the trial.

Results and discussion

Initially, great difficulty was experienced in identifying a suitable trial site. The original intention was to initiate the trial in October or November. However, due to low levels of mealybug, this was not possible. By reason of a history of high levels of mealybug infestation and signs of current infestation, two adjacent orchards on Sun Orange Farm were identified as a suitable trial site. After two weeks of evaluating trap catches and fruit infestation, it became clear that mealybug levels were insufficient (Table 3.4.6.1) to obtain meaningful results. The trial was therefore terminated at this site and the search for a more suitable trial site was resumed.

Table 3.6.4.1. Mean numbers of mealybug males per trap and mealybug infestation of fruit at Sun Orange Farm

Trap	Average number of mealybug males per trap		
	14 December	**21 December**	**28 December**
Large yellow delta	-	0.17	0.17
Small yellow delta	-	0	0.17
Small red delta	-	0	1.33
Small white delta	-	0	0.50
White card	-	0.33	0.33
Fruit infested with mealybug (%)	7	5	-

On 23 January, an appropriate trial site at Tierhok Farm was identified. Mealybug infestation of fruit was estimated at 56%. It was also confirmed that the majority of specimens observed were citrus mealybug, *P. citri*. Numbers of mealybug males caught on traps during the first three weeks, were considered to be high, averaging 19.48 individuals per trap per week. However, during the last three weeks of the trial, an average of only 2.36 individuals were recorded per trap per week. This was interpreted as an indication that the pheromone was being dispensed more weakly.

Mealybug infestation of fruit did not drop as drastically as did trap catches, from the first three weeks to the second three weeks. Infestation only dropped from an average of 48.7% fruit infested to an average of 30.7% fruit infested.

During only three of the six weeks of the trial, was there any statistical difference in mealybug catches between any of the trap types (Table 3.4.6.2). In the week ending 30 January, the small red delta trap caught the fewest individuals. The small yellow delta trap was the only trap which caught significantly more mealybug than did the red trap. During the week ending 14 February, the white card trap caught significantly more individuals than any other trap. During the following week (ending 20 February), the red delta trap was again the weakest, however, with only the large yellow delta trap catching significantly higher numbers.

Table 3.6.4.2. Mean numbers of mealybug males per trap and mealybug infestation of fruit at Tierhok Farm

Trap	Average number of mealybug males per trap*						
	23 Jan	30 Jan	5 Feb	14 Feb	20 Feb	27 Feb	6 Mar
Large yellow delta	-	26.50ab	25.00a	12.83b	6.33b	2.83a	0.17a
Small yellow delta	-	39.83b	27.00a	14.00b	4.17ab	2.00a	0.33a
Small red delta	-	10.50a	10.50a	12.00b	3.33a	1.67a	0.33a
Small white delta	-	15.00ab	17.83a	16.17b	4.00ab	2.67a	1.00a
White card	-	17.50ab	21.00a	26.50a	4.83ab	1.50a	1.00a
Fruit infested with mealybug (%)	56	50	40	34	29	29	24

*Values in the same column followed by the same letter are not significantly different (P>0.05; LSD multiple range test).

Over the full trial period at Sun Orange Farm – 23 January to 6 March – a higher average number of mealybug males were caught per small yellow delta trap than on any of the other trap types (Table 3). However, this was not statistically significant. It appeared that the pheromone dispensers were only strongly attractive for the first three weeks. Thereafter, catches (and therefore presumably attractiveness) declined dramatically. Consequently, mean numbers of mealybug males per trap were compared for only the first three weeks of the trial (Table 3.4.6.3). Again, the highest average numbers of mealybug males were caught on the small yellow delta traps. For this period, the small yellow delta trap was the only one which caught significantly more individuals than the weakest trap, the small red delta trap.

Table 3.6.4.3. Mean numbers of mealybug males per trap for the full trial period and the first three weeks of the trial at Tierhok Farm

Trap	Average mealybug males per trap	
	6 week trial period	**1st 3 weeks of trial**
Large yellow delta	12.28a	21.44ab
Small yellow delta	14.56a	26.94b
Small red delta	6.39a	11.00a
Small white delta	9.44a	16.33ab
White card	12.06a	21.67ab

*Values in the same column followed by the same letter are not significantly different (P>0.05; LSD multiple range test).

It was interesting to note that mean catches on the white card traps were not substantially or significantly lower than on the small yellow delta traps (Table 3.4.6.3). The white card traps had been previously used in similar trials and results were considered as disappointing (unpublished data). It was this finding which motivated the examination of the different delta trap types in this trial. However, differing results have been recorded with previous research. Zada *et al*. (2004) report that plate traps caught more *P. citri* males than did delta traps. Conversely, Millar *et al*. (2003) found that delta traps were more effective than double-sided sticky cards, for monitoring vine mealybug, *Planococcus ficus*. Walton *et al.* (2004) more specifically recommend the use of yellow delta traps for monitoring *P. ficus*. Zada *et al*. (2004) found that larger traps (both plate and delta shapes) caught more males than did smaller traps. This contradicts the finding in our trial, although the difference in catches between the large and small yellow delta traps was not significant.

The decline in trap catches over the trial period could perceivably be correlated with the declining infestation of fruit. However, this would most certainly be meaningless, as trap catches declined far more sharply than did infestation. It was therefore clear that the declining strength of the pheromone must have played a major role.

Conclusion

Further trials to compare the efficacy of the different trap types, might be useful in order to confirm the findings in this trial. If this is not done, it is recommended that any further work with *P. citri* trapping using pheromone traps, be done with small yellow delta traps. Not only did these traps catch the highest numbers of mealybug males, but they also tend to be more user friendly than the card traps, as their sticky surface is protected within the trap.

The logical follow on from this trial work will be to establish a relationship between trap catches and mealybug infestation in trees. If such a relationship exists, monitoring must be conducted at several sites over at least a couple of seasons. Pheromones should also be changed every third week, unless longer lasting dispensers can be developed.

Future research

During the 2006/07 season, trials will be conducted to investigate whether mealybug pheromone traps can be used to replace or supplement visual scouting for mealybug. This will be done by determining whether a relationship between trap catches and fruit infestation can be established.

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3.6.5 **Postharvest control of grain chinch bug (Heteroptera: Lygaeidae) on citrus using pyrethrum**
Experiment 871 by T. G. Grout and B. A. Tate (CRI)

Opsomming

Die graanstinkbesie (GSB), *Macchiademus diplopterus* (Distant), is nie bekend as 'n plaag wat skade op sitrus veroorsaak nie maar volwassendes kruip weg binne die nawel opening van nawel lemoene, *Citrus sinensis* L. Osbeck, wat na afkeurings van uitvoer vrugte lei. Biotoetse is met natuurlike piretrum (Xterminator® 2% SC) as 'n na-oes behandeling teen 5 ml/L water, gedoop vir 1 min en daarna afgespoel, gedoen. Díe behandeling het 98.1% mortaliteit van GSB veroorsaak. Die byvoeging van twee benatters aan die piretrum oplossing het nie die werking daarvan betekinsvol (P>0.05) verbeter nie. 'n Formulasie van Xterminator® met net 0.75% piretrum en meer plantolies bygoevoeg was net so doeltreffend as Xterminator® 2%, albei teen 5 ml/L. Albei formulasies het 100% mortaliteit veroorsaak nadat hulle 7 dae vroeër gemeng is. Dit was nodig om die 0.75% formulasie in oplossing na die 7 dae weer te meng. Xterminator® 0.75% is nou as 'n na-oes behandeling teen 5 ml/L water op sitrus in Suid-Afrika geregistreer.

Introduction

The grain chinch bug (GCB) *Macchiademus diplopterus* (Distant) is a little known lygaeid bug endemic to the Western Cape province of South Africa and is sometimes found as a contaminant of fruit destined for export to the USA. Due to concerns that this insect may become a cereal pest in the USA, infested consignments are rejected by US inspectors. Studies of the pest in and around pear orchards have shown that it moves from wheat fields when they are harvested in summer into orchards where it finds somewhere to hide (Addison, 2004). Cold disinfestation treatments are ineffective against this insect (Addison, 2005) and even hypobaric treatments at low temperature have not been efficacious (Tate, 2004). GCB does not damage citrus fruit but sometimes hides in the stylar opening of navel oranges, *Citrus sinensis* L. Osbeck (Figure 3.6.5.1). As the numbers of GCB found contaminating packed citrus are extremely low it was thought likely that an effective postharvest insecticide treatment would almost eliminate rejections. Natural pyrethrum was chosen for evaluation because it would not result in unacceptable residues and Dr Pia Addison at Stellenbosch University had conducted some preliminary trials that gave promising results.

Fig. 3.6.5.1. Grain chinch bug near the stylar opening of a navel orange.

Materials and methods

Laboratory experiments were conducted between March and June 2006 with GCBs acquired from behind *Eucalyptus* sp. bark on trees near Piketberg, Western Cape, South Africa. In all experiments, 5 GCBs were placed on a navel orange in a polyester gauze sleeve for 30 min to settle, then submerged for 1 min in the treatment solution (Figure 3.6.5.2) followed 15 s later by a dip in tap water (pH=7.2) for 30 s. Control insects were placed on fruit in sleeves in the same way and dipped in tap water for 1.5 min. After dipping, the bugs were left in the bag with the fruit to dry on a rack. Mortality was determined after 24 h. Eight replicates were used per treatment and three or four treatments were compared in each trial. The product Xterminator® (Agro-Organics, Somerset West, South Africa) containing 0.75% natural pyrethrum was registered in South Africa for the control of whiteflies (Aleyrodidae) on tomato at 500 ml/100 L water. Evaluations therefore centred on this dosage and also included the wetters WetCit® (Oro Agri SA, Somerset West, South Africa)

and Break-Thru S240® (Degussa Africa, Somerset West, South Africa). It was thought that the wetters might assist in reaching GCBs within the navel opening of the fruit. Two formulations of Xterminator® were provided by Agro-Organics containing 0.75% and 2% natural pyrethrum and denoted as Xterminator (0.75) and Xterminator (2), respectively.

Fig. 3.6.5.2. Infested fruit in gauze sleeve being dipped into a treatment mixture.

The bioassays conducted were as follows. Trial A: Water dip, WetCit 0.1%, Xterminator (2) 5 ml/L (0.01% a.i.). Trial B: Water dip, WetCit 0.25%, WetCit 0.5%, Xterminator (2) 5 ml/L. Trial C: Water dip, WetCit 0.1%, WetCit 0.1% plus Xterminator (2) 2.5 ml/L (0.005% a.i.), Xterminator (2) 2.5 ml/L. Trial D: Water dip, Break-Thru 0.1%, Break-Thru 0.1% plus Xterminator (2) 2.5 ml/L, Xterminator (2) 2.5 ml/L. Trial E: Water dip, Xterminator (0.75) 2.5 ml/L (0.001875% a.i.), Xterminator (2) 2.5 ml/L (0.005% a.i.). Trial F: Water dip, Xterminator (0.75) 5 ml/L (0.00375% a.i.), Xterminator (2) 5 ml/L (0.01% a.i.). Trial G: Water dip, Xterminator (2) 5 ml/L after the mixture in water stood for 0, 1, 2, or 3 days (d) indoors, Xterminator (2) 5 ml/L 3 d old followed by chlorine 0.02% a.i. in the rinse water. Trial H: Water dip, Xterminator (0.75) 5 ml/L 7 d old, Xterminator (2) 5 ml/L 7 d old.

A two-way analysis of variance was conducted on the data from each trial and when the F-ratio for treatments had a P-value of less than 0.05 the treatment means were compared further using Student-Newman-Keul's multiple comparison procedure. The software used was STATGRAPHICS PLUS ver. 5.1, (Manugistics, Inc., Rockville, Maryland, USA).

Results and discussion

By averaging results from all the trials, the mean percentage mortalities obtained were: dipping in tap water 23.4%, dipping in Xterminator (2) at 2.5 ml/L water 87.4% and at 5 ml/L 98.1%. The contribution to the mortality of the tap water dipping was not corrected for in the comparisons as citrus fruit goes through at least one water bath and usually high-pressure water jets as well, when being packed. The wetter WetCit® at 0.1% did not cause more than 30% mortality when used alone and did not increase mortality significantly ($P>0.05$) when combined with Xterminator (2) (Table 3.6.5.1). WetCit® alone at 0.5% only resulted in a mortality of 49%. Break-Thru S240® at 0.1% caused 57% mortality and slightly improved the efficacy of Xterminator (2), though not significantly ($P>0.05$) (Table 3.6.5.1).

Comparisons between the two Xterminator® formulations showed that Xterminator (0.75) caused significantly ($P<0.05$) less mortality (30%) at 2.5 ml/L but there was no significant difference between them at 5 ml/L (Table 3.6.5.2). Tap water mixtures containing Xterminator (2) did not show any loss of efficacy within 3 d and the addition of chlorine to rinse water did not reduce efficacy (Table 3.6.5.3). A comparison of both formulations after the diluted mixture stood for 7 d showed no loss of efficacy (Table 3.6.5.3), although the Xterminator (0.75) mixture had to be agitated because the plant oil component had floated to the surface.

These trials were not conducted on other citrus cultivars and possible effects on fruit quality or packhouse treatments were not evaluated. However, after further evaluations in packhouses by Agro-Organics, Xterminator® 0.75 SC was registered as a postharvest treatment on citrus in South Africa and will be effective in reducing the risk of rejection of packed citrus for export to the USA due to the presence of GCB.

Table 3.6.5.1. Percentage mortalities of grain chinch bug caused by Xterminator (2% pyrethrum) and two wetters after 24 hrs

Trial	Treatments (per litre)	Mortality (%)
A	Water dip	22.5 a
	WetCit 1 ml	22.5 a
	Xterminator (2) 5 ml	97.5 b
B	Water dip	24.0 a
	WetCit 2.5 ml	39.0 ab
	WetCit 5 ml	49.4 b
	Xterminator (2) 5 ml	100.0 c
C	Water dip	20.0 a
	WetCit 1 ml	46.7 b
	Xterminator (2) 2.5 ml + WetCit 1 ml/L	82.1 c
	Xterminator (2) 2.5 ml	86.7 c
D	Water dip	31.3 a
	Break-Thru 1 ml	56.7 b
	Xterminator (2) 2.5 ml + Break-Thru 1 ml	88.9 c
	Xterminator (2) 2.5 ml	75.4 bc

Within each trial, mortalities followed by the same letter are not significantly different at P=0.05 according to Student-Newman-Keuls test.

Table 3.5.6.2. Percentage mortalities of grain chinch bug caused by both Xterminator® formulations after 24 hrs

Trial	Treatments	Mortality (%)
E	Water dip	20.1 a
	Xterminator (0.75) 2.5 ml/L	70.1 b
	Xterminator (2) 2.5 ml/L	100.0 c
F	Water dip	29.1 a
	Xterminator (0.75) 5 ml/L	95.0 b
	Xterminator (2) 5 ml/L	100.0 b

Within each trial, mortalities followed by the same letter are not significantly different at P=0.05 according to Student-Newman-Keuls test.

Table 3.5.6.3. Percentage mortalities of grain chinch bug caused by both Xterminator® formulations after 24 hrs with solutions of variable age

Trial	Treatments	Solution age (d)	Mortality (%)
G	Water dip		28.3 a
	Xterminator (2) 5 ml/L	0	100.0 b
	Xterminator (2) 5 ml/L	1	97.9 b
	Xterminator (2) 5 ml/L	2	100.0 b
	Xterminator (2) 5 ml/L	3	100.0 b
	Xterminator (2) 5 ml/L with chlorine 0.02% a.i. in rinse	3	96.9 b
H	Water dip		32.1 a
	Xterminator (0.75) 5 ml/L	7	100.0 b
	Xterminator (2) 5 ml/L	7	100.0 b

Within each trial, mortalities followed by the same letter are not significantly different at P=0.05 according to Student-Newman-Keuls test.

Conclusion

Xterminator (0.75) is effective in reducing numbers of grain chinch bug infesting oranges when used as a post-harvest treatment and has been registered in South Africa for this purpose. Possible effects on quality of all citrus cultivars were not evaluated. A poster on this research was presented at the Citrus Research Symposium in August 2006.

Future research

No further work is planned.

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3.6.6 Using Break-Thru to improve corrective control of mealybug on citrus

Experiment 873 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

Dit wil voorkom dat Break-Thru S 240 'n ongelooflike vermoeë het om verspreiding te veroorsaak en sal daarom penetrasie van bespuitings tot in hoekies en gaatjies verbeter. As gevolg hiervan is dit moontlik dat die byvoeging van hierdie produk tot witluis behandelings die werking van korrektiewe behandelings vir witluis kan verbeter. 'n Boordproef is uitgevoer om hierdie te toets. Vier verskikllende produkte, naamliks Mevinphos, Applaud, Lannate en Ultracide, is gebruik om die beheer van witluis op sitrus te meet. Hierdie produkte is saam met of Agral 90 of Break-Thru toegedien. Geen verskil in vermindering van witluis besmetting is tussen die twee benatters met enige van die produkte gekry nie. Voordat Break-Thru aanbeveel kan word vir gebruik saam met hierdie produkte teen witluis op sitrus, moet verdere proewe uitgevoer word. Hierdie proewe moet teen hoër vlakke van witluis besmetting uitgevoer word en moet 'n reeks konsentrasies van Break-Thru toets.

Introduction

Good corrective control of mealybug is difficult to achieve. Once mealybug has packed underneath the calyx of the fruit and inside the navel end (of navel oranges), it is well protected against chemical sprays. Corrective spray trials conducted during the 2003/04 season with a range of insecticides, demonstrated that acceptable control was impossible with all treatments except Applaud (Moore & Kirkman, 2004). However, substantial reduction in infestation was only recorded for Applaud, eight weeks after application. Break-Thru S 240 is a polyether trisiloxane wetter and spreader. It appears to have an uncanny capacity to cause spreading and therefore improve penetration in nooks and crevices. For this reason it is possible that the addition of this product to mealybug treatments could improve their ability to correctively control mealybug. The trial described in this report was conducted to test this, and was performed at the commission of Degussa.

Materials and methods

A Midknight Valencia orange orchard with a conspicuous level of mealybug infestation was selected for this trial. This orchard was on Môreson Farm in Sundays River Valley, Eastern Cape. Before application of the trial, mealybug infestation on untreated control trees was evaluated by inspecting 10 randomly selected fruit on each of the 10 trees. Inspections were also conducted underneath the calyx of each fruit. The trial was laid out in single tree randomised block format, replicated 10 times. Four different products were used, either with Agral 90 (alkylated phenol-ethylene oxide) or with Break-Thru (Table 3.6.6.1). Sprays for mealybug control are commonly recommended to be applied with a wetter (Hattingh & Moore, 2003). Agral 90 is one of the wetters most commonly used. Treatments, for corrective control of mealybug, were applied on 19 April 2006, using a high pressure spray machine with hand guns, as full cover film sprays. A total of 15 L of spray mix was applied to each tree. Trees were spaced at 6 m x 1.5 m apart, making a total of 1111 trees per hectare. This would extrapolate to an application of 16665 L of spray mix per hectare.

Infestation was evaluated three weeks later on 11 May, by inspecting 10 fruit on each tree. Fruit was classified as clean or infested. Data was statistically analysed by comparing means for treatments using an ANOVA and the LSD multiple range test.

Table 3.6.6.1. Various treatments applied on 19 April 2006 for the corrective control of mealybug on Midknight Valencia orange trees at Môreson Farm

No.	Treatments	Active ingredient	Concentration in 100 L water	Wetter	Concentration in 100 L water
1	Untreated control	-	-	-	-
2	Mevinphos	Mevinphos	165 ml	Agral 90	18 ml
3	Mevinphos	Mevinphos	165 ml	Break-Thru	5 ml
4	Applaud	Buprofezin	30 g	Agral 90	18 ml
5	Applaud	Buprofezin	30 g	Break-Thru	5 ml
6	Lannate	Methomyl	20 g	Agral 90	18 ml
7	Lannate	Methomyl	20 g	Break-Thru	5 ml
8	Ultracide	Methidathion	150 ml	Agral 90	18 ml
9	Ultracide	Methidathion	150 ml	Break-Thru	5 ml

Results and discussion

On 19 April, the date on which the trial was applied, 23% of fruit on the untreated control trees was infested with mealybug. It was clear that infestation had been a lot higher, earlier in the season and had declined most probably due to natural biological control. This biocontrol trend was considered likely to increase towards harvest. The trial was therefore evaluated no later than three weeks after application, in order that infestation was still of such a level that differences between treatments could be detected. Despite this, infestation in the untreated control had already declined to 15% (Table 3.6.6.2). A marked decline in infestation was recorded for all treatments except Applaud. Applaud is recognised as the most effective corrective treatment for mealybug (Moore & Kirkman, 2004). However, being a slow acting insect growth regulator (IGR), three weeks was insufficient time for the full efficacy of the product to be shown. Reduction in infestation caused by Lannate was not statistically significant (Table 3.6.6.2). Lannate is recognised as possibly the weakest registered treatment for mealybug. Most importantly, in the case of this study, there was no significant difference in the efficacy of any of the products when used with either Break-Thru or Agral 90.

Table 3.6.6.2. Mealybug infestation of Midknight Valencia oranges on Môreson Farm on 11 May 2006, three weeks after various treatments were applied

No.	Treatments	Fruit infested (%)*
1	Untreated control	15a
2	Mevinphos + Agral 90	4bcd
3	Mevinphos + Break-Thru	6bcd
4	Applaud + Agral 90	10abc
5	Applaud + Break-Thru	11ab
6	Lannate + Agral 90	7abcd
7	Lannate + Break Thru	9abc
8	Ultracide + Agral 90	2cd
9	Ultracide + Break Thru	0d

*Values in the same column followed by the same letter are not significantly different (P>0.05, LSD multiple range test).

A total of 15 L of spray mix was applied to each tree, extrapolating to an application of 16665 L of spray mix per hectare. It is utterly imperative that treatments for mealybug control be applied as heavy full cover film sprays with good penetration. Break-Thru was mixed at a concentration of 5 ml per 100 L water. This means that Break-Thru was applied at 833 ml per hectare. The product label states that Break-Thru should not be applied at more than 500 ml per hectare but that it should be applied at a concentration of 25-50 ml/100 litres of water. It can therefore be argued that either too much or too little Break-Thru was applied. This points to the need for further work with different rates of Break-Thru and greater clarity on the product label as to the appropriate rates.

Conclusion

Four different products were used in a trial to measure the control of mealybug on citrus. These products were applied with either Agral 90 or Break-Thru. No difference in reduction in mealybug infestation was recorded between the two wetters, with each product. However, before Break-Thru can be recommended for use with these products, for control of mealybug on citrus, further trials should be conducted. These trials should be conducted against higher levels of mealybug and should investigate a range of concentrations of Break-Thru.

Future research

This work will be repeated and expanded during the 2006/07 citrus season. The objective will not only be to determine whether Break-Thru is adequately effective as a wetter with a corrective spray for mealybug control, but also to determine which is the most appropriate concentration of Break-Thru to use. This work will once again be funded by Degussa.

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3.7 PROJECT: PRODUCTION PESTS

Project coordinator: Tim Grout (CRI)

3.7.1 Project summary

For many years, red scale was the key pest affecting production but recently it has not been regarded as a research priority because it is effectively managed with oil sprays, neonicotinoids and pyriproxyfen, and assistance from natural enemies. Citrus psylla as the vector of Greening disease has been the most serious production pest for the last five years and research has been focused on developing IPM-compatible treatments for this pest. In 2006 a site was found with high populations of citrus psylla that allowed for research on chemical control alternatives and an evaluation of mass trapping. Unfortunately, no suitable alternatives to endosulfan were found that are not already registered and the efficacies of all prospective

products were inferior (3.7.2). Large Correx, sticky yellow traps were used in a horizontal position in a mass-trapping approach to try to control citrus psylla. Unfortunately, the blocks with the traps had significantly higher infestation levels of adults on new shoots than the untreated blocks. The traps therefore seemed to be drawing more adults into the block rather than reducing their numbers (3.7.3). Further research on citrus psylla control will not be conducted until new products or new techniques become available.

Projekopsomming

Rooi dopluis was vir jare 'n baie belangrike plaag in sitrusproduksie. Dit is egter nou nie meer 'n navorsingsprioriteit nie omdat dit effektief beheer word met olies, neonicotinioids, pyriproxyfen en ook met die hulp van natuurlike vyande. Navorsing is nou gefokus op die ontwikkeling van IPB-verenigbare behandelings teen die sitrus bladvlooi. Hierdie plaag, wat die vektor is van vergroeningsiekte, word die afgelope vyf jaar gereken as die belangrikste produksieplaag. Gedurende 2006 is 'n perseel gevind waar hoë populasies van die plaag teenwoordig was wat navorsing met alternatiewe vir chemiese beheer sowel as evaluasies van massa uitvangste moontlik gemaak het. Ongelukkig kon geen geskikte alternatief vir endosulfan gevind word anders as die middels wat reeds geregistreer is nie. Die moontlike produkte wat getoets is se effektiwiteit was almal laer as die van Endosulfan (3.7.2). In 'n poging om die sitrus bladvlooi te beheer met massa uitvangste is groot Correx, taai geel valle in horisontale posisies in blokke geplaas. Ongelukkig is daar noemenswaardige hoër vlakke van besmetting deur volwassenes op nuwe lote gevind in die blokke waar die valle geplaas is, as in die onbehandelde blokke. Dit blyk dus dit meer volwassenes na die blokke gelok het in plaas daarvan om die getalle te verminder (3.7.3). Daar sal nie verder met navorsing op die beheer van die sitrus bladvlooi voortgegaan word nie, tensy daar nuwe produkte of tegnieke beskikbaar kom.

3.7.2 IPM-compatible treatment options for citrus psylla *Trioza erytreae*

Experiment 586 by Tim Grout, Bruce Tate and Peter Stephen (CRI)

Opsomming

Daar bestaan 'n dringende behoefte vir alternatiewe blaar bespuitings, anders as die organofosfate vir die beheer van die sitrus bladvlooi. Hoë vlakke van besmettings is gevind gedurende die mid-somer op 'n plaas suid van Nelspruit. Twee proewe is op die plaas uitgevoer om moontlike bespuitings te evalueer. 'n Derde proef is ook later in die lente in dieselfde boord uitgevoer. In al die proewe is onvoldoende beheer verkry met die geregistreerde dosisse van endosulfan. Beter resultate is ook nie verkry met enige van die ander middels wat geëvalueer is nie. Die effektiwiteit van 1% olie en kaolien was swakker as in 2005. 'n Mate van onderdrukking is met Neem verkry wat vergelykbaar is met die onderdrukking wat verkry is met abamectin plus olie. Dit was dus nie moontlik om daarin te slaag om nuwe IPB-verenigbare behandelings te vind wat laat in die seisoen aangewend kan word met dieselfde effektiwiteit as organofosfate of endosulfan nie. Endosulfan mag nie meer later as blomblaarval in die geval van sitrus gebruik word nie. Geen verdere werk sal gedoen word op die chemiese beheer van die sitrus bladvlooi nie, tensy daar nuwe, belowende produkte beskikbaar kom.

Introduction

Periodically, citrus psylla populations increase dramatically and urgent control methods are required because the greening disease (*Liberobacter africanum)* that is transmitted by this vector can spread rapidly. In the past, organophosphates were often used to control these vectors but monocrotophos is no longer available in South Africa and the use of dimethoate has been reduced to one preblossom treatment on bearing trees due to the MRL in EU being lowered markedly. The neonicotinoid stem and soil treatments are effective but foliar spray options are few. The currently registered products are given in Nel et al. (2002) but not many of these can be used late in the season due to residue problems. Even endosulfan can no longer be used after petal fall on fruit going to certain markets. The only recent work that has been conducted on the chemical control of citrus psylla has been that by the authors in 2004 (Grout and Stephen 2005) and early in 2006 (Grout et al. 2006). A further trial was conducted in October 2006 and due to some similar treatments being used, all 2006 trials are included in the report below.

Materials and methods

In January 2006, extremely high populations of citrus psylla were found in an orchard of 12-year-old Empress mandarins on Brackenhill farm south of Nelspruit. A randomised block design was used with the orchard being split in half and each half being subdivided into seven treatment blocks. Each block comprised three rows and was at least seven trees long. Sprays were applied by hand using a high-pressure (30 bar) spray machine and applying 6-10 ℓ spray mixture per tree on 5 January 2006. Weather

conditions were dry, partly cloudy with a maximum temperature of 28°C. The treatments used are shown in Table 3.7.2.1. They were evaluated 8 days after treatment on 13 January 2006 with a further partial evaluation on 23 January. Data trees were primarily selected from the central row in each block and if there were insufficient trees in the centre row, the sides of trees closest to the central row were used as well. Counts were based on 10 branch terminals per data tree having new leaves suitable for citrus psylla. Each terminal was rated for the presence or absence of fresh eggs, live nymphs or adults. Data were transformed to the square root of the arc sine, then analysed by two-way ANOVA and means further compared using Student-Newman-Keul's test at α=0.05.

A second trial was conducted at the same site using exactly the same techniques after the whole orchard was sprayed with Phosdrin to slightly reduce the citrus psylla numbers. Treatments were applied on 16 February 2006 and evaluated on 24 February. Treatment details are provided in Table 3.7.2.3. Statistical analysis of results was as described above.

A third trial was conducted at Brackenhill Farm using the same orchard of Empress mandarins in October 2006. The treatments were expected to be less efficacious so smaller randomised blocks of only three trees in a row were used and replicated three times per treatment. Seven treatments were evaluated (Table 3.7.2.4), including an untreated control and a lower dosage of endosulfan registered for high spray volumes. The sprays were applied by hand to the point of run-off on 19 October 2006 in warm, overcast weather. The treatments were evaluated once on 30 October when 12 new growth terminals were selected from the inside tree and the inner sides of the two adjacent trees of each plot. The growth flush on each branch was then rated separately for infestation by eggs, nymphs or adults. Data were analysed as described above.

Results and discussion

In the first trial, numbers of citrus psylla were so high in this orchard that they were no longer ovipositing along the leaf margins but anywhere on the leaves. One-sided, yellow sticky traps (165 X 165 mm) were regularly catching over 300 psyllids per week. None of the treatments had any significant effect ($P>0.05$) on egg infestation (Table 3.7.2.1) and only the standard treatment of endosulfan had a significant effect on nymph infestation. There were more differences between treatments in adult infestation and Nemesis plus oil was significantly less infested than other treatments. For this reason, a second evaluation was conducted on 23 January to compare Nemesis with the control and the endosulfan standard. However, although the infestations of eggs and adults were the lowest in the Nemesis treatments they were not significantly different from the untreated control (Table 3.7.2.2). Neither the endosulfan treatment nor the Nemesis treatment caused a significant reduction in nymphal infestation. After the site was sprayed out and the treatments reapplied there were more differences in efficacy between the treatments (Table 3.7.2.3), although none could be considered to provide commercial control. Endosulfan was once again significantly better than all other treatments against nymphal life stages. Fruitcote and 1% medium grade horticultural oil appeared to be less effective than in the trial of the previous year (Grout & Stephen 2005) and were not significantly different from the control. The abamectin plus oil and the Bio-Neem treatments showed significantly better efficacy than the control against nymphs but did not reduce egg infestation significantly.

In the third trial conducted in October 2006, the numbers of psyllids were slightly lower as can be seen by the lower egg infestation levels in the control in Table 3.7.2.4. However, there were no significant differences between treatments based on infestation levels of eggs and adults. Based on nymphal infestation, endosulfan was once again the most effective treatment, even at the lower dosage used. Buprofezin plus WetCit had a slight but not significant ($P>0.05$) effect on nymphs, as did the increased dosage of neem. The wetter WetCit was ineffective on its own at 300 ml/hl water. The abamectin and buprofezin treatments with Break-Thru at 3 ml/hl were also ineffective.

The differences between the results in 2006 and 2005 can probably be attributed to the difference in infestation levels. This emphasises the need to maintain citrus psylla populations at low levels because once they get out of control they are difficult to stop without using very disruptive treatments. In the 2004-5 season, abamectin plus 0.3% oil was significantly different from the control when all types of infestation were considered (Grout & Stephen 2005) but in 2006 the combination was significantly more effective than the control only when nymphal infestation was compared and when used with BreakThru instead of oil it was ineffective. Unfortunately, none of the treatments were as effective as the old standard endosulfan, and this product can no longer be used on citrus after petal fall. Chemical control of psylla will therefore be dependent on soil or stem applications of methamidophos or neonicotinoids as the least IPM-disruptive control measures with perhaps mevinphos being used in late summer.

Table 3.7.2.1. The effect of various treatments against different life stages of citrus psylla at Brackenhill farm near Nelspruit.

Foliar treatments 5 Jan 2006	Infestation of shoot terminals 8 days after treatment		
	Eggs (%)	Nymphs (%)	Adults (%)
Untreated control	62.5 a	80.0 a	75.6 ab
Endosulfan (475 WP) 250 g/hl	77.5 a	21.3 b	68.8 b
Fruitcote (kaolin) 3 kg/hl	75.0 a	55.0 a	88.1 a
Biomectin (abamectin 18 EC) 20 ml plus BP Medium horticultural oil 300 ml/hl	69.4 a	51.3 a	75.6 ab
BP Medium horticultural oil 1%	73.8 a	46.9 a	80.6 ab
Nemesis (pyriproxyfen 100 EC) 30 ml plus BP Medium oil 300 ml/hl	53.1 a	45.6 a	51.9 c
Bio-Neem (azadirachtin 1.5 EC) 500 ml/hl	67.5 a	46.3 a	87.5 a

Means in the same column followed by the same letter are not significantly different (P>0.05 SNK).

Table 3.7.2.2. The longer-term effect of endosulfan and Nemesis plus oil against different life stages of citrus psylla at Brackenhill farm near Nelspruit.

Foliar treatments 5 Jan 2006	Infestation of shoot terminals 18 days after treatment		
	Eggs (%)	Nymphs (%)	Adults (%)
Untreated control	81.9 ab	86.3 a	53.1 ab
Endosulfan (475 WP) 250 g/hl	92.5 a	76.9 a	66.9 a
Nemesis (pyriproxyfen 100 EC) 30 ml plus BP Medium oil 300 ml/hl	73.8 b	89.4 a	40.6 b

Means in the same column followed by the same letter are not significantly different (P>0.05 SNK).

Table 3.7.2.3. The effect of various treatments against different life stages of citrus psylla in a second trial at Brackenhill farm near Nelspruit.

Foliar treatments 16 Feb 2006	Infestation of shoot terminals 8 days after treatment		
	Eggs (%)	Nymphs (%)	Adults (%)
Untreated control	63.8 a	41.9 a	51.3 a
Endosulfan (475 WP) 250 g/hl	35.6 b	4.4 c	27.5 c
Fruitcote (kaolin) 3 kg/hl	48.1 ab	40.0 a	46.9 ab
Biomectin (abamectin 18 EC) 20 ml plus BP Medium horticultural oil 300 ml/hl	44.4 ab	17.5 b	42.5 abc
BP Medium horticultural oil 1%	56.9 ab	37.5 a	52.5 a
Nemesis (pyriproxyfen 100 EC) 30 ml plus BP Medium oil 300 ml/hl	55.0 ab	26.9 ab	38.8 abc
Bio-Neem (azadirachtin 1.5 EC) 500 ml/hl	43.1 ab	18.1 b	29.4 bc

Means in the same column followed by the same letter are not significantly different (P>0.05 SNK).

Table 3.7.2.4. The effect of various treatments against different life stages of citrus psylla in a third trial at Brackenhill farm near Nelspruit.

Foliar treatments 19 Oct 2006	Infestation of shoot terminals 11 days after treatment		
	Eggs (%)	Nymphs (%)	Adults (%)
Untreated control	44.4 a	61.1 a	47.2 a
Applaud (buprofezin 500 WP) 30 g + Wetcit 100 ml/hl	52.8 a	38.9 ab	27.8 a
Biomectin (abamectin 18 EC) 20 ml + Break-Thru 3 ml/hl	47.2 a	61.1 a	44.4 a
Wetcit 300 ml/hl	52.8 a	72.2 a	27.8 a

Foliar treatments 19 Oct 2006	Infestation of shoot terminals 11 days after treatment		
	Eggs (%)	Nymphs (%)	Adults (%)
Endosulfan (475 WP) 100 g/hl	47.2 a	13.9 b	38.9 a
Applaud (buprofezin 500 WP) 30 g + Break-Thru 3 ml/hl	63.9 a	58.3 a	36.1 a
Bio-Neem (azadirachtin 1.5 EC) 750 ml/hl	19.4 a	38.9 ab	19.4 a

Means in the same column followed by the same letter are not significantly different (P>0.05 SNK).

Conclusion

Numbers of citrus psylla were high in these trials and the registered endosulfan spray could not provide adequate control. However, none of the alternatives tested were even as effective as the endosulfan.

Future research

Further research on citrus psylla is not planned but will be conducted if new, promising products become available.

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3.7.3 Development of an attract-and-kill system for citrus psylla

Experiment 794 by Tim Grout and Bruce Tate (CRI)

Opsomming

Daar is sistemiese plantbeskermingsprodukte beskikbaar vir die beheer van die sitrus bladvlooi, maar hierdie middels mag nie geskik wees vir gebruik laat in die seisoen op draende bome nie. Vanweë die beperkte aantal effektiewe blaarbespuitings sal 'n lok-en-dood sisteem die ideale oplossing wees. In vorige navorsing deur CRI is gevind dat sommige chemiese lokmiddels nie effektief was nie maar dat horisontale taai, geel valle wel tot drie maal meer vangste op die boonste oppervlak gehad het as op enige vertikale oppervlak. Die horisontale Correx taai, geel valle is gedurende 2006 geëvalueer as 'n massa uitvangs behandeling op hul eie of in bome wat met kaolien bespuit is. Die bevindinge was dat die besmettingsvlakke van die bladvlooie op nuwe groei nie verminder het nie, inteendeel dit het geblyk dat daar 'n verhoging was in die vlakke van besmetting deur volwassenes. Kaolien as 'n bespuiting het weereens die nimfale stadium van die bladvlooi besmettings onderdruk maar nie in dieselfde mate as endosulfan nie. Dit het ook geen noemenswaardige effek gehad op die besmetting van bladvlooi eiers en volwassenes nie. Geen verdere navorsing word beplan nie, tensy daar nuwe produkte of strategieë beskikbaar gestel word.

Introduction

Since 2001, when there was an upsurge in citrus psylla populations, the need for new IPM-compatible control options was highlighted because the greening disease (*Liberobacter africanum)* that is transmitted by this vector began to spread rapidly. Subsequently, the availability of chemical control options has declined. Monocrotophos is no longer available and the use of dimethoate has been reduced to one preblossom treatment on bearing trees due to the MRL in EU being lowered markedly. Systemic control options can be used as soil or stem treatments with little disruption of IPM but these are often not practical late in the season due to residues in fruit. Endosulfan can no longer be used after petal fall on fruit destined for certain markets. During summer growth flushes, sprays for citrus psylla can cause serious disruption to natural enemies of various pests and a more biorational technique such as an attract-and-kill system or mass trapping would be more appropriate, minimising or avoiding insecticide residues. Earlier research (Grout and Tate 2006) showed that horizontal Correx yellow traps caught more psyllids than vertical traps and caught fewer alate natural enemies when in the horizontal position. Large Correx yellow traps were therefore evaluated in the following research as a control method.

Materials and methods

An evaluation of mass trapping and a combination of traps and kaolin sprays to improve the contrast of yellow traps against white trees, was conducted at Brackenhill Farm using Empress mandarin trees of 12 years old. Five treatments were compared using two blocks of 60 trees each per treatment with each block comprising five rows. The orchard was divided in half and the treatment blocks were randomised within each half. The treatments were as follows:

1. Control
2. Endosulfan (475 WP) 250 g/hl water
3. Fruitcote kaolin 3 kg/hl water
4. Correx horizontal traps, yellow, 600 x 400 mm (20 traps per block)
5. Combination of treatments 3 and 4

The Fruitcote was sprayed on 28 August 2006 during fine, cloudy weather using high pressure (30 bar) hand guns. At this time the leaf buds of the spring growth flush were starting to expand. The yellow traps (600 x 400 mm) were installed the next day. Four traps were placed three trees apart in each of the five rows per block, giving a total of 20 traps per 60-tree block. The traps were mounted horizontally on wooden laths 2 m high inserted in the ground between trees in the row. Only the upper surface of the trap was coated with Ant-bar (polybutene). The endosulfan treatment was only applied on 29 September (again in fine weather) as it was felt that the kaolin and traps had to be on before the psylla moved onto the flush and it was only by late September that their numbers were getting high enough to conduct an evaluation.

On 18 October an assessment was made of the proportion of 10 new growth terminals per tree infested with eggs, nymphs or adults for each of eight data trees in the centre row of each block. Statistical analysis was by two-way ANOVA after arc sine square root transformation. If the F test showed significant differences between treatments at α=0.05, treatment means were further compared using Student-Newman-Keuls test at α=0.05.

Results and discussion

The results of mass trapping were disappointing and in the case of adult psyllid infestations, the traps seemed to significantly increase psyllid populations. The kaolin sprays alone did cause a significant reduction in infestation by nymphs but not adults or eggs. However, the reduction of nymphal infestation by endosulfan was significantly better than that by kaolin alone (Table 3.7.3.1). With minimal effect on eggs and adults, perhaps the kaolin is preventing the nymphs from settling on the leaf once they have emerged from the eggs.

Table 3.7.3.1. Effect of Correx mass trapping and traps plus kaolin on infestation by citrus psylla

Treatments 28-29 Aug[1] & 29 Sep[2] 2006	Infestation of shoot terminals on 19 Oct 2006		
	Eggs (%)	Nymphs (%)	Adults (%)
Untreated control	56.9 ab	56.9 a	25.0 bc
Endosulfan (475 WP) 250 g/hl[2]	40.6 b	4.4 c	13.8 c
Fruitcote kaolin 3 kg/hl[1]	50.0 ab	20.0 b	20.0 c
Correx sticky traps only[1]	68.1 a	65.6 a	41.3 a
Fruitcote 3 kg/hl and sticky traps[1]	48.1 ab	53.8 a	36.3 ab

Means in the same column followed by the same letter are not significantly different (P>0.05 SNK).

Conclusion

When horizontal Correx traps were evaluated as a control method they appeared to increase numbers of adult citrus psylla on new growth and therefore had a negative effect. Kaolin sprays did suppress nymphal infestation but had no effect on eggs or adults and were not as efficacious as endosulfan sprays.

Future research

No further research on biorational control methods for citrus psylla is planned.

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3.8 PROJECT: RESIDUE TRIALS FOR PHI AND MRL DEVELOPMENT

By Peter R Stephen and Wayne Kirkman (CRI)

3.8.1 Project summary

Residue trials were conducted with methamidophos, triflumuron, propargite, mercaptothion (in fruit fly baits) and buprofezin. In many cases the results are not yet available from SABS but where possible they have been included. Appropriate changes to the pre-harvest intervals will be communicated to growers via the Usage Restrictions for Plant Protection Products document produced periodically by Hattingh and Hardman.

Projekopsomming

Residu proewe is uitgevoer met methamidophos, triflumuron, propargite, mercaptothion (in vrugtevlieg lokmiddels) en buprofezin. Alhoewel al die resultate nog nie beskikbaar is vanaf die SABS nie, is dit waar moontlik ingesluit. Toepaslike veranderinge aan die weerhoudingsperiodes sal aan die produsente bekend gemaak word in die dokument - Beperkinge op die gebruik van Plantbeskermingsprodukte - wat periodiek deur Hattingh en Hardman beskikbaar gestel word.

3.8.1.1 Methamidophos residue trials

Experiment 851 by Wayne Kirkman and Peter Stephen (CRI)

Introduction

These trials were initiated to determine the decline of methamidophos (Citrimet) residues in citrus fruit when applied for control of citrus psylla. The study was started in the 2005/2006 season, with final trials planned for the 2006/2007 season.

Materials and methods

The trials were planned to be conducted over two citrus growing seasons. Eight trials were to be conducted in the first season, four each in the Mpumalanga and Eastern Cape production areas. All applications were made in such a manner as to simulate the worst-case scenario, i.e. the highest dosage rate applied with the shortest withholding period. The dosage rate to be applied was the same as currently registered for use on citrus in South Africa. The trials were conducted in accordance with the European Commission guidelines for residue trials. This included conforming to the principles of Good Laboratory Practice (GLP), requiring meticulous attention to detail, recording of all operations and independent auditing at all critical phases.

Treatments were applied separately to at least eight individual trees in a row in each trial. Control treatments consisted of untreated control trees, set out at a suitable distance from the treated area. All treatments were made using calibrated equipment. All relevant details of events, conditions and calibration verifications were noted in the field trial notebook, one per field trial. Wayne Kirkman (Port Elizabeth) and Peter Stephen (Nelspruit) were the appointed Principal Investigators for the study to control the trials in each area. Mr Vincent Nel (SABS) was the appointed Study Director and Mr Des van der Linde (Chromatographic Services) conducted the audits. Two further trials on mandarins are to be conducted by Wayne Kirkman in the Eastern Cape during the 2006/2007 citrus growing season.

The circumference of each tree trunk was measured using a verified tape measure at the intended point of application. The method of application was refined from the product label and verified syringes were used to apply the correct dose to each tree. Sites were located at Thornlands Farm in Swellendam, Crocodile Valley Citrus, Nelspruit and Golden Frontiers Citrus, Malelane. Two applications were made in 2005 and a third application was applied in February 2006 as seen in Table 3 . 8 . 1 . 1 . 1 .

Table 3.8.1.1.1. Application schedule for Citrimet to citrus.

Season	Location	Trial no	Cultivar	Treatment	Application time
2005/2006	Swellendam	Trial 1	Clementines	Treatment 1 3 applications	Application 1 September 2005
					Application 2 November 2005
					Application 3 13 February 2006
				Treatment 2 2 applications	Application 1 September 2005
					Application 2 November 2005
	Swellendam	Trial 2	Navels	Treatment 1 3 applications	Application 1 September 2005
					Application 2 November 2005
					Application 3 13 February 2006
				Treatment 2 2 applications	Application 1 September 2005
					Application 2 November 2005
2005/2006	Nelspruit	Trial 3	Valencia	Treatment 1 3 applications	Application 1 21 September 2005
					Application 2 23 November 2005
					Application 3 21 February 2006
				Treatment 2 2 applications	Application 1 21 September 2005
					Application 2 23 November 2005
2005/2006	Hectorspruit	Trial 4	Grapefruit	Treatment 1 3 applications	Application 1 22 September 2005
					Application 2 23 November 2005
					Application 3 21 February 2006
				Treatment 2 2 applications	Application 1 21 September 2005
					Application 2 23 November 2005

For the 2006/2007 citrus growing season, two sites were chosen on Carden Farm in the Sundays River Valley. These trials are not being conducted under GLP conditions, but all the principles will be applied. The first application was made to 5 trees in each site on the 17/11/06, and two applications followed at 60-day intervals.

During 2006, samples were collected at pre-determined intervals to determine residue decline and sample details are shown in Table 3.8.1.1.2. All samples were taken in duplicate. One sample was sent to SABS, and the duplicates kept in freezers at CRI Nelspruit or Port Elizabeth, and sent to the SABS at a later stage. Residue analyses were conducted by SABS.

Table 3.8.1.1.2. Fruit samples taken in 2006

Sample number	Sample type	Sample Time (DALA)	Cultivar	Season
05/536/001	Formulation sample	Before application	NA	NA
Trial 1 - Treatment 1 – Swellendam 3 applications				
05/536/002	Residue sample	T-1	Clementines	13/02/06
05/536/003	Control sample	T-1	Clementines	13/02/06
05/536/004	Residue sample	T5	Clementines	18/02/06
05/536/005	Residue sample	T10	Clementines	23/02/06
05/536/006	Residue sample	T21	Clementines	07/03/06
05/536/007	Control sample	T40	Clementines	26/03/06
05/536/008	Residue sample	T40	Clementines	26/03/06
Trial 1- Treatment 2 – Swellendam 2 applications				
05/536/009	Residue sample	T-1	Clementines	Not taken
05/536/010	Control sample	T-1	Clementines	Not taken
05/536/011	Residue sample	T5	Clementines	19/11/2005
05/536/012	Residue sample	T10	Clementines	24/11/2005
05/536/013	Residue sample	T21	Clementines	05/12/2005
05/536/014	Control sample	T40	Clementines	22/12/2005
05/536/015	Residue sample	T40	Clementines	22/12/2005
05/536/016	Control sample	T80	Clementines	02/02/06
05/536/017	Residue sample	T80	Clementines	02/02/06
05/536/018	Control sample	T120	Clementines	14/03/06
05/536/019	Residue sample	T120	Clementines	14/03/06
Trial 2 - Treatment 1 – Swellendam 3 applications				
05/536/020	Residue sample	T-1	Navels	13/02/06
05/536/021	Control sample	T-1	Navels	13/02/06
05/536/022	Residue sample	T5	Navels	18/02/06
05/536/023	Residue sample	T10	Navels	23/02/06
05/536/024	Residue sample	T21	Navels	07/03/06
05/536/025	Control sample	T40	Navels	26/03/06
05/536/026	Residue sample	T40	Navels	26/03/06
Trial 2 - Treatment 2 – Swellendam 2 applications				
05/536/027	Residue sample	T-1	Navels	14/11/2005
05/536/028	Control sample	T-1	Navels	14/11/2005
05/536/029	Residue sample	T5	Navels	19/11/2005
05/536/030	Residue sample	T10	Navels	24/11/2005
05/536/031	Residue sample	T21	Navels	05/12/2005
05/536/032	Control sample	T40	Navels	22/12/2005
05/536/033	Residue sample	T40	Navels	22/12/2005
05/536/034	Control sample	T80	Navels	02/02/06
05/536/035	Residue sample	T80	Navels	02/02/06
05/536/036	Control sample	T120	Navels	14/03/06
05/536/037	Residue sample	T120	Navels	14/03/06
Trial 3- Treatment 1 – Nelspruit 3 applications				
05/536/038	Residue sample	T-1	Valencia	21/02/06
05/536/039	Control sample	T-1	Valencia	21/02/06
05/536/040	Residue sample	T5	Valencia	26/02/06
05/536/041	Residue sample	T10	Valencia	03/03/06
05/536/042	Residue sample	T21	Valencia	14/03/06
05/536/043	Control sample	T40	Valencia	03/04/06
05/536/044	Residue sample	T40	Valencia	03/04/06
Trial 3- Treatment 2 – Nelspruit 2 applications				
05/536/045	Residue sample	T-1	Valencia	22/11/05
05/536/046	Control sample	T-1	Valencia	22/11/05
05/536/047	Residue sample	T5	Valencia	28/11/05
05/536/048	Residue sample	T10	Valencia	03/12/05

Sample number	Sample type	Sample Time (DALA)	Cultivar	Season
05/536/049	Residue sample	T21	Valencia	14/12/05
05/536/050	Control sample	T40	Valencia	03/01/06
05/536/051	Residue sample	T40	Valencia	03/01/06
05/536/052	Control sample	T80	Valencia	11/02/06
05/536/053	Residue sample	T80	Valencia	11/02/06
05/536/054	Control sample	T120	Valencia	23/03/06
05/536/055	Residue sample	T120	Valencia	23/03/06
Trial 4- Treatment 1 – Malelane 3 applications				
05/536/056	Residue sample	T-1	Grapefruit	21/02/06
05/536/057	Control sample	T-1	Grapefruit	21/02/06
05/536/058	Residue sample	T5	Grapefruit	26/02/06
05/536/059	Residue sample	T10	Grapefruit	03/03/06
05/536/060	Residue sample	T21	Grapefruit	14/03/06
05/536/061	Control sample	T40	Grapefruit	03/04/06
05/536/062	Residue sample	T40	Grapefruit	03/04/06
Trial 4- Treatment 2 – Malelane 2 applications				
05/536/063	Residue sample	T-1	Grapefruit	23/11/05
05/536/064	Control sample	T-1	Grapefruit	23/11/05
05/536/065	Residue sample	T5	Grapefruit	28/11/05
05/536/066	Residue sample	T10	Grapefruit	03/12/05
05/536/067	Residue sample	T21	Grapefruit	14/12/05
05/536/068	Control sample	T40	Grapefruit	03/01/06
05/536/069	Residue sample	T40	Grapefruit	03/01/06
05/536/070	Control sample	T80	Grapefruit	11/02/06
05/536/071	Residue sample	T80	Grapefruit	11/02/06
05/536/072	Control sample	T120	Grapefruit	23/03/06
05/536/073	Residue sample	T120	Grapefruit	23/03/06

Analysis of samples

The following is an extract from the report by SABS compiled by HV Garbers, Manager: Chromatographic Services and PFC van Zyl, Subject Specialist.

Eighty-one samples of citrus fruit (including 28 control samples) were submitted for analysis. The samples were in a frozen state when received. They were immediately placed in a deep-freeze and were kept under these conditions until the analysis commenced on 10 May 2006.

Method of test

The methamidophos residue content of the peel and the flesh was determined separately and the content on the whole fruit calculated. The fruit were peeled, the peel and flesh weighed and treated as separate samples. Each sample was shredded in a Stephan food cutter and was mixed thoroughly to render it homogeneous. The analysis was carried out employing method S19 as described in the *Manual of Pesticide Analysis Volume 1*. The Gel Permeation step was omitted. Recovery determinations were done by adding known amounts of methamidophos to portions of untreated control samples and analysing these concurrently with the samples.

Results

After consultation, only selected samples were analysed and the results are given in Table 3.8.1.1.3 below.

Table 3.8.1.1.3. Methamidophos residue content of samples tested

Sample description	SABS sample No.	No. of days after last application	Methamidophos residue content mg/kg		
			Peel	Flesh	Whole fruit
Treatment 1 Trial 1	05/536/002	T-1	-	-	ND
	05/536/004	T5	-	-	0.32

Sample description	SABS sample No.	No. of days after last application	Methamidophos residue content mg/kg		
			Peel	Flesh	Whole fruit
Swellendam 3 Applications	05/536/005	T10	-	-	0,26
	05/536/006	T21	-	-	0,13
	05/536/007	T40*	ND	ND	ND
	05/536/008	T40	0,04	0,04	0,04
Treatment 2 Trial 1 Swellendam 2 Applications	05/536/015	T40	-	-	ND
	05/536/017	T80	-	-	ND
	05/536/018	T120*	ND	ND	ND
	05/536/019	T120	ND	ND	ND
Treatment 1 Trial 2 Swellendam 3 Applications	05/536/025	T40*	ND	ND	ND
	05/536/026	T40	ND	ND	ND
Treatment 2 Trial 2 Swellendam 2 Applications	05/536/033	T40	-	-	ND
	05/536/034	T80*	ND	ND	ND
	05/536/035	T80	ND	ND	ND
	05/536/036	T120*	ND	ND	ND
	05/536/037	T120`	ND	ND	ND
Treatment 1 Trial 3 Nelspruit 3 Applications	05/536/043	T40*	ND	ND	ND
	05/536/044	T40	ND	ND	ND
Treatment 2 Trial 3 Nelspruit 2 Applications	05/536/045	T-1	-	-	0,01
	05/536/047	T5	-	-	1,3
	05/536/048	T10	-	-	0,12
	05/536/049	T21	-	-	0,70
	05/536/051	T40	ND	ND	ND
	05/536/053	T80	ND	0,01	ND
	05/536/054	T120*	ND	ND	ND
	05/536/055	T120	ND	ND	ND
Treatment 1 Trial 4 Hectorspruit 3 Applications	05/536/061	T40*	ND	ND	ND
	05/536/062	T40	ND	ND	ND
Treatment 2 Trial 4 Hectorspruit 2 Applications	05/536/068	T40*	ND	ND	ND
	05/536/069	T40	ND	ND	ND
	05/536/071	T80	ND	ND	ND
	05/536/072	T120*	ND	ND	ND
	05/536/073	T120	ND	ND	ND

* Control sample
ND Not detected/determined

Recovery determinations gave the following mean values.

Table 3.8.1.1.4. Methamidophos recovery at various levels of insertion

Recovery level (mg/kg)	Recovery (%)
0,01	98
0,05	73
0,10	63
0,20	100
2,0	62

The results reported were not corrected for recovery. Under the conditions of test employed the lowest limit of determination was 0,01 mg/kg.

Conclusion

These results will be available for the determination of a revised pre-harvest interval (PHI) for methamidophos.

3.8.1.2 **Triflumuron residue trials**
Experiment 867 by Peter Stephen and Wayne Kirkman (CRI)

Introduction

These trials were designed to evaluate the residue levels of the insecticide triflumuron after application of the formulated product Alsystin.

Materials and methods

A total of 16 trials were planned for two citrus-growing seasons, the 2005/2006 season and 2006/2007 season. This report covers 8 trials done during the first season, 4 in Mpumalanga on oranges and 4 in the Eastern Cape on soft citrus. All applications were made at the highest permitted dosage rate with the shortest possible withholding period. The trials were conducted in accordance with the European Commission guidelines for residue trials. These require meticulous attention to detail and recording of all operations, with independent auditing at all critical phases. Peter Stephen (Nelspruit) and Wayne Kirkman (Port Elizabeth) were appointed as Principal Investigators for the study and Pieter van Zyl (SABS) was appointed Study Director.

Treatments consisted of an untreated control and a 1X dosage rate of Alsystin equivalent to its current registration on citrus in South Africa. The compound was applied as a full cover spray simulating the applications for False Codling Moth control on citrus used by growers. Control treatments consisted of untreated trees of the same cultivar, but located at a suitable distance from the test trees. Before spraying, samples of the water used for spraying were checked for suspensibility, which could influence the integrity of the formulation. During spraying at each trial, samples of each tank mix were taken for analysis (Table 3.8.1.2.2). Applications were made using calibrated hand-gun spraymachines. The sprays were applied as “full cover” applications to wet the entire tree to the point of drip/run-off.

In the Eastern Cape, four sites of Clementines were selected – two in the Addo area and two in the Kirkwood area. In Mpumalanga four Valencia sites were selected at Friedenheim, Crocodile Valley Citrus (2) and Karino. The schedule of applications is given in Table 3.8.1.2.1.

Table 3.8.1.2.1. Application details

Citrus type	Cultivar	Trial site area	Date of application	Dosage Alsystin® mL/100 L
Oranges	Valencia	Site 1-	13/02/06	20 mL
		Site 2-	06/02/06	20 mL
		Site 3-	07/02/06	20 mL
		Site 4-	13/02/06	20 mL
Soft citrus	Clementine	Sites 1,2,3&4	07/02/06	20 mL

Table 3.8.1.2.2. Spray mix samples

Citrus type	Description	Trial site area	Date of sampling	Sample no
Oranges (Valencias)	Spraymix samples	Site 1-	13/02/06	06/23/105
		Site 2-	06/02/06	06/23/106
		Site 3-	07/02/06	06/23/107
		Site 4-	13/02/06	06/23/108
Soft citrus/Clementine	Spraymix samples	Site 1-	07/02/06	06/23/004
		Site 2-	07/02/06	06/23/005
		Site 3-	07/02/06	06/23/006
		Site 4-	08/02/06	06/23/007

Fruit samples were taken over a 79-day period to determine residue decline. All fruit samples were taken in duplicate. One sample was sent to SABS, and the duplicates kept in freezers at CRI Nelspruit or Port Elizabeth, and sent to the SABS at a later stage. Full details of samples taken are given in Table 3.8.1.2.3.

Table 3.8.1.2.3. Fruit Samples taken for triflumuron residue analysis.

Description	Trial Site	Mpumalanga (Valencias)		Eastern Cape (Clementines)	
		Date of sampling	Sample no.	Date of sampling	Sample no.
T-1 Control sample	Site 1-	13/02/2006	06/23/118	07/02/2006	06/23/008
	Site 2-	06/02/2006	06/23/109	07/02/2006	06/23/017
	Site 3-	07/02/2006	06/23/127	07/02/2006	06/23/026
	Site 4-	13/02/2006	06/23/136	08/02/2006	06/23/035
T0 Residue sample	Site 1-	2006/02/13	06/23/119	07/02/2006	06/23/009
	Site 2-	06/02/2006	06/23/110	07/02/2006	06/23/018
	Site 3-	07/02/2006	06/23/128	07/02/2006	06/23/027
	Site 4-	13/02/2006	06/23/137	08/02/2006	06/23/036
T7 Residue sample	Site 1-	20/02/2006	06/23/120	15/02/2006	06/23/010
	Site 2-	13/02/2006	06/23/111	15/02/2006	06/23/019
	Site 3-	14/02/2006	06/23/129	15/02/2006	06/23/028
	Site 4-	20/02/2006	06/23/138	15/02/2006	06/23/037
T14 Residue sample	Site 1-	27/02/2006	06/23/121	21/02/2006	06/23/009
	Site 2-	20/02/2006	06/23/112	21/02/2006	06/23/018
	Site 3-	21/02/2006	06/23/130	21/02/2006	06/23/027
	Site 4-	27/02/2006	06/23/139	21/02/2006	06/23/036
T30 Control sample	Site 1-	15/03/2006	06/23/122	09/03/2006	06/23/012
	Site 2-	08/03/2006	06/23/113	09/03/2006	06/23/021

Description	Trial Site	Mpumalanga (Valencias)		Eastern Cape (Clementines)	
		Date of sampling	Sample no.	Date of sampling	Sample no.
	Site 3-	09/03/2006	06/23/131	09/03/2006	06/23/030
	Site 4-	15/03/2006	06/23/140	09/03/2006	06/23/039
T30 Residue sample	Site 1-	15/03/2006	06/23/123	09/03/2006	06/23/013
	Site 2-	08/03/2006	06/23/114	09/03/2006	06/23/022
	Site 3-	09/03/2006	06/23/132	09/03/2006	06/23/031
	Site 4-	15/03/2006	06/23/141	09/03/2006	06/23/040
T45 Residue sample	Site 1-	30/03/2006	06/23/124	24/03/2006	06/23/014
	Site 2-	23/03/2006	06/23/115	24/03/2006	06/23/023
	Site 3-	24/03/2006	06/23/133	24/03/06	06/23/032
	Site 4-	30/03/2006	06/23/142	24/03/2006	06/23/041
T79 Control sample	Site 1-	03/05/2006	06/23/125	27/04/2006	06/23/015
	Site 2-	26/04/2006	06/23/116	27/04/2006	06/23/024
	Site 3-	26/04/2006	06/23/134	27/04/2006	06/23/033
	Site 4-	03/05/2006	06/23/143	27/04/2006	06/23/042
T79 Residue sample	Site 1-	03/05/2006	06/23/126	27/04/2006	06/23/016
	Site 2-	26/04/2006	06/23/117	27/04/2006	06/23/025
	Site 3-	26/04/2006	06/23/135	27/04/2006	06/23/034
	Site 4-	03/05/2006	06/23/144	27/04/2006	06/23/043

Analysis of samples

The following is an extract from the report by SABS compiled by HV Garbers, Manager: Chromatographic Services and PFC van Zyl, Subject Specialist.

Seventy-two samples of citrus, GLP study No. 06/23 were submitted for analysis during 2005/2006 citrus season. The samples were in a frozen state when received. They were immediately placed in a deep-freeze and were kept under these conditions until the analysis was commenced on 30 October 2006.

Method of test

The triflumuron residue content of the peel and the flesh was tested separately and the content on the whole fruit calculated. The fruit of each sample was peeled. The peel and the flesh were weighed and treated as separate samples.

Each sample was shredded in a Stephan food cutter and was mixed thoroughly to render it homogeneous. The analysis was carried out in duplicate employing method No. RA179 *viz Provisional Method for the determination of SIR 8514 Residues in Plants, Soil and Water by HPLC and GC.*

Recovery determinations were done by adding known amounts of triflumuron to portions of untreated control samples and analysing these concurrently with the samples.

Results

Table 3.8.1.2.4. Triflumuron residue content of fruit samples from Mpumalanga

Trial No.	No. of days after application	Triflumuron residue content, mg/kg		
		Peel	Flesh	Whole fruit
1	0 Control	0,02 ; 0,02 *	ND ; ND	ND
	0	1,4 ; 1,3	0,11 ; 0,08	0,45
	7	1,3 ; 1,3	0,09 ; 0,07	0,46
	14	1,2 ; 1,3	0,08 ; 0,07	0,41

Trial No.	No. of days after application	Triflumuron residue content, mg/kg		
		Peel	Flesh	Whole fruit
	28 Control	ND ; ND	ND ; ND	ND
	28	0,86 ; 0,94	0,05 ; 0,05	0,30
	35	0,97 ; 0,99	0,07 ; 0,07	0,33
	79 Control	ND ; ND	ND ; ND	ND
	79	0,82 ; 0,83	0,05 ; 0,06	0,29
2	0 Control	ND ; ND	ND ; ND	ND
	0	1,5 ; 1,5	0,17 ; 0,21	0,61
	7	1,5 ; 1,6	0,13 ; 0,12	0,57
	14	2,4 ; 2,3	0,22 ; 0,17	0,74
	28 Control	ND ; ND	ND ; ND	ND
	28	1,6 ; 1,9	0,09 ; 0,08	0,53
	35	2,0 ; 2,1	0,11 ; 0,15	0,61
	79 Control	ND ; ND	ND ; ND	ND
	79	1,5 ; 1,6	0,07 ; 0,07	0,45
3	0 Control	ND ; ND	ND ; ND	ND
	0	1,7 ; 2,2	0,16 ; 0,17	0,70
	7	1,3 ; 1,4	0,09 ; 0,11	0,45
	14	1,4 ; 1,4	0,11 ; 0,11	0,47
	28 Control	ND ; ND	ND ; ND	ND
	28	1,2 ; 1,2	0,06 ; 0,06	0,38
	35	1,2 ; 1,2	0,08 ; 0,07	0,38
	79 Control	ND ; ND	ND ; ND	ND
	79	1,1 ; 1,0	0,06 ; 0,06	0,33
4	0 Control	ND ; ND	ND ; ND	ND
	0	2,3 ; 2,2	0,17 ; 0,17	0,90
	7	1,7 ; 1,6	0,14 ; 0,16	0,64
	14	1,8 ; 1,8	0,17 ; 0,19	0,73
	28 Control	ND ; ND	ND ; ND	ND
	28	1,6 ; 1,6	0,11 ; 0,14	0,57
	35	1,6 ; 1,6	0,09 ; 0,07	0,54
	79 Control	ND ; ND	ND ; ND	ND
	79	1,8 ; 1,8	0,11 ; 0,10	0,64

ND = Not detected/determined
Note * = Duplicate sample will be re-analysed.

Table 3.8.1.2.5. Triflumuron residue content of fruit samples from the Eastern Cape

Trial No.	No. of days after application	Triflumuron residue content, mg/kg		
		Peel	Flesh	Whole fruit
1	1 Control	ND ; ND	ND ; ND	ND
	1	3,0 ; 2,8	0,30 ; 0,33	1,3
	7	2,4 ; 2,4	0,30 ; 0,28	1,1
	14	2,7 ; 2,7	0,26 ; 0,28	1,1
	28 Control	ND ; ND	ND ; ND	ND
	28	2,0 ; 2,0	0,15 ; 0,18	0,68
	35	1,6 ; 1,5	0,07 ; 0,07	0,51
	79 Control	ND ; ND	ND ; ND	ND
	79	1,3 ; 1,2	0,05 ; 0,05	0,34
2	1 Control	ND ; ND	ND ; ND	ND
	1	2,8 ; 2,9	0,39 ; 0,41	1,4
	7	2,4 ; 2,4	0,23 ; 0,30	1,1
	14	2,3 ; 2,4	0,16 ; 0,16	0,94
	28 Control	ND ; ND	ND ; ND	ND

Trial No.	No. of days after application	Triflumuron residue content, mg/kg		
		Peel	Flesh	Whole fruit
	28	2,0 ; 2,1	0,20 ; 0,21	0,79
	35	1,6 ; 1,7	0,09 ; 0,08	0,53
	79 Control	ND ; ND	ND ; ND	ND ; ND
	79	1,5 ; 1,4	0,07 ; 0,07	0,44
3	1 Control	ND ; ND	0,06 ; 0,06*	0,03
	1	3,0 ; 2,7	0,43 ; 0,45	1,5
	7	2,0 ; 2,0	0,22 ; 0,22	1,0
	14	2,0 ; 2,2	0,23 ; 0,20	0,95
	28 Control	ND ; ND	ND ; ND	ND
	28	1,4 ; 1,4	0,14 ; 0,16	0,58
	35	1,2 ; 1,1	0,01 ; 0,01	0,39
	79 Control	ND ; ND	ND ; ND	ND
	79	1,1 ; 1,1	0,04 ; 0,04	0,35
4	1 Control	ND ; ND	ND ; ND	ND
	1	2,0 ; 1,9	0,27 ; 0,27	0,91
	7	1,8 ; 1,7	0,22 ; 0,21	0,79
	0,79	2,0 ; 2,1	0,17 ; 0,20	0,80
	28 Control	ND ; ND	ND ; ND	ND
	28	2,1 ; 2,0	0,13 ; 0,12	0,67
	35	1,3 ; 1,3	0,08 ; 0,09	0,46
	79 Control	ND ; ND	ND ; ND	ND
	79	1,2 ; 1,2	0,05 ; 0,05	0,34

ND = Not detected/determined
Note * = Duplicate sample will be re-analysed

Recovery determinations gave the following mean values.

Table 3.8.1.2.6. Triflumuron recovery at various levels of insertion

Recovery level (mg/kg)	Recovery (%)	
	Peel	Flesh
0,05	102	108
0,10	108	104
0,15	-	97
0,50	107	100
1,0	98	92
2,5	90	-

The results reported were corrected for recovery using the appropriate value.

Under the conditions of test employed the lowest limit of determination was 0,01 mg/kg.

Conclusion

These trials will continue for a further season and the results then used for the determination of a revised PHI for triflumuron (Alsystin).

3.8.1.3 **Propargite Residue Trials**
Experiment 868 by Peter Stephen and Wayne Kirkman (CRI)

Introduction

The trials are designed to determine the decline of propargite residues in citrus fruit when applied using the critical GAP for control of citrus red mite with the application of Omite 30 WSB.

Materials and methods

These trials were planned to be conducted over two citrus-growing seasons, the 2005/2006 season and 2006/2007 season. This report covers 8 trials done during the first season, 4 in Mpumalanga on oranges and 4 in the Eastern Cape on soft citrus. All applications were made at the highest permitted dosage rate with the shortest possible withholding period. The trials were conducted in accordance with the European Commission guidelines for residue trials. These require meticulous attention to detail and recording of all operations, with independent auditing at all critical phases. Peter Stephen (Nelspruit) and Wayne Kirkman (Port Elizabeth) were appointed as Principal Investigators for the study and Pieter van Zyl (SABS) was appointed Study Director.

Treatments consisted of an untreated control and a 1X dosage rate of Omite equivalent to its registration on citrus in South Africa. Control treatments consisted of untreated trees of the same cultivar, but located at a suitable distance from the test trees. Before spraying, samples of the water used for spraying were checked for suspensibility, which could influence the integrity of the formulation. During spraying at each trial, samples of each tank mix were taken for analysis. Applications were made using calibrated hand-gun spraymachines. The sprays were applied as "medium cover" applications to wet the entire foliage canopy to the point of drip/run-off, simulating the applications for mite control used by growers.

Residue data will be collected after the treatment.

Table 3.8.1.3.1. Application details

Citrus type/area	Cultivar	Trial site	Date of application	Dosage Omite mL/100 L
Oranges Mpumalanga	Valencias	Site 1,2,3 & 4	09/05/2006	200 g
Soft citrus Eastern Cape	Clementine	Site 1,2,3 & 4	04/04/2006	200 g

Fruit samples were taken over a 28-day period to determine residue decline. All fruit samples were taken in duplicate. One sample was sent to SABS, and the duplicates kept in freezers at CRI Nelspruit or Port Elizabeth, and sent to the SABS at a later stage. Full details of samples taken are given in Table 3.8.1.3.2.

Table 3.8.1.3.2. Details of fruit samples taken

		Eastern Cape		Mpumalanga	
Description	**Trial site**	**Date of sampling**	**Sample no**	**Date of sampling**	**Sample no**
T0 Control sample	Site 1-	04/04/06	06/24/008	09/05/2006	06/24/109
	Site 2-	04/04/06	06/24/017	09/05/2006	06/24/118
	Site 3-	04/04/06	06/24/026	09/05/2006	06/24/127
	Site 4-	04/04/06	06/24/035	09/05/2006	06/24/110
T0 Residue sample	Site 1-	04/04/06	06/24/009	09/05/2006	06/24/119
	Site 2-	04/04/06	06/24/018	09/05/2006	06/24/119
	Site 3-	04/04/06	06/24/027	09/05/2006	06/24/128
	Site 4-	04/04/06	06/24/036	09/05/2006	06/24/136
T1 Residue sample	Site 1-	05/04/06	06/24/010	10/05/2006	06/24/111
	Site 2-	05/04/06	06/24/019	10/05/2006	06/24/120
	Site 3-	05/04/06	06/24/028	10/05/2006	06/24/129
	Site 4-	05/04/06	06/24/037	10/05/2006	06/24/138
T3 Residue sample	Site 1-	07/04/06	06/24/011	12/05/2006	06/24/112
	Site 2-	07/04/06	06/24/020	12/05/2006	06/24/121
	Site 3-	07/04/06	06/24/029	12/05/2006	06/24/130
	Site 4-	07/04/06	06/24/038	12/05/2006	06/24/139
T7 Residue sample	Site 1-	11/04/06	06/24/012	16/05/2006	06/24/113
	Site 2-	11/04/06	06/24/021	16/05/2006	06/24/122

	Site 3-	11/04/06	06/24/030	16/05/2006	06/24/131
	Site 4-	11/04/06	06/24/039	16/05/2006	06/24/140
T14 Control sample	Site 1-	18/04/06	06/24/013	23/05/2006	06/24/115
	Site 2-	18/04/06	06/24/022	23/05/2006	06/24/124
	Site 3-	18/04/06	06/24/031	23/05/2006	06/24/133
	Site 4-	18/04/06	06/24/040	23/05/2006	06/24/142
T14 Residue sample	Site 1-	18/04/06	06/24/014	23/05/2006	06/24/114
	Site 2-	18/04/06	06/24/023	23/05/2006	06/24/123
	Site 3-	18/04/06	06/24/032	23/05/2006	06/24/132
	Site 4-	18/04/06	06/24/041	23/05/2006	06/24/141
T28 Control sample	Site 1-	02/05/06	06/24/015	06/06/2006	06/24/117
	Site 2-	02/05/06	06/24/024	06/06/2006	06/24/126
	Site 3-	02/05/06	06/24/033	06/06/2006	06/24/135
	Site 4-	02/05/06	06/24/042	06/06/2006	06/24/044
T28 Residue sample	Site 1-	02/05/06	06/24/016	06/06/2006	06/24/118
	Site 2-	02/05/06	06/24/025	06/06/2006	06/24/125
	Site 3-	02/05/06	06/24/034	06/06/2006	06/24/134
	Site 4-	02/05/06	06/24/043	06/06/2006	06/24/143

Conclusion

Due to the fact that this material is now no longer being manufactured, this study was terminated before fruit samples were analysed.

3.8.1.4 **Malathion residue trials**
Experiment 872 by Peter Stephen and Wayne Kirkman (CRI)

Introduction

This study is being conducted to determine the decline of mercaptothion residues in Citrus fruit when applied for control of fruit fly with the application of Avi Gard.

Materials and methods

For this study 16 trials were to be conducted over two Citrus-growing seasons i.e. 2006 and 2007. This report covers 8 trials done during the first season, 4 in Mpumalanga on oranges and 4 in the Eastern Cape on mandarins. All applications were made at the highest possible dosage rate with the shortest possible withholding period. The trials were conducted in accordance with the European Commission guidelines for residue trials. These require meticulous attention to detail and recording of all operations, with independent auditing at all critical phases. Peter Stephen (Nelspruit) and Wayne Kirkman (Port Elizabeth) were appointed as Principal Investigators for the study and Vincent Nel (SABS) was appointed Study Director.

The applications were made through the use of a baiting method. In this system, fruit fly attractant is made up in water as a mixture with a pesticide. The tank mixture is then applied as coarse droplets to the trees at an application rate of 50 to 150 ml per one side of the tree. A knapsack was used with a 1 bar pressure regulator and a 56 whirler core with a D3 disc. Hym-Lure was used as the fruit fly attractant and AviGard (malathion) as the insecticide. Applications were made once a week for 5 weeks.

Spray mixture and dosage rates

The applications were made using the equivalent spray mixture concentration rate of 175 ml AviGard and 400 ml Hym-Lure per 100 ℓ of water (Table 3.8.1.4.1).

Table 3.8.1.4.1. Application schedule for Mercaptothion to citrus.

Citrus growing season	Citrus type	Cultivar	Application no	Date of application	Dosage Avi Gard® mL/10 L
2006/2007	Mandarin	Orr and Affourer	1	15/05/2006	17.5 ml
			2	22/05/2006	17.5 ml
			3	30/05/2006	17.5 ml
			4	06/06/2006	17.5 ml
			5	12/06/2006	17.5 ml
2006/2007	Oranges	Valencia	1	17/05/2006	17.5 ml
			2	25/05/2006	17.5 ml
			3	01/06/2006	17.5 ml
			4	07/06/2006	17.5 ml
			5	15/06/2006	17.5 ml

Tank mix samples were taken for each application at each site, frozen and sent to SABS.

Fruit sampling

Fruit samples were taken over a 14-day period to determine residue decline. All fruit samples were taken in duplicate. One sample was sent to SABS, and the duplicates kept in freezers at CRI Nelspruit or Port Elizabeth, and sent to the SABS at a later stage. Full details of samples taken are given in Table 3.8.1.4.2.

Table 3.8.1.4.2. Samples taken in 2006

Cultivar	Sample time	Sampling date	Sample number			
			Site 1	Site 2	Site 3	Site 4
Orr and Affourer	T-1 Residue	12/06/06	06/144/007	06/144/018	06/144/029	06/144/040
	T-1 Control	12/06/06	06/144/008	06/144/019	06/144/030	06/144/041
	T0 Residue	12/06/06	06/144/009	06/144/020	06/144/031	06/144/042
	T3 Residue	15/06/06	06/144/010	06/144/021	06/144/032	06/144/043
	T7 Residue	19/06/06	06/144/011	06/144/022	06/144/033	06/144/044
	T14 Residue	26/06/06	06/144/012	06/144/023	06/144/034	06/144/045
Valencia	T-1 Control	14/06/06	06/144/052	06/144/062	06/144/073	06/144/084
	T-1 Residue	14/06/06	06/144/051	06/144/063	06/144/074	06/144/085
	T0 Residue	15/06/06	06/144/053	06/144/064	06/144/075	06/144/086
	T3 Residue	18/06/06	06/144/054	06/144/065	06/144/076	06/144/087
	T7 Residue	21/06/06	06/144/055	06/144/066	06/144/077	06/144/088
	T14 Residue	29/06/06	06/144/056	06/144/067	06/144/078	06/144/089

Analysis of samples

The following is an extract from the report by SABS compiled by HV Garbers, Manager: Chromatographic Services and PFC van Zyl, Subject Specialist.

Forty-eight samples of citrus, GLP study No. 06/144, were submitted for analysis during 2006. The samples were in a frozen state when received. They were immediately placed in a deep-freeze and were kept under these conditions until the analysis was commenced on 11 December 2006.

Method of test

To determine the malathion (mercaptothion) residue content, the peel and the flesh were analysed separately and the content on the whole fruit calculated. The fruit of each sample was peeled. The peel and the flesh weighed and treated as separate samples. Each sample was shredded in a Stephan food cutter and was mixed thoroughly to render it homogeneous. The analysis was carried out in duplicate employing *SABS In-house Method No. 019/2000 Multi residue Analysis in Citrus.*

Recovery determinations were done by adding known amounts of malathion to portions of untreated control samples and analysing these concurrently with the samples.

Results

Table 3.8.1.4.3. Mercaptothion residue content of fruit samples from Mpumalanga

Trial No.	No. of days after application	Malathion residue content, mg/kg		
		Peel	Flesh	Whole fruit
5	T-1	0,02 ; 0,02	ND ; ND	0,006
	T-1 Control	ND ; ND	ND ; ND	ND
	0	0,17 ; 0,18	ND ; ND	0,05
	3	0,07 ; 0,07	ND ; ND	0,02
	7	0,04 ; 0,04	ND ; ND	0,01
	14	0,03 ; 0,03	ND ; ND	0,008
6	T-1	0,03 ; 0,03	ND ; ND	0,008
	T-1 Control	ND ; ND	ND ; ND	ND
	0	0,18 ; 0,18	ND ; ND	0,05
	3	0,11 ; 0,11	ND ; ND	0,03
	7	0,09 ; 0,08	ND ; ND	0,02
	14	0,04 ; 0,03	ND ; ND	0,009
7	T-1	ND ; ND	ND ; ND	ND
	T-1 Control	ND ; ND	ND ; ND	ND
	0	0,13 ; 0,11	ND ; ND	0,03
	3	0,06 ; 0,06	N ; ND	0,02
	7	0,02 ; 0,02	ND ; ND	0,005
	14	0,01 ; 0,01	ND ; ND	0,003
8	T-1	0,03 ; 0,03	ND ; ND	0,008
	T-1 Control	ND ; ND	ND ; ND	ND
	0	0,18 ; 0,18	ND ; ND	0,05
	3	0,06 ; 0,07	ND ; DN	0,02
	7	0,05 ; 0,05	ND ; ND	0,01
	14	0,02 ; 0,02	ND ; ND	0,005

ND = Not detected/determined

Table 3.8.1.4.4. Mercaptothion residue content of fruit samples from the Eastern Cape

Trial No.	No. of days after application	Malathion residue content, mg/kg		
		Peel	Flesh	Whole fruit
1	T-1	0,15 ; 0,15	ND ; ND	0,04
	T-1 Control	ND ; ND	ND ; ND	ND
	0	0,66 ; 0,67	ND ; ND	0,17
	3	0,15 ; 0,15	ND ; ND	0,04
	7	0,11 ; 0,10	ND ; ND	0,03
	14	0,07 ; 0,07	ND ; ND	0,02

Trial No.	No. of days after application	Malathion residue content, mg/kg		
		Peel	Flesh	Whole fruit
2	T-1	0,83 ; 0,83	ND ; ND	0,23
	T-1 Control	ND ; ND	ND ; ND	ND
	0	1,4 ; 1,3	ND ; ND	0,36
	3	0,89 ; 0,92	ND ; ND	0,24
	7	0,94 ; 0,85	ND ; ND	0,24
	14	0,61 ; 0,60	ND ; ND	0,16
3	T-1	1,2 ; 1,1	ND ; ND	0,34
	T-1 Control	0,78* ; 1,0*	ND ; ND	-
	0	2,6 ; 2,7	ND ; ND	0,76
	3	2,0 ; 2,0	ND ; ND	0,57
	7	0,95 ; 0,96	ND ; ND	0,27
	14	1,6 ; 1,4	ND ; ND	0,42
4	T-1	1,8 ; 1,8	ND ; ND	0,48
	T-1 Control	0,47* ; 0,34*	ND ; ND	-
	0	2,8 ; 2,1	ND ; ND	0,66
	3	2,0 ; 1,8	ND ; ND	0,51
	7	0,95 ; 1,0	ND ; ND	0,30
	14	1, 0 ; 1,1	ND ; ND	0,27

ND = Not detected/determined
Note * = Duplicate sample will be re-analysed

Recovery determinations gave the following mean values:

Table 3.8.1.4.5. Mercaptothion recovery at various levels of insertion

Recovery level (mg/kg)	Recovery (%)	
	Peel	Flesh
0,01	92	76
0,05	91	84
0,10	73	80
0,50	84	83
1,0	92	78

The results reported were corrected for recovery using the value of 86 % (mean).

Under the conditions of test employed the lowest limit of determination was 0,01 mg/kg.

Conclusion

These trials will be repeated in 2007 and the results then used for the determination of a revised PHI for Mercaptothion.

3.8.1.5 **Applaud Residue Trials**
Experiment 892 by Wayne Kirkman and Peter Stephen (CRI)

Introduction

The trials were designed to evaluate the residue levels of the insecticide buprofezin after application of the formulated product Applaud.

Materials and methods

The trials were conducted over the 2006/2007 season only. A total of 2 trials were conducted on oranges: one in the Eastern Cape and one in Mpumalanga.

Treatments consisted of an untreated control and single and double applications of Applaud as per schedule of application. A 1X dosage rate of Applaud equivalent to its current registration on citrus in South Africa

was used. The compound was applied to at least 4 trees per treatment/date as a full cover spray simulating the applications for red scale and mealybug control as used by growers.

Control treatments consisted of untreated trees of the same cultivar, but located at a suitable distance from the test trees.

The applications were made using a suitable, calibrated hand-gun type applicator. The sprayer had suitable agitation and was suitable for accurate measuring and calibration. The sprayer was adjusted to deliver coarse droplets at 20 - 30 bar (2000 – 3000 kPa) pump pressure to enable application of a full cover spray.

The Applaud was applied in a mixture with spray oil, Latron B-1956 (replaced with Agral 90 as Latron B not available) and water as registered. The equivalent of 30 g Applaud, 500 ml Medium range oil and 10 ml Agral 90 per 100 L water was used as the highest registered dosage rate. Application was as a "full cover" spray to wet all above ground parts of the tree.

The spray mixtures were prepared on the day of application. The required amount of Applaud was premixed in a bucket of water to a homogenous slurry and then added to about half the required water in the spray tank. The tank was agitated while the Agral 90 and oil are added and the tank filled to the required volume. The application was made as a "full cover spray" to the point of run-off.

The schedule of applications can be seen in Tables 3.8.1.5.1 and 2.

Table 3.8.1.5.1. Schedule of applications for trial 1 (Mpumalanga)

Treatment number	**Treatment description**	**Date of Application**
1	Single application in **October**	12/10/2006
2	Application in **October** + **November**	12/10/2006 & 09/11/2006
3	Single application in **November**	09/11/2006
4	Single application in **February**	13/02/2007

Table 3.8.1.5.2. Schedule of applications for trial 2 (Eastern Cape)

Treatment number	**Treatment description**	**Date of Application**
1	Single application in **December**	17/12/06
2	Application in **December** and **January**	17/12/06 + 18/01/07
3	Single application in **January**	18/01/07
4	Application in **January** and **February**	08/01/07 + 21/02/07
5	Single application in **February**	21/02/07

Buprofezin residue samples (a minimum of 2 kg) will be taken at the following times after the **last** application in each area.

T0 – After application allowing for drying time. (Including Control)
T30 – 30 days after application
T60 – 60 days after application
T90 – 90 days after application (Including Control)

Conclusion

Residue results will allow for the determination of a PHI and the reintroduction of this product for the control of mealybug after petal fall.

4 PROGRAMME: DISEASE MANAGEMENT

4.1 PROGRAMME SUMMARY

By Paul H. Fourie (Programme Manager)

During 2006, very good progress was made in all the projects in the Disease Management programme. Apart from research, the Project Graft Transmissible Diseases provided essential services for the Citrus Improvement Programme through re-indexing of foundation block trees, shoot tip grafting and pre-immunisation of new entries. For the latter aspects, significant improvements were made to the infrastructure at CRI Nelspruit, which resulted in improved throughput and higher success rates. In 2007, budwood for several new selections will be submitted to the Citrus Foundation Block for multiplication. Trials are under way to select and evaluate new mild CTV strains for pre-immunisation of grapefruit and Valencia oranges. Significant progress was made at UP in developing molecular detection techniques for endemic and exotic virus and viroid pathogens of citrus. These technologies will become essential tools in maintaining biosecurity of the citrus industry in Southern Africa. Moreover, the developed technologies will in future be evaluated for implementation in CIP services. New projects were initiated to investigate host resistance to citrus greening as well as methods of eradicating citrus greening in existing orchards, such as chemical or heat treatment.

In the Citrus Black Spot project, several experiments were completed during this report year. Several projects focused on detection of *Guignardia citricarpa*, specifically through use of a selective growth medium, PCR techniques, and leaf wilting to enhance sporulation of the pathogen. These techniques are integrated in a protocol that is being developed for determination of the CBS status of nursery plants or orchards. Important epidemiological aspects were studied in an attempt to understand and forecast ascospore discharge, host susceptibility and disease development. Good progress is being made in this regard and our improved understanding of this complex disease will aid us in improving management strategies. Strategies that are being studied to improve CBS control include inoculum management and improved spray programmes.

Post Harvest Diseases remain a very high priority and several experiments were directly aimed at improving post harvest disease management in packhouses. Good progress was made, most notably the double application of imazalil (in fungicide bath, and in wax treatment), which resulted in effective residue levels (>1 ppm) that inhibit sporulation of green and blue mould without exceeding the permitted MRL of 5 ppm. Effective imazalil concentrations on fruit will limit post harvest decay, and it will limit the effect of imazalil resistance development. Preliminary screenings have indicated incidences of imazalil and/or guazatine resistance in *Penicillium* spp. and research is continuing to characterise resistance levels and to determine whether these levels constitute practical resistance (i.e. loss of control). Registration trials with a new fungicide to the citrus post harvest arena, pyrimethanil, have shown promising results against post harvest control of blue and green mould. Pyrimethanil is of unrelated chemistry to imazalil and guazatine and will prove invaluable in anti-resistance management strategies. Various trials were also conducted with sanitisers, fungicides and biocontrol agents, and yielded mixed results. Research with regards to alternatives to chemical control is ongoing. Alternatives to 2-4 D were also investigated and certain plant growth regulators showed promising results.

New spray programmes were evaluated in the Fruit and Foliar Diseases Project for control of Alternaria brown spot (ABS) in summer and winter rainfall areas. Effective control was obtained and stippling was reduced by applying a mixture of copper fungicide at reduced concentration in combination of Sporekill. A very exciting development was the effective control of ABS with only three applications of strobilurin fungicides, compared with the standard 8 applications of contact fungicides. Since strobilurin fungicides are high-risk fungicides with regard to resistance development, further studies will be done to investigate effective alternatives to be recommended in an anti-resistance strategy. Collar rot of Clementines in the southern Cape is caused by *Phytophthora citrophthora* and was effectively inhibited through 3 bi-monthly foliar applications of phosphonate in summer, followed by 3 bi-monthly trunk sprays with a Sporekill and captan mixture during winter.

In the Soilborne Diseases Project, several contract trials were conducted. From these trials, invaluable information was obtained regarding the control of nematodes with alternative products. An exciting experiment aims at stimulating nematode egg hatching, which would improve the efficacy of nematicides. Phytotoxicity as a result of foliar application of phosphonate compounds is being investigated, but we are no closer to resolving this mysterious phenomenon. Indications of resistance in *Phytophthora* populations to metalaxyl have indicated the need for evaluation of alternative chemistry for management of root rot in nurseries.

PROGRAMOPSOMMING

Gedurende 2006 is baie goeie vordering in die projekte binne die Siektebestuurprogram gemaak. Buiten navorsing, het die Projek Entoordraagbare Siektes onmisbare dienste aan die Sitrusverbeteringskema gelewer, hoofsaaklik deur herindeksering van moederbome in die grondvesblok, groeipunt-enting en pre-immunisering van nuwe seleksies. Vir laasgenoemde het aansienlike verbeterings aan die toepaslike infrastruktuur by CRI-Nelspruit tot verhoogde deurvloei en beter sukseskoerse gelei. In 2007 sal enthout van verskeie nuwe seleksies aan die grondvesblok gestuur word. Proewe is verder geplant om nuwe matige Tristeza virusrasse vir die pre-immunisasie van pomelo's en Valencia lemoene te selekteer en evalueer. Baie goeie vordering is by UP in ontwikkeling van molekulêre opsporingstegnieke van endemiese en eksotiese virus- en viroied-patogene van sitrus gemaak. Buiten vir toepassing in navorsing, sal hierdie tegnologie gepas vir handhawing van bio-sekuriteit van die sitrus-industrie in Suider-Afrika, asook in die Sitrusverbeteringskema gebruik kan word. Nuwe projekte wat gasheerweerstand van sitrus teen vergroening ondersoek, asook chemiese en hitte-behandelings om hierdie siekte in bestaande boorde te beheer, is begin.

In die Sitrus Swartvlek Projek is verskeie projekte in hierdie jaar voltooi. Van hierdie projekte fokus op die opsporing van *Guignardia citricarpa* deur gebruik te maak van selektiewe groeimedia, PKR tegnieke, en blaarverwelking om sporulasie van die swam te induseer. Hierdie tegnieke word in 'n protokol vir bepaling van swartvlekstatus van kwekerybome en boorde geïntegreer. Belangrike epidemiologiese aspekte word bestudeer om askospoorvrystelling, gasheervatbaarheid en siekte-ontwikkeling te moduleer. Goeie vordering word gemaak en sal tot beter siektebestuurstrategieë lei. Strategieë wat tans ondersoek word behels inokulumbestuur en verbeterde spuitprogramme.

Na-oessiektes is steeds 'n belangrike prioriteit en verskeie eksperimente word op verbeterde beheer in pakhuise gemik. Goeie vordering is veral gemaak met dubbelaanwending van imazalil (in swamdoderbad, en in waksaanwending) om effektiewe residuvlakke (>1 dpm), wat sporulering van groen- en blouskimmel op vrugte inhibeer sonder om die MRL vlakke van 5 dpm te oorskrei, te verseker. Effektiewe imazalilvlakke sal na-oes bederf asook ontwikkeling van fungisiedweerstand verminder. Voorlopige resultate het gewys dat imazalil en/of guazatine weerstand in *Penicillium* spp. voorkom. Navorsing om weerstandsvlakke te karakteriseer en te bepaal of hierdie vlakke tot praktiese weerstand (verlies aan beheer) sal lei, duur voort. Registrasie-proewe met 'n nuwe swamdoder in die sitrus na-oes arena, pyrimethanil, lyk belowend. Pyrimethanil is nie chemies verwant aan imazalil of guazatine en sal dus 'n belangrike produk in teen-weerstand strategieë wees. Verskeie ander proewe is ook met saniteerders, swamdoders en biologiese beheeragente met gemengde resultate uitgevoer. Navorsing rakende alternatiewe tot chemiese produkte gaan voort. Alternatiewe tot 2-4 D is ondersoek, en sekere plantgroeireguleerders lyk belowend.

In die Vrug en Blaarsiekte Projek is nuwe spuitprogramme teen Alternaria bruinvlek in somer en winterreëngebiede ge-evalueer. Effektiewe beheer is met 'n mengsel van Sporekill met koper teen verlaagde dosis verkry, en boonop is koper-stippeling ook voorkom. 'n Deurbraak is vir beheer van hierdie siekte gemaak deur slegs 3 toedienings met strobilurien-middels, in plaas van 8 kontakmiddels. Siende dat strobiluriene hoë-weerstandsrisiko produkte is, sal verdere werk fokus op effektiewe alternatiewe vir gebruik in 'n teen-weerstand strategie. Kraagvrot van Clementines in die suid-Kaap is veroorsaak deur *Phytophthora citrophthora* en is effektief gestuit met 3 twee-maandelikse blaarbespuitings met 'n fosfonaat in die somer, gevolg deur 3 twee-maandelikse stambespuitings met 'n Sporekill+kaptan mengsel gedurende die winter.

Verskeie kontrakproewe is in die Grondgedraagde Siektes projek gedoen. Uit hierdie proewe is waardevolle inligting oor beheer van aalwurms met alternatiewe produkte versamel. 'n Uitdagende eksperiment ondersoek die stimulering van aalwurmeiers om sodoende beheer met aalwurmdoders te verbeter. Fitotoksisiteit na aanleiding van blaartoedienings met fosfonate is ondersoek, maar ons is steeds nie nader aan 'n antwoord op hierdie probleem nie. Aanduidings van weerstand in *Phytophthora* populasies teen metalaxyl dui op die behoefte om alternatiewe chemie vir beheer van wortelvrot in kwekerye te ondersoek.

4.2 PROJECT: GRAFT TRANSMISSIBLE DISEASES

Project co-ordinator: S.P. van Vuuren (CRI)

4.2.1 Projekopsomming

'n Fitosanitêre program vir entoordraagbare siektes is uiters noodsaaklik vir die sitrusbedryf aangesien siektes die produktiewe lewe van bome verkort. Hulle het ook 'n nadelige uitwerking op die gehalte van vrugte wat bemarking en inkomste beïnvloed. Daar is meer as 40 entoordraagbare siektes in die wêreld bekend waarvan ongeveer 16 in Suid Afrika voorkom. Van die uitheemse siektes is uiters gevaarlik vir die bedryf en dit is noodsaaklik dat hulle uit die land gehou word. Die mees belangrikste siektes in Suid Afrika is

Citrus tristeza virus (CTV), Sitrus viroide (eksokortis, cachexia, gomsaksiekte), Psorose virus, Appelstam groefvirus (voorheen "Citrus tatter leaf" virus), Impietratura en Sitrusskroei ("Citrus Blight"). Indeksering (vasstelling van die teenwoordigheid) van die siektes word hoofsaaklik biologies gedoen deur indikatorplante (cultivars wat gevoelig is). Indikatorplante van elk van die siektes vereis spesiale temperature wat dit genoodsaak om indeksering in temperatuurbeheerde glashuiskamers te doen. Die indekseringstyd varieer van 6 tot 12 maande, afhangende van die siekte, en vertraag beheermaatreëls.

'n Roetine opsporingsmetode van CTV is gevestig en die volgende Polimerase-ketting reaksie (RT-PKR) sisteme is ge-optimiseer vir die opsporing van CTV in plante: 1) 'n RT-PKR sisteem wat die vermoëns het om alle virusse wat in die *Closteroviridae* familie, waaronder CTV sorteer, op te spoor; 2) 'n multipleks RT-PKR gemik teen die p23 geen van CTV wat rasse kan onderskei in ligte, strawwe of atipiese vorms; 3) 'n stel van 23 PKR sisteme wat CTV in vier genotipes onderskei. Suksesvolle 5' ent-kleuring van CTV amplikone met die kleurstof Cy3 het toegelaat dat hibridisasie van rasse CTV-T36 (straf) en CTV-T30 (lig) deur middel van 'n "micro-array" skyfie, ontwerp in 2005, gedemonstreer kon word (afdeling 4.2.2). CTV virus partikels is gesuiwer met die doel om 'n antiserum te berei en ELISA te ontwikkel, maar die virus is nie in hoog genoeg konsentrasie en suiwerheid verkry vir die doel nie (afdeling 4.2.2). Twee multipleks PKR sisteme is ontwikkel om die opsporing van Liberibacter sp. bakterieë te verbeter. Rutaceae plante is getoets as alternatiewe gasheer bronne vir *"Candidatus."* L. africanus (Afrika vergroening). Geen van die plante het tot dusver positief getoets nie. 'n Groot aantal monsters wat tydens 'n landwye opname van vergroening versamel is, is getoets. Geen bronne van "Ca" L. americanus (Brasiliaanse vergroening) is gevind nie. Die konsentrasie van amplikone verkry uit verskeie bronne het dramatiese gewissel en dit was moeilik om tussen *"Ca."* L. africanus sowel as *"Ca."* L. asiaticus (Asiatiese vergroening) in 'n multipleks PKR reaksie te kon onderskei (afdeling 4.2.2). 'n PKR met breë spesifisiteit teen Ophio virusse is ge-implementeer vir die opsporing van sitrus psorose virus. Dit is slegs teen DNA getoets en moet aangepas word om die virus in plante te kan opspoor (afdeling 4.2.2).

Biologiese indeksering is gebruik om die CTV strafheid in die moederbome van die Sitrus Grondvesblok te bepaal. Strawwe rasse is in sommige moederbome geïdentifiseer. Die bome sal getermineer word as enthoutbronne. 'n Groot aantal moederbome (hoofsaaklik losskil tipes) het negatief getoets. Die rede is onbekend maar dit kan wees dat die gashere die spesifieke CTV isolaat onderdruk en sodoende vermeerdering en beweging beperk. Ondersoek word ingestel na 'n alternatiewe kruisbeskermings-isolaat. Sitrus material wat van kwekers af ontvang is, of wat tydens besoeke versamel is, is ook op indikatorplante sowel as met die Kol Klad serologies metode geïndekseer (afdeling 4.2.3). Twee en twintig nuwe cultivars is gedurende die jaar by die 213 cultivars van die genebron gevoeg. 'n Groot aantal cultivars en seleksies wag op groeipunt-enting (afdeling 4.2.4). Die Marsh en Star Ruby boompies wat in Swaziland en die Nkwaleni Vallei geplant is, is nou 3 jaar oud en daar is aanduidings dat van die sub-isolate groei strem en met ander is daar interaksies tussen die bostamme. Sommige van die Marsh bome is soortgelyk aan die bome met die GFMS 12 isolaat wat 'n strawwe isolaat huisves. Die Marsh bome met die GFMS 35 isolaat, wat gebruik word om rooi pomelos te preïmmuniseer, lyk beter as bome met die GFMS 12 (isolaat vir wit pomelos) isolaat. Die eksperiment bevestig dat GFMS 12 nie 'n geskikte isolaat vir Star Ruby pomelos is nie. Oor die algemeen lyk bome met B390/3 sub-isolaat die beste (afdeling 4.2.5). Star Ruby boompies wat met dieselfde isolate en sub-isolate as in die proewe in afdeling 4.2.5 geïnokuleer is, is in die Kakamas omgewing geplant. Die bome toon tans geen effek van die verskillende CTV inokulasies nie (afdeling 4.2.6). Nuwe belowende ligte isolate wat in verskillende pomelo produksiegebiede versamel is word gebruik om Marsh en Star Ruby boompies te preïmmuniseer. Die boompies is gereed om geplant te word (afdeling 4.2.7). Agt-jaar oue bome van sewe rooi pomelo seleksies, nl. Star Ruby, Rio Red, Henderson, Nel Ruby, Flame, Ruben en Oran Red op Swingle citrumelo onderstam, het baie eenvormig gereageer met vier ligte *Citrus tristeza virus* (CTV) isolate (GFMS 12, GFMS 35, GFMS 67, GFMS 73) as kruisbeskermings-agente. Tussen die CTV isolate was daar nie verskille in boomgrootte nie. Tussen die pomelo seleksies was Nel Ruby bome die grootste en Oran Red bome die kleinste. Dit is moontlik dat Oran Red 'n genetiese dwerg eienskap het. Daar is aanduidings van interaksies tussen sommige CTV isolate en pomelo seleksies (Rio Red met GFMS 12; Flame met GFMS 35). Die strawwe isolaat (GFSS 5) het nie die kroon volume van die Ruben bome geaffekteer nie, wat 'n aanduiding is dat die seleksie tolerant is teen CTV. Die kumulatiewe produksie oor 3 jaar toon dat Star Ruby bome met GFMS 35 swakker is as bome met die ander ligte isolate. Dit is in teenstelling met verskeie vorige bevindings (afdeling 4.2.8).

Dit is voorheen genoem dat kruisbeskerming voordelig is by die sogenaamde tolerante sitrus cultivars. Studies om geskikte CTV isolate vir kruisbeskerming vir hierdie sitrusgroep te identifiseer, gaan voort. Vruggrootte is 'n groot probleem by Clementines in die Oos– en Wes-Kaap en daar was 'n versoek om die invloed van kruisbeskerming op Clementines te bepaal. 'n Proef is by die Addo Navorsingstasie gevestig waar gepreïmmuniseerde en virusvrye bome van sewe Clementine seleksies en 'n Satsuma seleksie vergelyk word. Boomgroottes is bepaal en alhoewel die proef nog te jonk is om gevolgtrekkings te maak, wil dit voorkom of al die Clementine seleksies nie dieselfde reageer op CTV besmetting nie (afdeling 4.2.9). In

die nawel proef op Addo Navorsingstasie het bome wat met LMS 6 isolaat (huidige kruisbeskermings-isolaat) gepreïmmuniseer was, die beste presteer oor 'n 7-jaar tydperk. Bome met hierdie isolaat het 'n 32% hoër kumulatiewe produksie oor die laaste 3 jaar gehad as bome wat virus-vry geplant was (afdeling 4.2.10). Daar is gevind dat Turkey Valencia blykbaar meer gevoelig is vir CTV en daarom word daar gepoog om 'n geskikte CTV isolaat vir hierdie soetlemoen seleksie te kry. Preïmmunisasie met nuwe CTV isolate wat vanaf soetlemoene versamel is, is deur middel van ELISA bevestig en die boompies is gereed om uitgeplant te word (afdeling 4.2.11). Die effek van verskillende ligte CTV isolate word in drie Valencia bostamme (Delta, McClean, McClean Saadloos) ge-evalueer. Boomgrootte van McClean Valencia was betekenisvol kleiner as die van Delta – en McClean Saadloos Valencias. Oor die algemeen was produksie weer swak met uitermatige klein vrugte. Die bome toon egter herstel na die swak toestand waarin hulle was die vorige jaar. Die data van hierdie jaar varieer egter nog baie en daar kan nie onderskeid getref word tussen goed en swak nie (afdeling 4.2.12). Ligte CTV isolate vanaf soetlemoenbome word in vyf Valencia bostamme ge-evalueer (Delta, Midknight, McClean, McClean Saadloos, Turkey). Preïmmunisasie is deur middel van ELISA bevestig en die boompies is gereed om uitgeplant te word (afdeling 4.2.13).

In die proef waar 17 onderstamme ge-evalueer word vir sitrusskroei ("Blight") toleransie, toon bome op Sun Chu Sha en Orlando tangelo onderstamme die meeste toleransie en kwekers moet hierdie onderstamme oorweeg in sitrusskroei gebiede. Bome op C35 en Sunki mandaryn onderstamme is die meeste ge-affekteer deur die siekte (afdeling 4.2.14).

Daar word gepoog om Haunglongbing (vergroening) weerstandbiedendheid te verkry deur embrio's uit gesonde chimeras van vergroende vrugte te verwyder en op kunsmatige medium te kweek. Twee klone, E2 en T2, is in 2006 geïdentifiseer as simptoomloos na blootstelling aan die vektor. PKR is nog nie op die plante toegepas nie. Die klone is op onderstamme vermeerder en afsonderlik met twee CTV isolate ge-preïmmuniseer om in die boord ge-evalueer te word. Vier nuwe klone is die afgelope jaar gegenereer. Sodra hulle groot genoeg is, word die klone vermeerder en aan die vektor blootgestel. Die eksperiment is ook daarop gemik om die gene wat weerstandbiedendheid of toleransie teen vergroening aan die plante verleen te identifiseer (afdeling 4.2.15). Twee potproewe en 'n boord proef is begin om chemiese en/of hittebehandeling in vergroeningsbesmette bome te evalueer. Die proewe is nog aan die gang en geen resultate is beskikbaar nie. Die finale evaluasies sal gedurende die komende winter en lente gedoen word. Die resultate sal bepaal wat die opvolg navorsing sal behels (afdeling 4.2.16).

Project summary

A phytosanitary programme for graft transmissible diseases is essential for the citrus industry since diseases reduce the productive life of trees. They also have a detrimental effect on fruit quality that influences the market and income. There are more than 40 graft transmissible diseases known in the world of which approximately 16 occur in South Africa. Some of the foreign diseases are very dangerous for the citrus industry and it is important that they should be kept out of the country. The most important diseases in South Africa are *Citrus tristeza virus* (CTV), Citrus viroids (Exocortis, Cachexia, Gum pocket), Psorosis virus, Apple stem grooving virus (formerly Citrus tatter leaf virus), Impietratura and Citrus Blight. Indexing (determination of the presence) of the diseases is mainly done biologically through the use of indicator plants (cultivars that are sensitive). Indicator plants for each of the diseases require specific temperatures, which necessitates the use of temperature-controlled glasshouse rooms. The indexing time varies from 6 to 12 months and delays the application of control measures.

A routine detection method has been established and the following polymerase chain reaction (RT-PCR) systems capable of detecting CTV RNA in plants could be implemented,: 1) a RT-PCR system that is able to detect all members of the *Closteroviridae* (the Family to which CTV belongs); 2) a multiplex RT-PCR directed at the p23 gene and capable of differentiating CTV strains into severe, mild and atypical categories; 3) a 23 PCR system used to classify CTV into 4 recognised genotypes. Successful 5' end-labeling of CTV amplicons with Cy3 allowed hybridisation of CTV-T36 and CTV-T30 strains to the micro-array chip designed in 2005 to be tested (section 4.2.2). CTV particles were purified to initiate immunisations of animals but insufficient virus of the required purity was obtained in order to obtain antisera for ELISA (section 4.2.2). Two multiplex PCR systems were developed to improve the detection of "*Candidatus*" Liberibacters. Rutaceae plants were tested as alternate hosts for *"Ca."* L. africanus (African greening). None of the plants tested thus far, contained this bacteria. A large number of samples collected during a country-wide survey of greening were tested. No sources of *"Ca."* L. americanus (Brazilian greening) were found. Amplicons obtained in the samples varied widely in concentration and no differentiation of *"Ca."* L. africanus and *"Ca."* L. asiaticus (Asiatic greening) could be made in a single multiplex PCR (section 4.2.2). A PCR system with broad specificity to Ophio viruses was implemented for the detection of citrus psorosis virus. This was tested against DNA and still needs to be refined (section 4.2.2).

Biological indexing was used to determine the severity of CTV in mother trees at the Citrus Foundation Block. Severe strains were identified in some mother trees. These trees will be terminated as budwood sources. A large number of mother trees (the majority soft citrus types) tested negative. The reason is unknown but it is possible that the hosts restrict the multiplication and movement of the specific CTV isolate. An investigation to find an alternative cross-protecting isolate is in progress. Citrus samples received from growers or collected during visits, were also indexed using indicator plants as well as a serological Dot Blot system (section 4.2.3). Twenty-two new cultivars were added to the 213 cultivars in the gene source this year. A large number of cultivars and selections await STG (section 4.2.4). The Marsh and Star Ruby trees that were planted in Swaziland and the Nkwaleni Valley are now 3 years old and there are indications that some sub-isolates reduced growth and with others, and there are some interactions with the scions. Some sub-isolates suppressed growth of the Marsh trees and these trees are similar to the trees with GFMS 12, which is known to carry a severe strain. The Marsh trees with GFMS 35, which is the red grapefruit pre-immunising isolate, are better than trees with GFMS 12, the white grapefruit pre-immunising isolate. The experiment confirms that GFMS 12 is not a good pre-immunising isolate for Star Ruby grapefruit. Trees with B390/3 sub-isolate performed the best overall (section 4.2.5). Star Ruby trees that were pre-immunised with the same isolates and sub-isolates as the trials in section 4.2.5 were planted in the Kakamas region. The trees are not showing any affect of the CTV inoculations yet (section 4.2.6). New promising mild isolates that were collected in different grapefruit production areas, are being used to pre-immunise Marsh and Star Ruby trees. The trees are ready for planting (section 4.2.7). Seven-year-old trees of seven red grapefruit selections, viz. Star Ruby, Rio Red, Hendersen, Nel Ruby, Flame, Ruben and Oran Red reacted very similar to four CTV isolates (GFMS 12, GFMS 35, GFMS 67, GFMS 73) as cross-protecting agents. . Between the grapefruit selections the Nel Ruby trees were the largest and the Oran Red trees the smallest. It appears that Oran Red contains a genetic dwarfing characteristic. There are indications of interactions between CTV isolates and the grapefruit selections (Rio Red with GFMS 12; Flame with GFMS 35). The severe isolate (GFSS 5) did not affect the canopy volumes of the Ruben trees and it is an indication of CTV tolerance. The cumulative production over three years shows that the Star Ruby trees with GFMS 35 were poorer than trees with the other isolates. This is in contradiction of several previous findings (section 4.2.8).

It was mentioned previously that cross-protection is advantageous for the so-called tolerant citrus cultivars (sweet oranges and mandarins). Studies to obtain suitable CTV isolates for pre-immunisation are continuing. Fruit size is a great problem with Clementines in the eastern and western Cape and there was a request to evaluate the effect of pre-immunisation on fruit size. A trial was established at Addo Research Station where pre-immunised trees of seven Clementine selections are compared to trees planted virus-free. Tree sizes were determined but it is too early to make any conclusions, however, it appears that the different Clementine selections do not re-act the same to CTV infection (section 4.2.9). In the navel trial at Addo Research Station, trees pre-immunised with LMS 6 (the present pre-immunising agent for the industry) performed the best over a 7-year period. Trees with this isolate had a 32% better cumulative production than trees that were planted virus-free (section 4.2.10). It was found that Turkey Valencia is apparently more sensitive to CTV than other Valencia cultivars. CTV isolates that were derived from sweet orange trees will be evaluated as cross-protectors. Pre-immunisation has been confirmed by Elisa and the trees are ready for planting in the field (section 4.2.11). The effect of different CTV isolates, derived from sweet orange, is being evaluated in three Valencia scions (Delta, McClean, McClean Seedless). Tree size of McClean was significantly smaller than those of Delta and McClean Seedless. Generally the yield was poor again with an excessive production of small fruit. The trees show recovery after the poor condition they were in during the previous year. The present data still vary a lot and differentiation between good and poor can not be made (section 4.2.12). Mild CTV isolates derived from sweet orange trees will be evaluated in five Valencia scions (Delta, Midknight, McClean, McClean Seedless, Turkey). Pre-immunisation has been confirmed by ELISA and the trees are ready for planting in the field (section 4.2.13).

In the trial where 17 rootstocks are evaluated for Citrus Blight tolerance, trees on Sun Chu Sha and Orlando tangelo rootstocks appear to exhibit the most tolerance and growers should consider these rootstocks in CB areas. C35 citrange and Sunki mandarin rootstocks appear to be the most sensitive (section 4.2.14).

Attempts are made to generate resistant plants against Huanglongbing (greening) by the rescuing of immature embryos out of healthy chimeric sectors in fruits infected with greening. Two clones, E2 and T2 were identified in 2006 as being symptomless after challenging them with the vector. PCR has not been employed on the plants as yet. These clones were multiplied on rootstocks and pre-immunised separately with two *Citrus tristeza virus* isolates for orchard evaluation. Four new clones were generated in 2007, which will be multiplied and challenged with the vector. The experiment also aims to identify the gene/genes that assist the plant to be tolerant or resistant against greening (section 4.2.15). Two pot trials and a field trial to assess chemical and/or heat treatments of infected trees were initiated. The trials are ongoing and no results are available. The final evaluations will be done during winter and when spring commences. The results will determine if any future research is necessary (section 4.2.16).

4.2.2 **Establish diagnostic capabilities to graft transmissible pathogens of Citrus at CRI-UP, with emphasis on *Citrus tristeza virus* variants**

Experiment 783 by Prof. G. Pietersen, Katherine Stewart, Baby Phaladira and J. Fouche (CRI at UP)

Opsomming

Hierdie verslag dek die vordering in die tweede jaar van 'n twee-jaar projek om vinnige, betroubare opsporing- en identifisering metodes te ontwikkel teen entoordraagbare patogene van sitrus. Die hoofdoel was om 'n plaaslike diagnostiese kapasiteit te vestig wat hoofsaaklik gebaseer is op die polimerase ketting reaksie (PKR) tegniek teen hierdie siektes. Die klem val op die opsporing van *Citrus tristeza virus* (CTV) en Liberibacter spp. bakterieë wat Huanglongbing (vergroening) op Sitrus veroorsaak, aangesien hierdie die twee belangrikste plaaslike siektes van Sitrus is, maar daar word ook diagnostiese tegnieke ontwikkel teen belangrike eksotiese sitrus patogene. 'n Tweede doelwit was om die variasie wat bestaan in die kruisbeskermende CTV isolate in Suid-Afrika te bepaal m.b.v. PKR en dan ook om 'n "Micro-array" te ontwikkel en te evalueer vir gebruik vir dieselfde doel. Dit is belangrik om CTV op verskeie taksonomiese vlakke te kan opspoor (Familie, Genus, spesie, ras), bv. na virus reiniging moet geen vorm van CTV meer voorkom nie en moet alle rasse, selfs die waarvan die nukleotied volgorde nie bekend is nie, ook opgespoor kan word. Daarenteen, studies oor die dinamiek tussen kruisbeskermende en ander rasse verg dat hulle gemonitor moet word met ras spesifieke PKR tegnieke. Om hierdie doelwitte te bereik is verskeie gepubliseerde PKR tegnieke teen CTV tydens 2005 gevestig en geïmplementeer vir opsporing teen DNA. Roetine opsporing van die virus in plante kon egter nie uitgevoer word nie aangesien die cDNA sintese stap nie betroubaar was nie. Sedertdien is 'n nuwe, betroubare cDNA sintese sisteem gevestig en kon die volgende PKR sisteme geoptimiseer word vir die opsporing van CTV in plante: 1) 'n RT-PKR sisteem wat die vermoëns het om alle virusse wat in die *Closteroviridae* familie sorteer (insluitend CTV) op te spoor. Die vermoë van die sisteem is bevestig deur te bewys dat beide CTV sowel as "grapevine leafroll associated virus type 3" (GLRaV-3), van die Ampelovirus genus binne in die *Closteroviridae*, deur die metode opgespoor kon word; 2) 'n multipleks RT-PKR gemik teen die p23 geen van CTV wat rasse kan onderskei in ligte, strawwe of atipiese vorms; 3) 'n stel van 23 PKR sisteme wat CTV in vier genotipes onderskei. Die laaste twee sisteme is gebruik om verskeie plaaslike CTV bronne te toets en te klassifiseer. Ligte, strawwe sowel as atipiese rasse is verkry vanaf plaaslike "ligte" kruisbeskermingsisolate en sub-isolate daarvan. Verder is verteenwoordigers van elk van die 4 bekende genotipes se verskeie molekulêre merkers plaaslik gevind. Hierdie is gereeld in kombinasies gevind wat dui op die teenwoordigheid van meer as een ras in die plant of van rekombinasie tussen verskeie genotipes. Suksesvolle 5'ent-kleuring van CTV amplikone met die kleurstof Cy3 het toegelaat dat hibridisasie van rasse CTV T36 en CTV T30 met 'n "micro-array" skyfie, ontwerp in 2005, gedemonstreer kon word. CTV virus partikels is ook op vier geleenthede gesuiwer met die doel om 'n antiserum te maak en ELISA te ontwikkel, maar 'n hoog genoeg konsentrasie en suiwerheid van die virus is nie verkry vir die doel nie. Verdere suiwerings word beoog in 2007. Twee multipleks PKR sisteme is ontwikkel om die opsporing van Liberibacter sp. bakterieë te verbeter. In die eerste sisteem is voorvoerders wat die vermoëns het om 'n proteïen wat universeel in plante voorkom te kan opspoor gekombineer met die A2/J5 voorvoerders wat beide *"Ca."* L. africanus sowel as *"Ca."* L. asiaticus kan amplifiseer. Aangesien die sisteem altyd 'n amplikon teen plant monsters sal lewer, dien hierdie as 'n kontrole dat DNA ekstraksie suksesvol was en dat daar nie plant inhibitore is wat die PKR affekteer nie. Negatiewe resultate teen Liberibacters kan dus vertrou word. Die sisteem is gebruik vir die analise van Rutaceae alternatiewe gasheer bronne vir *"Ca."* L. africanus. Geen van die Rutaceae plante het tot dusver positief getoets nie. Die tweede mulipleks PKR sisteem kombineer voorvoerders A2/J5 saam met GB1/GB3 voorvoerders wat "*Ca*." L. americanus spesifiek opspoor (Bové persoonlike kommunikasie). Al drie Liberibacter spesies kan dus saam opgespoor word in een toets. Hierdie sisteem is gebruik om 'n landwye opname uit te voer vir die voorkoms van vergroening. Geen bronne van "Ca." L. americanus is gevind nie. Die konsentrasie van amplikone verkry uit 197 bronne het dramatiese gewissel en dit was moeilik om tussen *"Ca."* L. africanus sowel as *"Ca."* L. asiaticus te kon onderskei op jelle. 'n Alternatiewe tegniek sal in 2007 hiervoor gebruik word. 'n PKR met breë spesifisiteit teen Ophioviruses is ge-implementeer vir die opsporing van sitrus psorose virus. Dit is slegs teen DNA getoets en moet aangepas word om die virus in plante te kan opspoor. Reagense, positiewe DNA kontroles en voorvoerders teen sitrus viroïede is verkry en ontwikkeling van die PKR word kortliks beoog.

Introduction

This progress report covers the second year of a two year project to develop rapid, reliable detection and identification methods to graft transmissible pathogens of citrus. These techniques are fundamental to establishing strategies for the management and control of the pathogens, whether by phytosanitary legislation, certification schemes, vector control, resistance selection and breeding, or cultivation practices. Development of such techniques is also an essential first step in most research actions involving such pathogens, and as such is regarded as the initial aims of the Citrus Virology programme of CRI at the

University of Pretoria. As an increasing number of PCR's with their primer sequences are being described in the literature. These techniques can be adapted for local use and implemented relatively rapidly. A first aim of this project is therefore to establish a local diagnostic capability (mainly PCR-based) to detect the major graft transmissible diseases of citrus. In this regard the emphasis is on techniques to detect *Citrus tristeza virus* (CTV) and Liberibacter spp., the bacteria causing Huanglongbing (Citrus greening), and then secondarily serious exotic pathogens. In addition, to address the control of CTV by cross protection, and to exploit the probable underlying mechanism of RNA-silencing, it is imperative that information on the variability of CTV be obtained locally. The new technique of micro-array analysis is ideally suited to this. The second aim of this project therefore was to assess the variability of the mild strain cross protecting CTV isolates used in South Africa, using PCR and to assess the usefulness of a micro-array based system, which will ultimately differentiate strains utilizing the entire viral genome.

Materials and methods

The nature of this project, viz. primarily the development, establishment and implementation of various detection techniques makes a lumping together of disparate aims within one Methods and Materials as well as a separation of "Methods and material" and "Results and discussion" confusing and cumbersome. Furthermore the implementation and development nature often makes the information very technical, therefore in this report, methods and materials are incorporated within the results and discussion sections for each separately defined aim.

Results and discussion

Detection of *Citrus tristeza virus* (CTV)

Citrus tristeza virus (CTV) is the most important virus of citrus and its detection on different taxonomic levels (virus Family, genus, species and strains) is required to develop increasingly effective methods of control. For example, a method capable of detecting all members of the *Closteroviridae*, the family to which CTV belongs, allows theoretical detection of all variants of CTV, irrespective of whether their sequences are known in advance. This technique would be suited for use in the certification scheme in testing plants following virus-elimination. In contrast, detection of specific strains of CTV will allow studies on strain cross protection dynamics to be performed, and allow increasingly effective cross protection strains to be selected or improved control strategies to be employed. Use of CTV ELISA expands the repertoire of tests, allowing the testing of large numbers of samples simultaneously, and is ideal for the large-scale routine screening of citrus mother-block trees.

1) Aim: Establish and assess PCR for the group specific detection of closteroviruses (for the wider range detection of CTV strains)

This task was entrusted to a Hons. student, Mr. Johan Fouche, during 2006. The PCR method is that of Dovas & Katis (2003), originally utilised in detection of viruses in grapevines related to CTV. In order to test the efficacy of locally synthesised primers and the local preparations of reagents, amplification was tested on CTV-T30 and CTV-T36 DNA templates (plasmids obtained from Dr. Gowda, CREC,IFAS, University of Florida) both in the first and second of the nested PCR steps. Amplification of products of the expected size was successfully obtained (Fig. 4.2.2.1) and sequencing of the nucleotides of these products confirmed the specificity of the product. The system was also optimised for local use with regard to the required magnesium chloride concentration and the appropriate annealing temperature (Fig. 4.2.2.2). The reverse transcriptase step of this PCR, however, initially proved to be problematic and a fair amount of time was spent adapting this. The problem was overcome through the use of a cDNA synthesis protocol developed by Herron (2003), which was adapted for local use by Katherine Stewart, as well as the use of a two-step RT-PCR test. The procedure follows: 12 ul of total RNA is used as template for the reverse-transcriptase step. The RNA is heated for 15 minutes at 65°C, 10 minutes at 55°C and 5 minutes at room temperature. Thereafter a mix consisting of 50 pmol reverse primer, 5U of RNAsin (Promega, USA), 10U AMV reverse transcriptase (Roche, Germany), 1x AMV buffer (Roche, Germany) and 0.2 mM dNTP was added to the RNA to make a final volume of 26 µl. The reaction is heated to 47°C for 1 hour, and 12 ul of molecular grade water added afterwards.

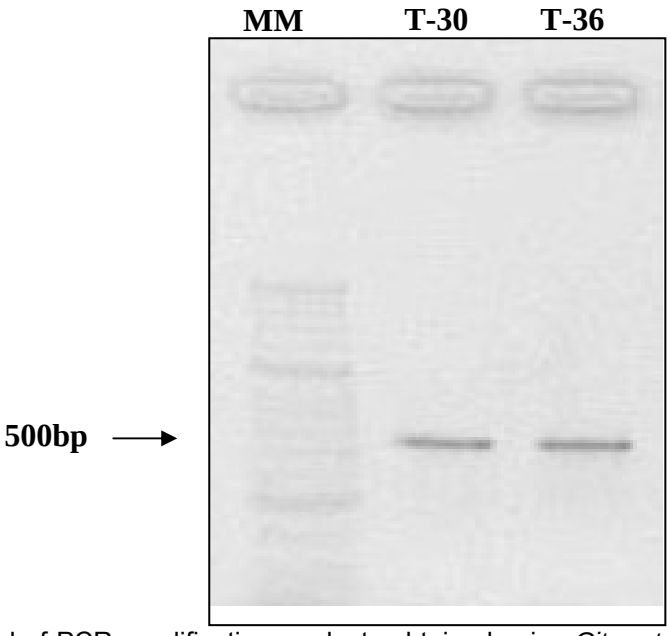

Figure 4.2.2.1. Agarose gel of PCR amplification products obtained using *Citrus tristeza virus* T30 and T36 containing plasmid clones as templates.

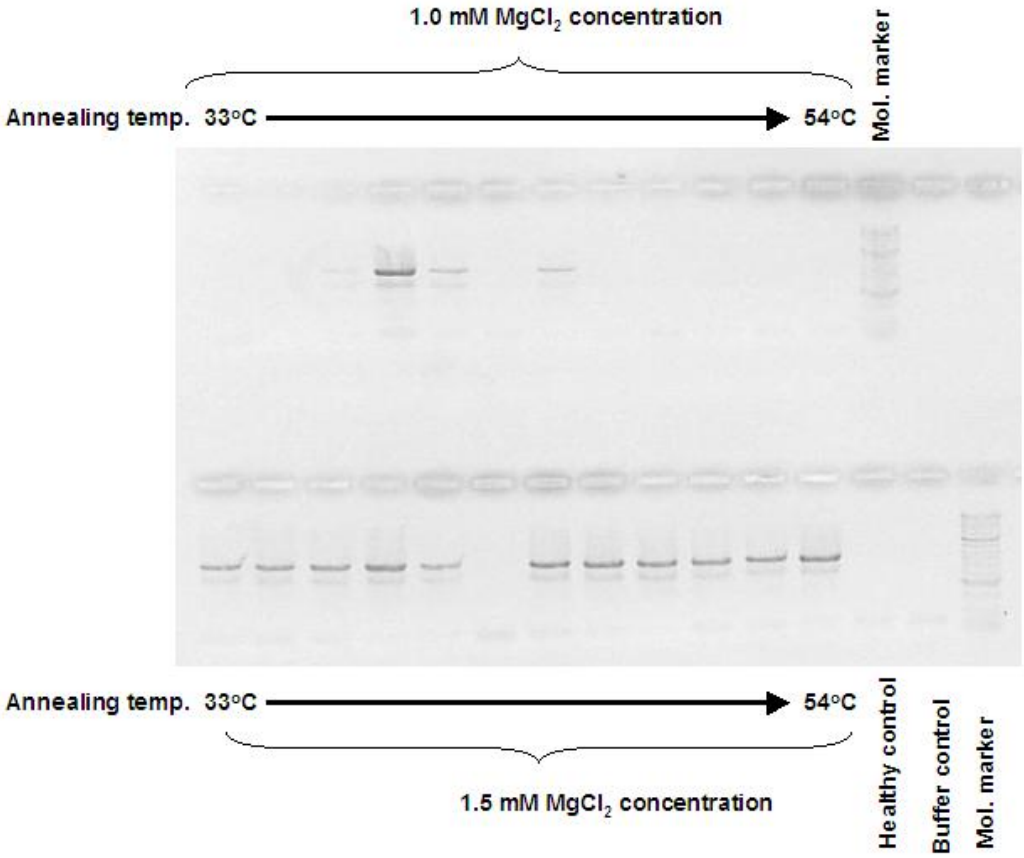

Figure 4.2.2.2. Agarose gel of amplicons obtained following PCR under differing annealing temperatures on either 1mM (upper wells) or 1.5mM (lower wells) magnesium chloride.

The very broad specificity of the PCR system was confirmed by testing its ability to detect two diverse members of the *Closteroviridae* viz. grapevine leaf roll associated virus type 3 (GLRaV-3, an Ampelovirus), and CTV, a Closterovirus. Bands of the expected size were obtained with some preparations of both viruses but not of all (Fig. 4.2.2.3). Furthermore, some smaller, unexpected bands were also obtained. The origin of

these could not be determined during the student's project by sequencing, but they may represent the products of virus-specific primer binding to defective interfering particles or regions of partial homology within the virus. The technique can thus be used for routine testing but the anomalous results need further investigation.

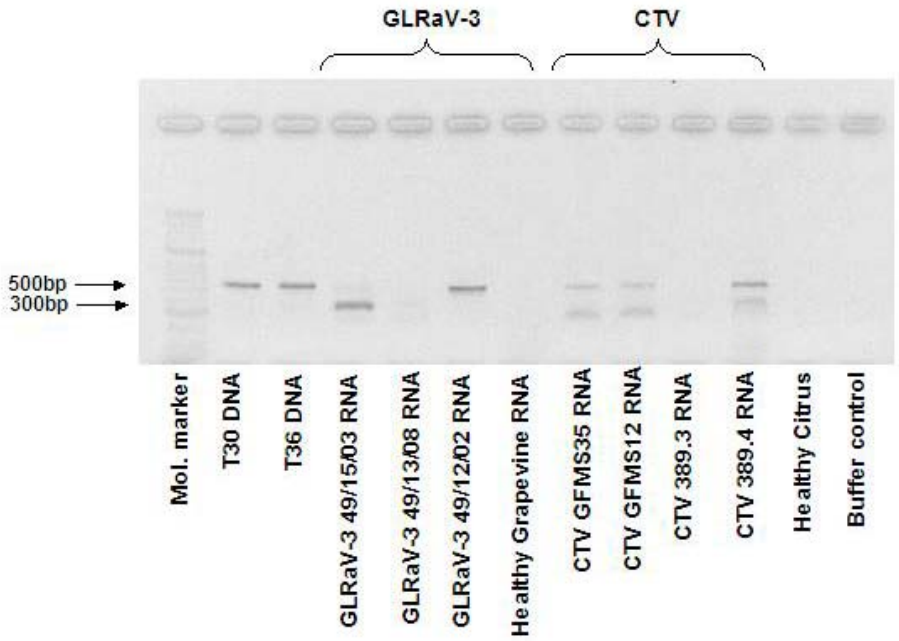

Figure 4.2.2.3. Agarose gel of PCR amplicons obtained with a Closterovirus-wide primer set. Range of detection illustrated by the ability to detect both grapevine leaf roll associated virus type 3 (GLRaV-3, an ampelovirus) and *Citrus tristeza virus* (the type member of the Closteroviruses).

2) Aim: Establish a functional, routine use CTV-specific PCR

With the implementation of a successful reverse transcriptase step for *Citrus tristeza virus* (see above), the primer set of Huang *et al.* (2005) can now be utilised for routine use. This specific RT-PCR was not assessed during the report period. However, the bi-directional RT-PCR system of Sambade *et al.* (2003), was implemented by Me. Katherine Stewart. This PCR detects all known CTV isolates, but also differentiates them into mild, severe and atypical groups based on differences in nucleotides that code for amino acid in positions 78-80 of the p23 gene. Two local cross protecting isolates, GFMS 12 and GFMS 35, and eight single aphid sub-isolates were tested and classified into strain groups (Fig. 4.2.2.4). The local isolates were also sequenced w.r.t. the p23 gene and compared to reference isolates in a phylogenetic study (Fig. 4.2.2.5). The predicted amino acid sequences were also compared for areas of possible variability for further strain differentiation. RSA isolates 390-3 and 390-5 were atypical; 390-4, 389-4 and 389-3 were mild; GFMS 35 had mild and atypical isolates; GFMS12, 12-7 and 12-9 had mild and severe isolates and; 12-5 was severe.

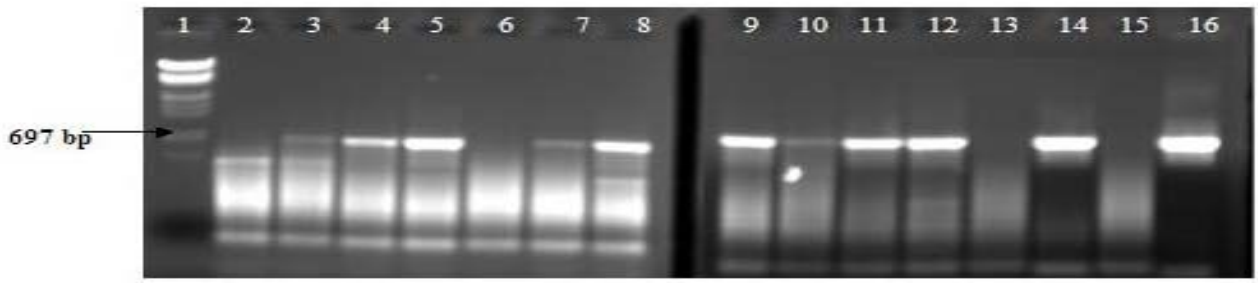

Figure 4.2.2.4. Agarose gel photo of CTV samples amplified with conserved p23 gene primers (Sambade, López *et al*., 2003). Lanes: (1) Molecular Marker 100 bp Lambda; (2) T30 plant; (3) GFMS 35; (4) 390-3; (5) 389-4; (6) 390-4; (7) 389-3; (8) 390-5; (9) 12-7; (10) 12-5; (11) 12-9; (12) GFMS 12; (13) Virus Free; (14) T36 DNA clone control; (15) Negative control; (16) Positive cDNA control**.**

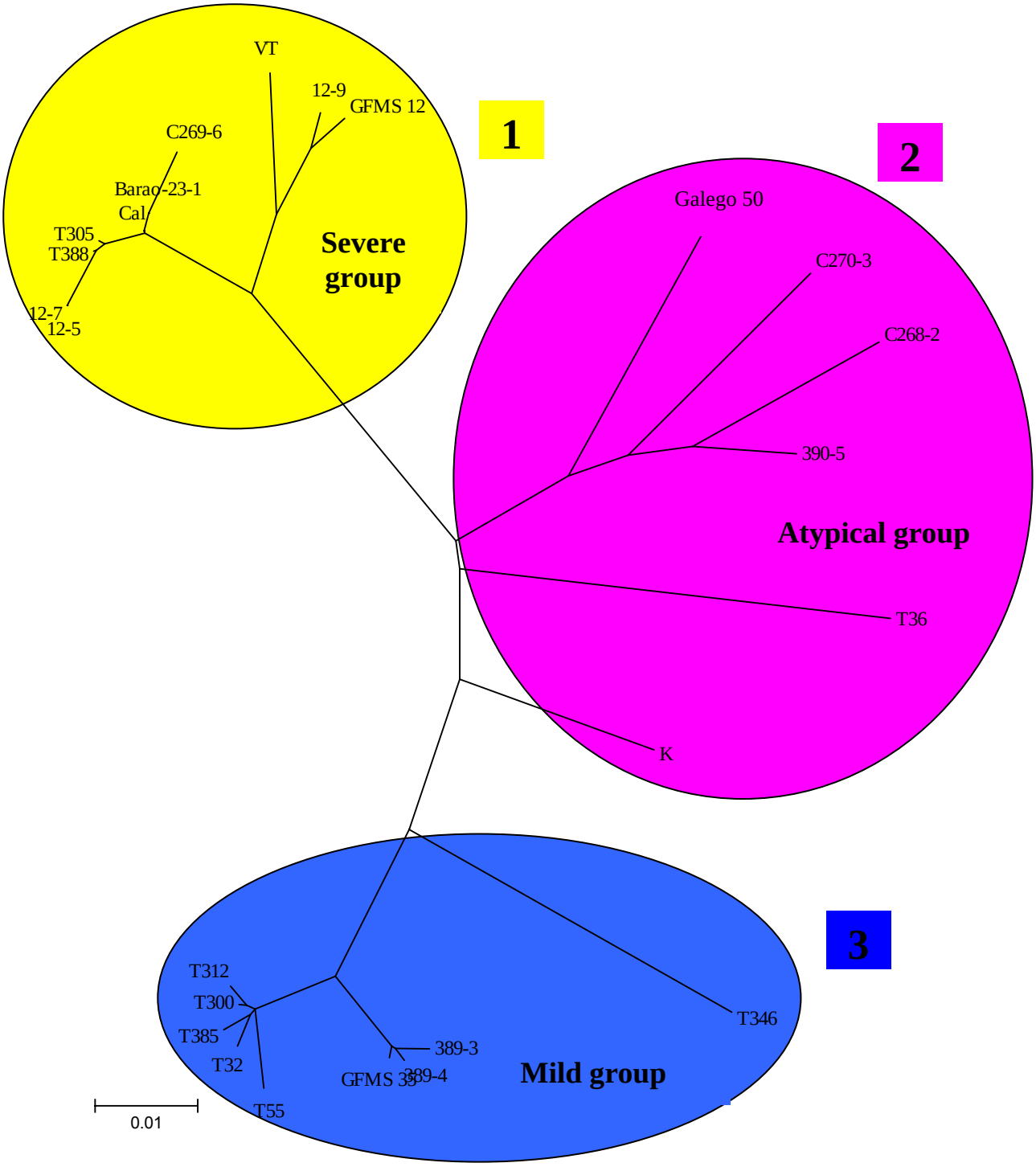

Figure 4.2.2.5. An unrooted phylogenetic tree obtained by the neighbour joining method with the nucleotide sequences of the p23 gene of RSA and other reference CTV isolates.

3) Aim: Development of CTV strain differentiation and detection techniques

Three approaches to strain differentiation were followed by a MSc. student, Me. Katherine Stewart: 1) differentiation of strains into mild, severe or atypical based on the p23 gene specific PCR (Sambade *et al.*,

2003) as discussed above; 2) use of a set of differentiating primers (Hilf & Garnsey, 2000;Hilf *et al.*, 2005) in PCR to classify CTV isolates to genotype level; 3) development of a micro-array chip to detect variability of sequences amongst isolates.

In order to establish the 23 primer set PCRs used to differentiate four CTV genotypes (VT, T36, T30, T3) as developed by Hilf *et al*. (2000), the necessary primers were synthesised locally and each primer system individually optimised using DNA of known CTV strains obtained from a number of international collaborators (S. Gowda, USA; M. Hilf, USA; M. Bar-Joseph, Israel; J.R. Fischer, USA; O. Batuman, Israel).

TAS-ELISA and virus particle immunocapture or total RNA extractions were performed on 11 RSA isolates currently or potentially useful in the cross-protection programme in South Africa. Each isolate was tested with RT-PCR using the 23 individually optimised genotype-specific primer sets (Hilf & Garnsey, 2000) (Fig. 4.2.2.6 and 4.2.2.7). The most common genotype detected was T30 and the least common was T3. The GFMS 35, T30 plant and 389-3 isolates had a defined homogenous T30 genotype profile and isolate 12-5 had a VT genotype profile. The 389-4, 390-3 and 390-4 isolates had a predominantly T30 genotype profile and isolate 12-7 had a predominantly VT genotype profile. Isolate GFMS 12 had a mixed genotype profile indicative of a mixed infection while isolates 390-5 and 12-9 appeared to have mixed genotypes of VT, T30 and T36 as well. Based on the existing markers, isolate 12-9 appeared to have a predominantly VT genotype and isolate 390-5 had a predominantly T36 genotype within the mixed genotypes. Isolates 390-3, 390-4 and 390-5 had many regions with no amplification and appear to be highly variable isolates or possible recombinants. The T3 genotype-specific markers were found in region 2 of a few isolates and could be a cross-reacting primer set to the T30 genotype. The presence of the non-amplification regions and possible cross-reacting primers to incorrect genotypes remains a problem that requires further investigation. Furthermore, it is difficult to identify an isolate displaying mixed genotype-specific markers without sequencing the isolate. This could be a disadvantage to the use of this system in South Africa. While the system was difficult to standardise and optimise, significant information can be garnered about isolates. Isolates with a homogenous strain type are easily identified to genotype level, and possible variability or recombination identified.

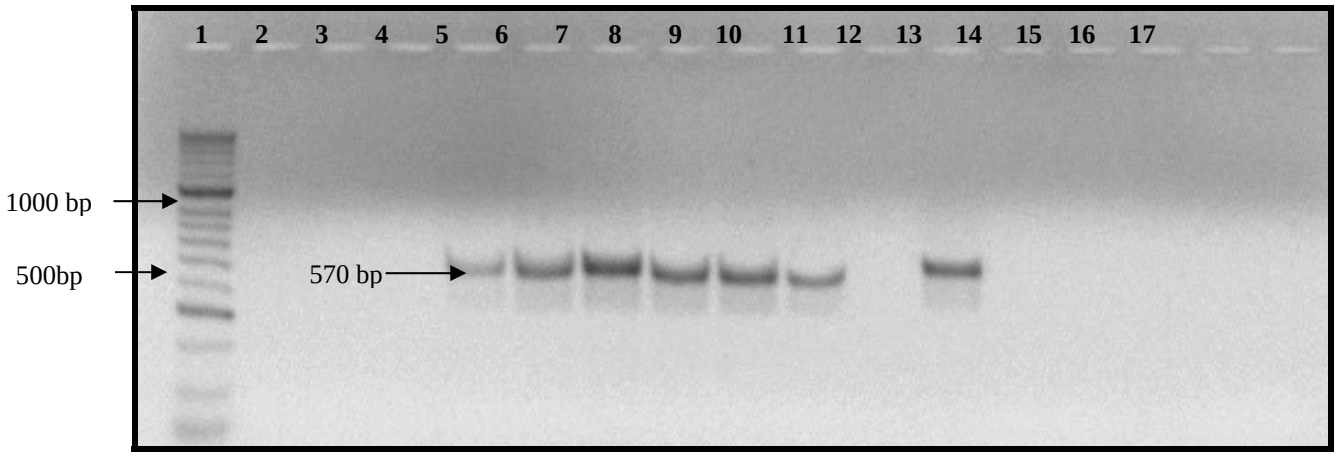

Figure 4.2.2.6. Example of results obtained with genotype differentiating PCR's ({Hilf, 2000 4103 /id}. 1% Agarose gel electrophoresis of the T30 1+/- molecular marker PCR primer set T30 1+ and T30 1-. Expected PCR product is 570 bp in size. Lanes: (1) 100 bp Molecular marker Hyperladder II (Bioline); (2) 12-5; (3) 12-7; (4) 12-9; (5) GFMS12; (6) GFMS35; (7) T30; (8) 389-3; (9) 389-4; (10) 390-3; (11) 390-4; (12) T30 clone; (13) T36 clone; (14) T3 clone; (15) VT clone; (16) 390-5 and (17) Negative control.

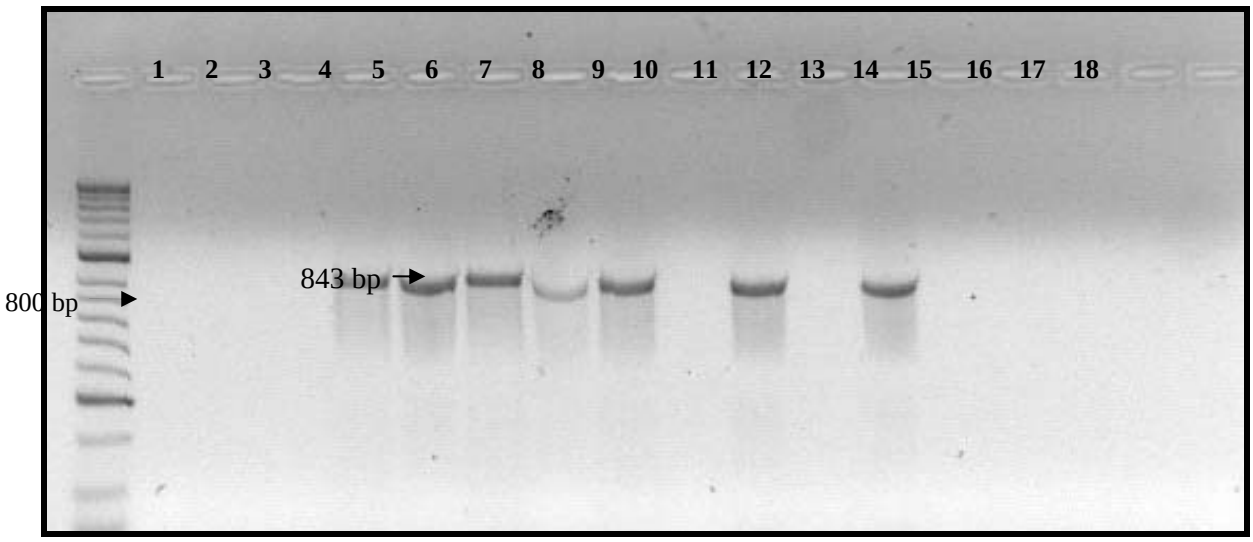

Figure 4.2.2.7. A 1% agarose gel electrophoresis of the T30 2+/- molecular marker PCR primer set T30 2+ and T30 2-. Expected PCR product is 843 bp in size. Lanes: (1) 100 bp Molecular marker Hyperladder II (Bioline); (2) 12-5; (3) 12-7; (4) 12-9; (5) GFMS12; (6) GFMS35; (7) T30; (8) 389-3; (9) 389-4; (10) 390-3; (11) 390-4; (12) 390-5; (13) T30 clone; (14) T36 clone; (15) T3 clone (16) VT clone; (17) Virus Free Control and (18) Negative control.

A microarray chip was designed to differentiate CTV strains of T36 and T30 types as a proof-of-concept pilot study. Oligonucleotides were designed to be 1) primarily T36 specific, 2) have differing amounts of positions of mismatch relative to strain CTV T30, 3) have a Tm of above 60°C and 4) a GC content of above 65%. It consists of 23 CTV-T36 strain specific, 7 CTV-T30 strain specific, 2 CTV conserved and 3 negative control olignucleotides. A few oligonucleotides were modified to include Locked nucleic acids (LNA) instead of DNA in an attempt to increase specificity. Some oligonucleotides proved to be more specific at 42°C while others were so at 52°C. The first half of the CTV genome of T36 was amplified using 5'-Cy3-labelled strain specific primers and the conventional forward primer. Amplified products were added to a hybridisation mix of 60 ul 20x SSc, 4 ul 50x Denhardt's solution, 2 ul 10% SDS and ultrapure water cleaned on a Milli-Q-Synthesis A10 (Millipore). The mix was denatured at 96°C for 10 min and cooled on ice. The slide was pretreated with a mix of 3.5x SSC, 0.2% SDS and 1% BSA and incubated at 60°C for 20 min. The hybridisation took place at 42°C overnight. The 3 washing steps were at 42°C: (2x SSC, 0.2 % SDS for 6 min), (0.2x SSC, 0.2% SDS for 2 min) and finally (0.075x SSC for 2 min). The slide was scanned (Fig. 4.2.2.8) and analysed using Genepix 5.1.

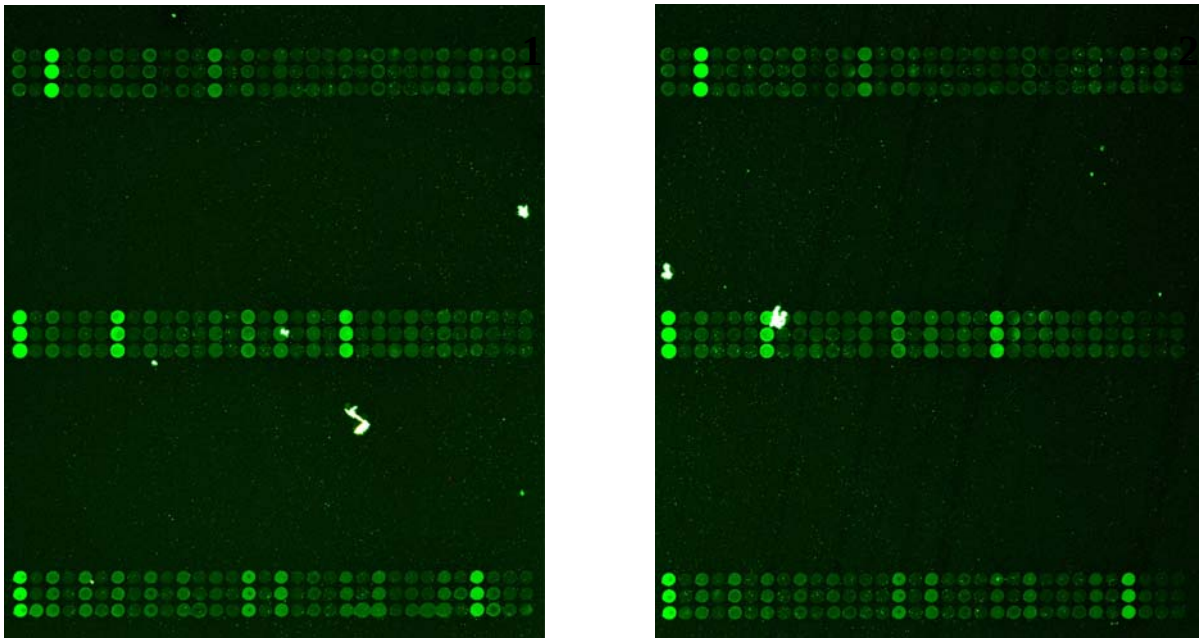

Figure 4.2.2.8. Hybridisation results of the T36 strain at 42°C on a two-dimensional array. The Cy3-labelled DNA amplicons are hybridised oligonucleotide capture oligonucleotides that bound onto glass slides. Fluorescent patterns were recorded with a Genepix 400B micro-array scanner (Molecular Devices, USA) at a wavelength of 532 nm. Block 1 represents the replicate on the left side and Block 2 represents the replicate on the right side of the slide.

The micro-array system was optimised using different temperatures and washing steps. Experimental variation was quantified and after normalising data through the removal of outliers obviously due to experimental error, statistically significant differences were apparent. The secondary structures of the amplicons were determined by mfold software. The micro-array spots showing a SNR (Signal to Noise ratio) above 3 were considered true positive. Of the 9 expected features to show hybridisation for T36, 7 were above this SNR. The other 2 showed moderate hybridisation. The 2 conserved features did not yield good hybridisation. All expected negative features showed a SNR of below 0.4.

The results of the hybridisation revealed that the method allowed a clear differentiation of strain T36 with 11 of the T36-specific oligonucleotides at their more optimal hybridisation temperature. A few oligonucleotides showed cross-hybridisation to strain T30 and were not used in further analysis. It was found that generally oligonucleotides with 21% or more mismatches were successful differentiating oligonucleotides, whereas ones that had 18% or less mismatches had cross-hybridisation. Two of the three LNA modified oligonucleotides increased specificity compared to the unmodified DNA oligonucleotides. The successful differentiation of strains by hybridisation to strain specific oligonucleotides opens paths for highly parallel, yet specific, assays of CTV strains and a more thorough insight into those circulating in RSA and in the cross-protection scheme.

4) Aim: Production of antisera and the development of a TAS ELISA for the large-scale routine detection of CTV

While PCR is an extremely sensitive detection technique it has a number of negative aspects. In the current protocol utilised at CRI at UP (requiring gel electrophoresis) the test is not amenable to testing large numbers of samples, it is prone to false positives due to contamination if care is not taken, and it is a complex technique requiring well trained personnel for reliable use. ELISA is much more robust and is amenable to automation. Although it is less sensitive than PCR, it is currently still the method of choice for the routine testing of large numbers of samples.

Permission to conduct this experiment was required due to the use of experimental animals. This was obtained after the aims of the experiment and envisaged immunisation protocols were reviewed by ARC-Onderstepoort Veterinary Institute (ARC-OVI) ethics committee as well as the ethics committee of the University of Pretoria. A New Zealand White rabbit and a goat were purchased and were maintained at the ARC-OVI. Furthermore the services of veterinarians at ARC-OVI were secured for the immunisation of the experimental animals.

Grapefruit cv. Flame twig tissue (5 kg) inoculated with CTV mild isolate GFMS35 was obtained from Mr. Thys du Toit (Citrus Foundation Block [CFB], Uitenhage), from plants kept in screen-houses under insect-free conditions. Bark shavings were prepared and 35 x 100 g packages were stored at –30^{o}C, pending virus purification of CTV (10-12 months). Purification was performed at the ARC-Plant Protection Research Institute at Roodeplaat (PPRI). The purification method of Lee *et al.* (1987) was initially used and is as follows: 100 g of bark shavings from CTV infected plants were frozen in a large mortar with liquid nitrogen and pulverised with a pestle. The finely macerated tissue was transferred to a blender (Waring, USA) at room temperature and further macerated in 0.1M Tris-HCl buffer, pH 8.4 containing 0.1% (v/v) Triton X-100 at a final ratio of extraction buffer to fresh weight of tissue of 5ml/g. The extract was centrifuged at 10 000 g for 20 min (8500 rpm in a Beckman JA-14 rotor) and the supernatant collected. Proteins were precipitated using Polyethylene glycol (PEG) (MW 6000) and NaCl at 4% and 0.8% (w/v), respectively, for 1 hour at 4^{o}C. The preparation was centrifuged at 10 000 g for 20 min (8500 rpm with a Beckman JA-14 rotor) and the precipitate collected and re-suspended in 0.04 M potassium phosphate buffer, pH 8.0 at a ratio of 1.2 ml/g of original tissue. After stirring for 1 hour at 4^{o}C, the preparation was centrifuge at 5000 g for 10 min (5700 rpm with a Beckman JA14 rotor). The supernatant was collected and adjusted to a final concentration of 5% PEG (w/v) and 1%NaCl (w/v). The solution was stirred for 1 hour at 4^{o}C, centrifuged at 10 000 g for 15 min and the pellet re-suspended in 30 ml 0.05 M Tris-HCl, pH 8.0 for 1 hour at 4^{o}C. This was followed by a low speed centrifugation step of 5000 g for 10 min. Six isopycnic gradients were prepared in centrifuge tubes using steps of 10%, 15% and 30% Caesium sulphate (Cs_2SO_4) in 10% sucrose in 0.05 M Tris-HCl, pH 8.00 buffer. Partially purified viral extract (5 ml) was layered on top of each gradient tube. The gradients were centrifuged overnight in a Beckman SW41 rotor at 30000 rpm at 4^{o}C using a Beckman Optima L-100 XP Ultracentrifuge. Light-scattering zones were recorded with a digital camera under overhead lighting conditions and the bands collected. These were dialysed overnight against 0.05 M Tris-HCl, pH 8.00 buffer. The protocol was modified after the first purification by the addition of a centrifugation step to concentrate and purify the virus prior to the Cs_2SO_4 gradient centrifugation step. This was done by layering of the partially purified virus over 10 ml of a 30% sucrose (w/v) layer and centrifugation of 30 000 rpm for 2.5 hours using a Beckman 50.2Ti rotor. The pellet was selected for further purification on the Cs_2SO_4 gradient.

All steps of the purification were monitored for virus presence and relative purity by electron microscopy by Mr. K. Kasdorf of ARC-PPRI. Samples from each step were negatively stained using either 2% uranyl acetate, pH 3.9 or 2% potassium phosphotungstate, pH 5.00 and a number of EM fields were monitored using an ABT10 Electron Microscope (ARC-PPRI). Virus yield and purity were also determined by spectrophotometry from 320 nm to 220 nm wavelength.

One CTV purification, using the standard method of Lee *et al.* (1987), was done during October, 2006. Final purified virus preparations obtained from two serial caesium sulphate gradients (Fig. 4.2.2.9) were monitored with the Electron microscope and shown to contain relatively pure preparations of CTV. Yields were, however, relatively low. Unfortunately, preparations for the impending greening survey precluded further purifications or immunisation of experimental animals. Two further purifications were performed in January, 2007, with the standard procedure but yielded only partially pure, low yield virus preparations. The technique was modified to include centrifugation through a sucrose pad prior to gradient centrifugation. Relatively large numbers of virus particles could be observed after this step. In order to obtain sufficient virus for the entire immunisation schedule of both experimental animals, a number of purifications are scheduled for early 2007, up to the step just prior to gradient centrifugation. These will be pooled and gradient centrifugation and fractionation performed to obtain pure virus. This will be aliquot and used for immunisation.

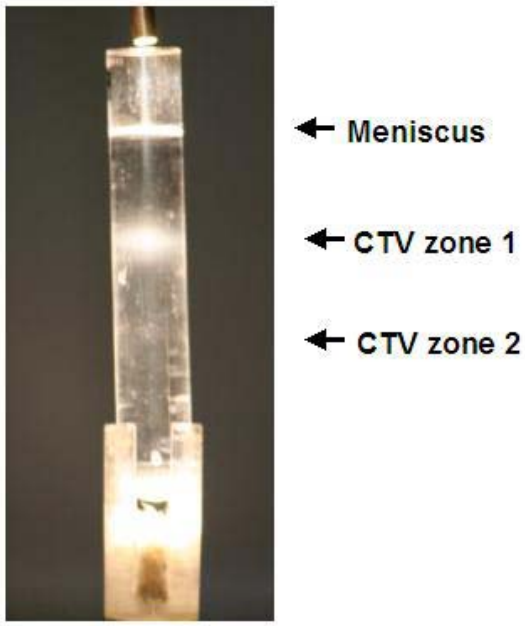

Figure 4.2.2.9. High speed Caesium sulphate density gradient loaded with partially purified *Citrus tristeza virus* preparation, showing separation of purified virus following overnight centrifugation of 30 000 rpm in a SW27 Beckman rotor.

Detection of "*Candidatus*" Liberibacter africanus, the causal organism of African Greening

Aim: Established a functional, routine-use PCR for greening organisms

The standard A2/J5 primer-based PCR of Hocquellet, *et al.* (1999) is now routinely used at CRI at UP as well as at CRI in Nelspruit (Jacolene Meyer). It was used in 2006 to test more than fifty samples submitted as *ad hoc* samples by various producers and researchers for testing for "*Ca*." L. africanus *or "Ca."* L. asiaticus from areas as diverse as the Eastern and Western Cape, Rustenburg, Nelspruit and White River.

When testing non-citrus potential host plants (Rutaceae) for "*Ca*." L. africanus, false negative results may be obtained as these hosts potentially contain sap components or inhibitors that prevent successful DNA extraction or PCR amplification. To overcome this in studies on these hosts of the Liberibacters ("*Ca*." L. africanus and "*Ca*." L. asiaticus), a unique multiplex PCR was developed by Me. Baby Phahladira. The Liberibacter specific primers set remains the standard A2/J5 primer system of Hocquellet, *et al.* (1999), but the PCR contains a second primer set to serve as an internal control for DNA extraction, and potential PCR inhibition by foreign plant components. This second primer set (RBCL-C705 and RBCL-H535 of Nassuth *et al.* (2000)) is directed to a conserved segment of the gene coding for ribulose-biphosphate carboxylase oxygenase (Rubisco), a protein universally found in plants and involved in the Calvin cycle. All plant extracts should theoretically yield this amplicons if DNA extraction was successful and PCR inhibitors are absent, therefore Liberibacter-specific negative results with respective Rubisco positive results can be considered true negatives for Liberibacter (Fig. 4.2.2.10).

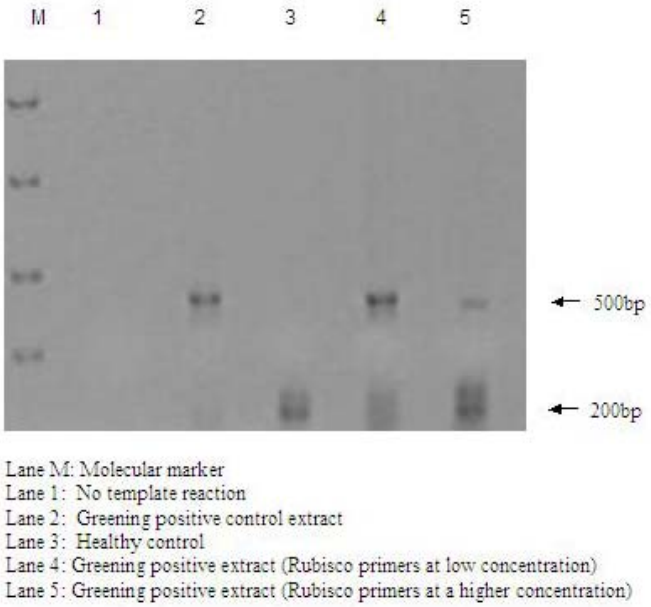

Figure 4.2.2.10. Agarose gel of PCR products obtained with A2/J5 + Rubisco multiplex PCR.

Samples of *Strychnos* sp. with Psylla marks and mottling resembling greening were observed and collected in the close proximity of greening infected citrus groves near Nelspruit with the help of Dr. van Vuuren. These were tested using the multiplex PCR but, while the internal controls worked, L. africanus or L. asiaticus were not detected. Similarly, a *Vepris lanceolata* plant collected in Pretoria with Psylla damage also tested negative for the bacteria. Further samples were collected by Dr. van Vuuren at the Nelspruit Botanical Gardens and tested for *"Ca."* L. africanus and L. asiaticus by Me. Phahladira and include: *Calodendrum capense, Citrus jambhiri, Clausena anisata, Murraya paniculata, Techlia natalensis, Toddalia asiatica, Toddaliopsis tremicampes, Vepris reflexa, Zanthoxylum capense* and *Z. chalydum*. No clear amplicons indicative of Liberibacter infection was obtained with any of the samples. The *T. asiatica* sample yielded a very faint band/smear between 400 and 850bp and requires further investigation.

During a visit to Brazil to attend a Huanglongbing workshop, a visit to Fundecitrus was used to gain insight into methods of DNA extraction used by them on large numbers of samples submitted for greening tests. The technique employed is similar to that used at CRI at UP and like CRI at UP, Fundecitrus is currently unable to test large numbers of samples per researcher and laboratory. However, some steps were done more effectively at Fundecitrus through the use of alternate apparatus (mainly the maceration and CTAB extraction steps) and these have now been implemented at CRI.

A 2-week visit by two Brazilian researchers from Fundecitrus working on Huanglongbing along with Prof. J. Bové, a world authority on Huanglongbing, was exploited to conduct a survey of greening infected trees in the major citrus production areas of South Africa. The aim of the survey was two-fold, firstly to determine whether either *"Ca."* L. asiaticus or *"Ca."* L. americanus, which are found in other countries, occurs in South Africa in addition to the known "*Ca*." L. africanus species, and secondly to collect and establish *"Ca."* L. africanus sources from various production areas throughout South Africa in order to study the molecular variability within this species.

A multiplex PCR utilising the A2/J5 primer system of Hocquellet *et al*. (1999) and the GB1/GB3 primer system of Bové (personal communication) was established for the country-wide greening survey. This technique is capable of detecting *"Ca."* L. africanus, L. asiaticus and L. americanus in one test and was used for routine tests on nine Citrus samples from Mpumalanga, collected and submitted by M.C. Pretorius for greening testing. Clearly, "*Ca.*" L. africanus infected as well as weakly positive results were obtained. No infection by *"Ca."* L. asiaticus or *"Ca."* L. americanus was revealed with these PCR tests.

A total of 249 samples with greening-like symptoms were collected from 57 orchards with the help of the three overseas visitors as well as a dedicated number of local researchers, extension officers, growers and orchard managers, with thanks. DNA extraction was performed on all samples using the slightly modified Fundecitrus modifications of the procedure of Doyle and Doyle (1990). The A2/J5=GB1/GB3 multiplex PCR,

optimised to simultaneously detect the three Liberibacter species, was used in duplicate to test all DNA extracts for the three bacterial species. None of the samples yielded amplicons of 1027 bp size, which would have been indicative of *"Ca."* L. americanus infection. Amplicons in the size region of 669-703 bp were obtained for 197 samples. This relatively high number of positives is indicative of the collective expertise the group in correctly identifying greening-like symptoms during a sub-optimal time of year. The concentration of amplicons obtained, and therewith electrophoresis gel band intensity and width, varied dramatically from sample to sample. This made resolution of specific band size between "*Ca.*" L. africanus (669 bp) and *"Ca."* L. asiaticus (703 bp), a 40bp difference, near-impossible (Fig. 4.2.2.11). Further tests to differentiate these two bacterial species and to confirm that samples contain only "*Ca.*" L. africanus are scheduled for the first quarter of 2007. Samples yielding low amplicon levels were re-tested in duplicate using a fresh DNA extract to confirm the initial result. With the cooperation of S.P. van Vuuren and J.H.J. Breytenbach (CRI-Nelspruit), all samples were budded onto sweet orange plants in order to establish greenhouse cultures of each. Mandatory collection of buds on branches with symptoms and availability generally of poor quality buds at the time of collection, suggests that bud-take and subsequent bacterial transfer is likely to be low. No clear greening-like symptoms were visible when assessed at the end of January 2007. Bacterial transmission will be confirmed by PCR on the sweet orange plants.

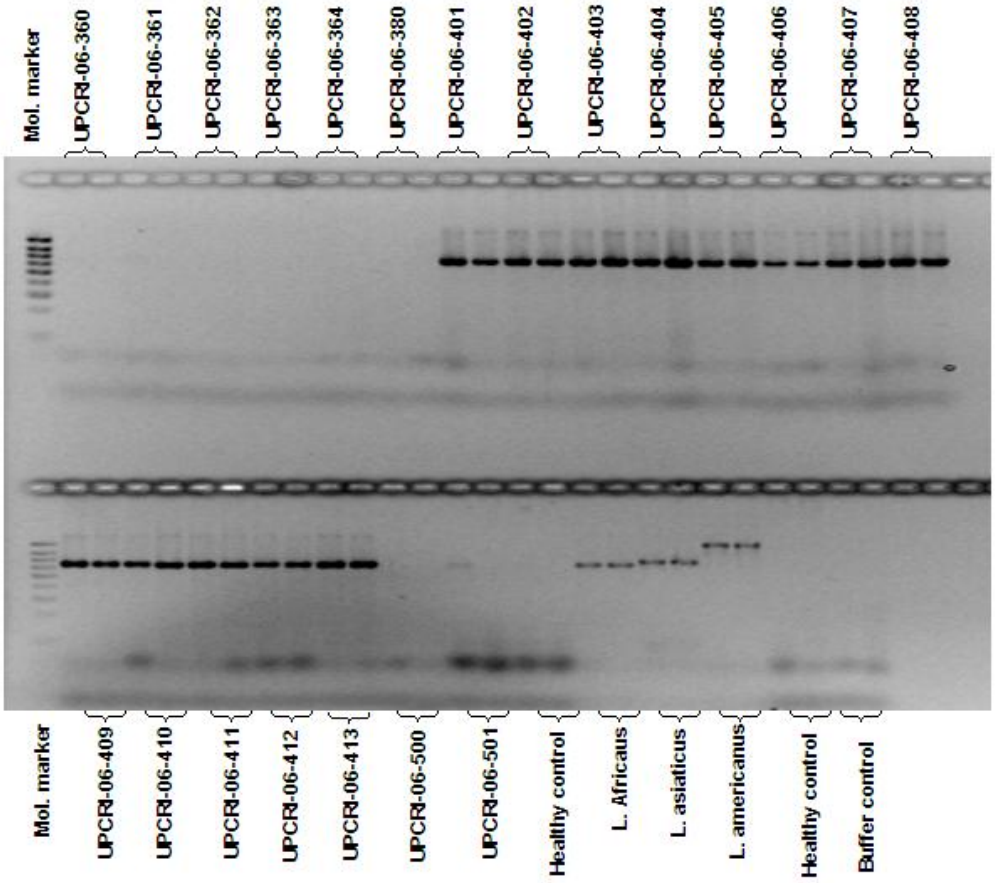

Figure 4.2.2.11. Example of typical results obtained with the A2/J5 and GB1/GB3 multiplex PCR for the detection of *"Ca."* L. africanus, L. asiaticus and L. americanus. Note that intensity/width of bands precludes differentiation of *"Ca."* L. asiaticus and L. africanus amplicons.

During the Huanglongbing Symposium held in Rebeiro Preto, Brazil, the possibility of collaboration with Dr. Hong Lin, USDA was discussed. His research involves generating new sequence information on *"Ca."* Liberibacter asiaticus, for which he has developed novel methodology (Lin *et al*., 2006) and his laboratory facilities are equipped to do large sequencing projects. It was agreed that a collaborative effort would be made to generate more novel sequence data on *"Ca."* L. africanus as well as to characterise the diversity on a molecular level. To this end, total DNA was extracted from a known *"Ca."* L. africanus sources (UPCRI 06-0071) and submitted to Dr. Lin. He has processed these and has generated sequence data on the locus not previously sequenced for *"Ca."* L. africanus. This sequence data is current under review at Genbank and should become public directly after publication.

Detection of other graft transmissible pathogens of citrus

1) Citrus viroids

In anticipation of implementing the set of PCR tests that were recently developed by Bernad & Duran-Vila (2006) to detect the five established Citrus viroids (Citrus exocortis viroid, citrus bent leaf viroid, hop stunt viroid and Citrus viroids III and IV), positive controls were requested from Dr. Duran-Vila, Spain. These were kindly sent by her in the form of DNA clones. Unfortunately, some spillage occurred and labels were obliterated. Care will have to be taken when using these positive controls to prevent contamination. Viroid infected Etrog seedlings were also kindly donated by Drs. B. Manicom, ITSC and S.P. van Vuuren, CRI. Furthermore, the 15 primers required to detect these five viroids in either a one or two-step PCR were synthesised by IDT, USA, and obtained locally. Implementation and optimisation of the PCR systems to detect all Citrus viroids is imminent. During the course of 2006, Citrus Viroid II was detected locally by Drs. B. Manicom and S.P. van Vuuren based on PCR. The amplicons were submitted to CRI@UP where they were sequenced by Me. Stewart and the isolate identified as CVd-IIa (non-cachexia inducing). This viroid does not cause disease symptoms but it has been shown to reduce tree size (ideal for high density planting) and enhance production.

2) Citrus psorosis virus

The OP1 and OP2 (Vaira, *et al.*, 2003) primer set directed against Ophioviruses, which include citrus psorosis virus, have been synthesised locally and tested against citrus psorosis DNA obtained from Texas (Fig. 4.2.2.12). Reverse transcriptase steps and detection of local isolates can now be attempted. Dr. van Vuuren is currently establishing psorosis infected budwood onto new rootstocks for this purpose. Interestingly, South African isolates of psorosis could not be detected internationally by a citrus psorosis specific PCR (Martin *et al.*, 2004) and may prove to differ regarding nucleotide sequence.

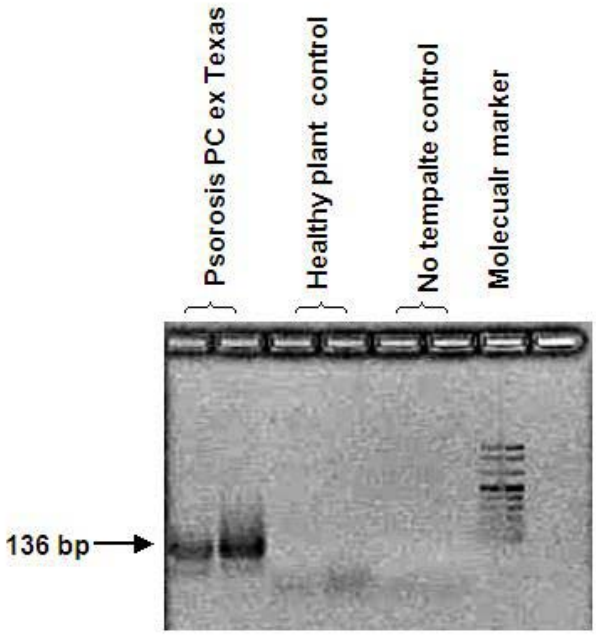

Figure 4.2.2.12. Ethidium bromide stained electrophoresis gel of PCR products from Ophiovirus specific PCR (Vaira, Accotto et al., 2003).

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4.2.3 **Diagnostic services for graft transmissible diseases**

Experiment 796 by J.H.J. Breytenbach & S.P. van Vuuren (CRI)

Opsomming

Die sukses van die sitrusverbeteringskema (CIP) berus op 'n fitosanitêre program wat gebaseer is op 'n diagnostiese bepaling van die teenwoordigheid van skadelike patogene, die eliminering daarvan en die onderhou en verpreiding van gesonde voortplantingsmateriaal. Indeksering, of die bepaling of entoordraagbare siektes teenwoordig is in plantmateriaal, word tans hoofsaaklik deur biologiese indikatorplante gedoen. Serologiese tegnieke soos ELISA en Kol Klad word hoofsaaklik gedoen om pre-immunisering met *Citrus tristeza virus* (CTV) te bevestig, asook om die teenwoordigheid van Sitrusskroei te bevestig. Molekulêre tegnieke vir indeksering (bv. PKR) van verskeie sitrussiektes word tans ge-optimiseer en sal baie tyd bespaar (sien 4.2.2). Behalwe vir indeksering wat op CIP material gedoen word, word daar ook indeksering op materiaal wat vanaf kwekers ontvang of versamel word gedoen. Dit is verder nodig om die oorsaak van siektetoestande te bevestig om sodoende sinvolle aanbevelings te maak vir beheer. Spesifieke virusvrye indikatore is in die glashuis gekweek vir elk van die entoordraagbare siektes waarvoor ge-indekseer word. Die moederbome by die Sitrus Grondvesblok is ge-indekseer om te bepaal of enige strawwe CTV rasse in die moedermateriaal voorkom. 'n Totaal van 164 moederbome is getoets waarvan 18 bome met strawwe CTV geïdentifiseer is en 61 sonder CTV. Die rede vir laasgenoemde scenario is huidiglik onbekend. Enthout wat vanaf kwekers ontvang of versamel is, is op verskeie indikatorplante ge-inokuleer en by optimale temperature in die glashuis gehou sodat siektesimptome kan ontwikkel indien dit teenwoordig is. Agt monsters is onderskeidelik vir CTV, sitrus viroide, psorose, sitrus skroei en Appelstam groef virus (voorheen Tatterleaf) getoets. Drie monsters het positief getoets vir sitrus viroide terwyl geen van die ander siektes opgespoor kon word nie.

Introduction

As with any commercial tree crop, citrus species are subjected to various graft transmissible diseases (GTD) caused by viruses, viroids, bacteria and in some cases, unknown organisms. The GTD affect the vigour, longevity of the trees, as well as the yield and quality of fruit. The framework of disease-free planting material

is a phytosanitary programme based on diagnosis, detection and elimination of causal agents and maintenance and distribution of healthy propagation material. Shoot tip grafting (STG) is used to eliminate these diseases (Navarro, 1976) and is used in South Africa since 1977 (de Lange, *et al*., 1981). Mainly biological indexing is used for the detection of GTD in STG material while ELISA is used to confirm pre-immunisation (Roistacher, 1991). Since *Citrus tristeza virus* (CTV) and its vector, *Toxopthera citricida*, is endemic in South Africa, virus-free material should be protected by pre-immunisation with a suitable virus isolate (Müller & Costa, 1987). Currently three CTV isolates are used in the southern African Citrus Improvement Scheme (CIS) depending on the scion material to be protected (van Vuuren, *et al.*, 1993a; van Vuuren, *et al*., 1993b; van Vuuren, *et al*., 2000; von Broembsen & Lee, 1988). The STG and pre-immunisation procedures have been improved to suite South African conditions (Fourie & van Vuuren, 1993). Re-indexing to establish that mother material at the Citrus Foundation Block remains free of graft transmissible diseases and that the pre-immunising agent remained mild is done on an annual basis for CTV and greening, bi-annually for Citrus viroids (CVd) and every 10 years for the other GTD. Indexing for GTD where growers have problems of disease infections is necessary to recommend proper control measures. Indexing includes biological as well as serological techniques (ELISA, Dot Blot, PCR) (Roistacher, 1991).

Materials and methods

Virus-free indicator plants for the different graft transmissible diseases (GTD) are propagated from seed in an insect-free glasshouse and kept in stock until needed (except for Citrus leaf blotch virus, which does not occur in South Africa, GTD are not seed transmissible). When budwood from the source to be indexed is received, two buds are budded on each of three indicator seedlings for each disease. Hereafter the plants are cut back to force new growth and kept in the glasshouse at a temperature required for symptom expression of the specific disease. Positive and negative control samples are included. A minimum indexing time of 6 months is required for CTV, CVd, Apple stem grooving virus (tatter leaf) and greening, while 12 months are required for psorosis virus and impietratura disease. Details on indexing procedures and disease symptoms on indicator plants are given in Section 4.2.4, Appendix 4.2.4.1.

Field material is usually not suitable for the serological or molecular techniques (ELISA, PCR, etc.) since the organisms are usually present in low concentrations or are poorly distributed and therefore false results may be obtained. Such material is multiplied on suitable plants at optimal temperatures in the glasshouse and can be collected 3 months after inoculation. Results can be obtained quicker and is a confirmation of the biological result.

When indexing is completed the client is informed of the result.

Results and discussion

The Citrus Foundation Block (CFB): The CTV status of 164 mother trees at the CFB is presented in Table 4.2.3.1. The same trees are currently being indexed for citrus viroids (awaiting results). The Mexican lime indicators showed the presence of severe CTV in 18 of the mother trees. It is suggested that these trees should be terminated as budwood sources. There is also evidence that 61 mother trees are CTV-free. These trees were all pre-immunised with isolate LMS 6, which should have been detected by the biological indexing. The reason for this is unknown. At first, it was speculated that the isolate is sensitive to high temperatures, but glasshouse tests as well as the erratic occurrence of the virus in newly pre-immunised soft citrus, showed that the problem is not due to high temperature. It is possible that multiplication and movement of isolate LMS 6 is restricted in some cultivars.

During re-indexing of Clemenpons for all graft transmissible diseases, it was found that the Mexican lime (ML) indicators (CTV induce vein clearing and stem pitting) were negative for these symptoms, while Duncan grapefruit (DG) indicators (CTV induce stem pitting and seedling yellows [CTV-SY]) showed the presence of CTV-SY. ELISA was performed on the 5 DG indicators as well as samples of the ML indicators used to assess the CTV status of the 6 Clemenpons mother trees at the CFB. Three out of 5 DG indicators were positive by ELISA and only one of the 6 ML indicators of the mother trees was positive. However, stripping the bark of the ELISA positive ML plant, no stem pitting could be observed. Following on this result, it was decided to do ELISA on all the sources of the soft citrus CFB mother trees. The results are included in Table 4.2.3.1. The ELISA results show that some indicators that showed no stem pitting (SP) but very mild or no vein clearing, are CTV positive. This is again evidence that the LMS 6 isolate is filtered out completely or that the titer is reduced significantly by some cultivars and selections.

The difficulty to pre-immunise soft citrus with LMS 6 has been reported in 2006 at the Cultivar Committee Meeting. An instruction was received to investigate the matter and also to find an alternative CTV isolate to pre-immunise soft citrus. A glasshouse trial is currently in progress.

Table 4.2.3.1. *Citrus tristeza virus* status of pre-immunised mother trees of different cultivars at the CFB.

Cultivar	Number of mother trees	Number of trees with severe SP	Number of trees negative for SP	Number of trees negative for CTV by ELISA
Afourer	6	0	2	2
Or4	6	0	6	4*
Nova SL	6	0	5	5
Bay Gold	3	3	0	0
Cami	3	1	0	0
W Murcott	5	0	5	5
Murcott X Clem	3	0	3	3
Nova	3	0	2	2
Total Mandarins	**35**	**4**	**23**	**21**
Salustiana	6	0	4	nt**
Raratonga	3	1	2	nt
Tarocco 57	3	0	1	nt
Tarocco Gallo	3	1	1	nt
Total Midseasons	**15**	**2**	**8**	**-**
Bahihaninha	6	0	0	nt
Navelina	6	0	0	nt
Palmer	8	0	0	nt
Atwood	3	1	0	nt
Autumn Gold	3	3	0	nt
Barnfield Summer	3	0	0	nt
Chislett Summer	3	0	1	nt
Powell Summer	3	1	0	nt
Summer Gold	3	0	0	nt
Cambria	3	0	0	nt
Dream	3	1	1	nt
Washington	3	0	0	nt
Total Navels	**47**	**6**	**2**	**-**
Turkey	6	0	2	nt
McClean SL	5	1	2	nt
Total Valencias	**11**	**1**	**4**	**-**
Kuno	4	0	1	nt
Total Satsumas	**4**	**0**	**1**	**-**
Clemenpons	6	0	6	5*
Nour	6	0	6	5*
Tardif de Janvier I	3	0	3	3
Esbal	3	0	3	1*
Marisol	3	0	3	0
Total Clementines	**21**	**0**	**21**	**14**
Eureka	2	0	2	nt
Total Lemons	**2**	**0**	**2**	**-**
Marsh	6	3	0	nt
Star Ruby	6	2	0	nt
Flame	6	0	0	nt
Nelruby	3	0	0	nt
Rio Red	3	0	0	nt
Henderson	5	0	0	nt
Total Grapefruit	**29**	**5**	**0**	**-**
Grand Total	**164**	**18**	**61**	**-**

* Some indicator plants that were ELISA positive but displayed no stem pitting symptoms.
** nt = not tested.

Shoot tip grafted (STG) material: Citrus material that underwent STG is indexed firstly for CTV and CVd. When the material indexed negative for these two graft transmissible diseases, the material is released to the CFB to establish mother trees. Indexing for psorosis virus, apple stem grooving virus (tatter leaf) and impietratura disease continues. Huanglongbing (greening) is continuously monitored since all the cultivars and selections, except the trifoliate types, are self indexed.

Biological indexing of 7 STG plants for CTV and CVd's are presented in Table 4.2.3.2. The results are not available yet.

Table 4.2.3.2. The number of STG plants indexed for CTV and CVd's.

Cultivar	Number of plants	Bio-indexing Result
Navel	5	Results pending
Midseason	2	Results pending

The number of trees indexed for psorosis virus, apple stem grooving virus and impietratura disease are given in Table 4.2.3.3. They all indexed negative for the different diseases.

Table 4.2.3.3. The number of STG plants that were indexed for psorosis virus, apple stem grooving virus and impietratura disease.

Cultivars	Number of selections indexed	Bio-indexing Result
Navels	1	Negative
Midseasons	1	Negative
Valencias	1	Negative
Reticulatas	2	Negative
Rootstocks	4	Negative

General indexing: Citrus material that was sent in by growers or collected during visits was indexed for specific diseases as indicated in Table 4.2.3.4. The results are also indicated.

Table 4.2.3.4. Indexing for graft transmissible diseases for various clients.

Client	Number of samples	Disease	Indexing	Result
Citrusdal grower	1	Psorosis virus	Biological	Awaiting results
Vaalharts Nursery	1	Citrus Viroids	Biological	Positive
F. Veldman	1	Citrus Viroids	Biological	Positive
CRI (Croc Valley – X639)	1	Citrus Viroids	Biological	Positive
CRI (Grower request)	2	CTV Citrus Viroids Apple stem grooving virus Greening bacterium Impietratura	Biological Biological Biological Biological Biological	Negative Negative Negative Negative Negative
La Rhyn Citrus Nursery	1	Citrus Viroids	Biological	Awaiting results
Kakamas grower	1	Citrus Blight	Blight protein test	Negative

Conclusion

The diagnosis of graft transmissible diseases is a continuous service and results are reported to the parties involved.

Future Research

Annual indexing of mother trees at the Citrus Foundation Block (every year for CTV severity and every third year for CVd).

Indexing of STG plants for tristeza virus, citrus viroids, psorosis virus, apple stem grooving virus (tatter leaf) and impietratura disease.

Index suspected budwood from growers and institutions using ELISA, PCR, Dot Blot and biological indicators.

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4.2.4 Citrus virus-free gene source

Experiment 790 by J.H.J. Breytenbach & S.P. van Vuuren (CRI)

Opsomming

Groeipunt enting word gebruik om sitrus materiaal te vrywaar van ent-oordraagbare patogene. Virusvrye boompies van verskillende cultivars en seleksies word in 'n insekvrye tonnel by CRI bewaar. Die virusvrye bron by die LNR-ITSG is gedupliseer by CRI en 'n totaal van 213 cultivars en seleksies is gevestig. 'n Verdere 22 cultivars en seleksies wat deur 'n indekseringsproses is, is gedurende 2006 bygevoeg in die bron. Virusvrye materiaal word gepre-immuniseer met 'n geskikte *Citrus tristeza virus* isolaat voordat dit aan die Sitrus Grondvesblok te Uitenhage verskaf word.

Introduction

The overall objective of the southern African Citrus Improvement Programme is to enhance the productivity of the industry by making the highest quality propagation material available. Graft transmissible diseases (GTD) have detrimental effects on the growth and production of citrus trees since they are responsible for stunting, decline and small fruit. Shoot tip grafting (STG) is the standard method for the elimination of pathogens (Navarro *et al*., 1975). Some pathogens are more difficult to eliminate and heat therapy should be incorporated with the STG process (Roistacher, 1977). The STG technique was developed by Murashige *et al.* (1972) and improved by Navarro *et al.* (1975) and de Lange (1978).
The virus-free gene source at the ARC-ITSC has been duplicated at CRI Nelspruit as a safety measure. Shoot tip grafting (STG) facilities were established at CRI and new virus-free cultivars and selections will be added to the gene source after STG and indexing.

Materials and methods

In vitro cultured rootstocks
The standard method used for *in vitro* cultured rootstocks is to expose the cotyledons by removing the testa of Troyer citrange seed and surface sterilise in 1% sodium hypochlorite (NaOCl) for 10 minutes followed by three rinses in sterile deionised water. Three to four seeds are planted in growth tubes containing sterile Murashige and Skoog (MS) agar medium (Murashige and Skoog, 1962). Germination takes place at a constant temperature of 28ºC in continuous darkness. When the seedlings have reached a height of 30 to 40 mm, they can be stored at 4ºC in darkness.
Scion preparation
Method 1. Buds of the plant that should go through STG are budded on a standard rootstock in the glasshouse. After the buds have grown and matured (approximately 3 – 4 months), the contaminated plants are defoliated by hand to induce flushing. Ten to 14 days later, the new shoots are harvested and surface sterilised on a flow bench for 5 minutes in 0.52% NaOCl and then rinsed three times in sterile deionised water.

Method 2. Bud sticks from the mother plant are cut in 50 mm lengths and surface sterilised in 70% ethanol for 5 seconds followed by immersion for 10 minutes in 1% NaOCL containing a wetting agent (what???). After 3 rinses in sterile deionised water the bud sticks are cultured in 250 ml glass bottles containing sterile wet sand. The cultures are incubated at 32ºC and exposed to 16 h light/day. Ten to 14 days later new shoots are harvested and treated as in method 1.

STG

The seedling rootstock is aseptically decapitated about 10 mm above the cotyledons. The cotyledons and their auxiliary buds are removed and an inverted T incision is made, 1mm vertically and 1 – 2 mm horizontally. The cuts are made through the cortex to reach the cambium. A shoot tip consisting of the apical meristem and 2 to 3 leaf primordia is excised from the growth point of the collected shoots under a stereo microscope. The growth point with primordia is placed on the horizontal cut of the incision. The grafted plant is transferred to sterile MS liquid medium and cultured at constant 28ºC exposed to 16 h light/day.

STG plant care

The shoot tip will start growing 3 to 4 weeks after STG. The seedling rootstock with the shoot tip is now grafted onto a vigorous-growing virus-free rootstock in the glasshouse. After grafting, it is closed by a plastic bag for 8 days.

Virus indexing

Elimination of graft transmissible pathogens is confirmed by indexing the STG material on sensitive biological indicators as described by van Vuuren and Collins (1990). Virus-free plants are multiplied and kept in an aphid-free tunnel containing the gene sources from where material is taken, multiplied and pre-immunised (van Vuuren and Collins, 1990) with suitable *Citrus tristeza virus* (CTV) isolates prior to release to the Citrus Foundation Block at Uitenhage.

Results and discussion

STG activities for 2006

STG:

1) Contaminated cultivars and selections were transferred from the ARC-ITSC to CRI (Table 4.2.4.1).
2) In addition, 42 new cultivars and selections were submitted by clients (Table 4.2.4.1). STG has been initiated on the latter additions.
3) Cultivars and selections that went through STG previously but of which the CTV status was unknown were transferred from a tunnel at the ARC-ITSC to a glasshouse at CRI. ELISA has been performed on these 23 cultivars and selections to establish if they are CTV-free. One Reticulata was found to be CTV positive and STG should be repeated. The others were added to the nucleus block (Table 4.2.4.2).

Table 4.2.4.1. Cultivars and selections in the pipeline for graft transmissible disease elimination by STG.

Cultivar	STG'd (await ELISA)	Dirty transfers from ARC-ITSC	New Introductions
Clementine	8	0	4
Diverse (Citron, Sour orange, etc.)	1	0	6
Ellendale	0	0	0
Grapefruit	1	1	1
Kumquat	0	0	0
Lemon	0	1	0
Lime	3	0	0
Midseason	1	2	3
Navel	7	0	21
Pummelo	0	0	0
Reticulata	2	0	3
Rootstock	0	0	0
Satsuma	0	0	0
Valencia	0	5	4
Total	**23**	**9**	**42**

Establishing and maintaining a virus-free gene source

The virus-free gene source at the ARC-ITSC was duplicated in an aphid-free tunnel at CRI. The cultivars and the number of selections of each cultivar are listed in Table 4.2.4.2. New additions are made continuously as STG plants are found virus-free. This year 22 new cultivars and selections were added to the virus-free block.

Table 4.2.4.2. The number of virus-free selections of different cultivars that were established at the Nucleus Block at CRI.

Cultivar	Number of selections		
	Virus-free 2005	New additions 2006	Total
Clementine	15	8	23
Diverse (Citron, Sour orange, etc.)	1	1	2
Ellendale	4	0	4
Grapefruit	16	1	17
Kumquat	1	0	1
Lemon	20	0	20
Lime	1	3	4
Midseason	22	1	23
Navel	33	7	40
Pummelo	7	0	7
Reticulata	31	1	32
Rootstock	20	0	20
Satsuma	8	0	8
Valencia	34	0	38
Total	**213**	**22**	**235**

General

Manuals and reporting: Cleansing of citrus material from GTD is a long-term process and the minimum time from receiving material until it is released to the client is approximately 2.5 years. During this time several steps must be planned carefully to avoid a delay in the release of virus-free material. To make the process more streamline, several documents were compiled this year to assist in the planning and execution of the different processes. Firstly, a document on practicalities has been compiled (Appendix 4.2.4.1). This document summarises the indicator plants needed, where to obtain seed and plant material and the preparation of the indicator plants. It also gives a brief description of each disease and the indexing thereof. Secondly, a detailed tabled document was compiled of the whole STG and preimmunisation process along with a time schedule (Appendix 4.2.4.2). This document is supplied to a client when material is submitted for STG. The client will then be able to follow the STG process of his/her material when quarterly reports are submitted to him/her. Thirdly, a document was compiled to report to the client on a quarterly basis on the progress of the submitted material (Appendix 4.2.4.3).

Workshop: A workshop on STG was held during May 2006 to find improved methods that will enhance the release of citrus budwood to the industry. Points that arose that may contribute to meet the objective were the following:

1. *Change of phytosanitary act*. Submit a re-application that STG should be excluded when material is imported from **specified** STG laboratories. Declaration should be made by these laboratories of the GTD status of the material. Indexing should still be done. It will only save the STG time (± 10 mo).
2. *The Citrus Improvement Programme (CIP) should continue with the cultivar numbering system as maintained by the ARC-ITSC*. Information of a new cultivar / selection that was submitted for STG at CRI should be supplied to Dr. Manicom who will give it an ARC-ITSC # as well as a CIS #. Necessary information on indexing and release at CRI should be supplied to the ARC-ITSC from time to time to keep the data base complete and the database must be accessible to the CIP manager.
3. *Use rough lemon rootstocks to establish a source*. Rough lemon is a more vigorous rootstock and growth of the buds may be enhanced.
4. *When enough budwood is available, bud ten rootstocks with two buds each*. Two rootstocks are used to establish the source while the shoots of the others are used for STG (may save 3-4 mo). Instead of waiting for the buds to grow, harden off and stripping of leaves to force new shoots for STG, shoots from some buds are taken for STG as soon as they start to grow from the rootstocks. Usually two trees are made as source plants for STG, but instead, ten (or more) are made with multiple buds on each. This is an alternative method for the sterile sand technique where contamination is sometimes a problem. However, this method will take longer (10 d vs. 40 d). It can also be done in combination. Shoots of buds on at least two rootstocks should be left to grow to serve as back-ups (source plants).

5. *Use of tissue culture medium to preserve buds*.
6. *The use of tissue culture medium to remove GTD*. Instead of putting the shoot tip on an etiolated rootstock, put the shoot tip on an artificial medium, let it callus and develop a shoot, which is then micro-grafted on a strong virus-free rootstock in the glasshouse. This may be an improvement where incompatibility and oxidation are problems.
7. *The use of antioxidant solutions in sterile distilled water rinses to prevent oxidation and slime development associated with some mandarin types*. For example, dip knife in antioxidant solution before cut of tip. Concentration should be established for citrus. The following were found in the literature on antioxidant solutions: a) Cysteine @ 50 mg/l, b) Ascorbic acid @ 10-150 mg/l, c) Citric acid @ 10-150 mg/l, d) AA @ 50-150 mg/l + CA @ 100-200 mg/l.
8. *Leave cotyledons on etiolated rootstock to enhance growth of shoot tip*. (According to Dr. Manicom the MS liquid medium contains all the nutrients that are necessary and actually replaces the cotyledons).
9. *Do not use rootstocks in a dormant stage (especially trifoliate types)*. When these rootstocks are kept in an environment where artificial lighting extends daylight during winter, dormancy will not be a problem.
10. *Do not cut budwood during winter*. If budwood is selected behind specific fruit, label the branch / twig and cut in spring or summer.
11. *Develop molecular techniques for indexing*. Especially for psorosis and impietratura that take a year.
12. *Investigation for an alternative pre-immunising CTV isolate for mandarin types*. The mild LMS 6 CTV isolate appears to move slowly and/or un-evenly in some mandarin types. Currently there are no experiments to identify suitable CTV isolates for mandarins. The sweet orange “mild” isolates could possibly be evaluated in a glasshouse experiment.

Re-indexing of Clemenpons Clementine: Budwood of the Clemenpons Clementine selection was received from the Citrus Foundation Block (CFB) for re-indexing of graft transmissible diseases. It was pre-immunised material and therefore it was expected to detect the LMS 6 isolate that contains both components of *Citrus tristeza virus* viz. stem pitting (CTV-SP) and seedling yellows (CTV-SY). No CTV-SP was detected in the Mexican lime indicators but a strong CTV-SY reaction was observed in 3 of 5 Duncan grapefruit indicators (Fig. 4.2.4.1). There is not a direct comparison of the CTV-SY in LMS 6 and the CTV-SY in Clemenpons but it appears that it is more severe in the latter source. According to the literature CTV-SY is never found on its own but always with the CTV-SP component. This needs further investigation.

Furthermore, with the re-indexing to assess the CTV severity in the Clemenpons mother trees at the CFB, only one tree shows the presence of CTV 3 months after indexing. It is therefore recommended that CCP-PO1, CCP-PO2, CCP-PO3, CCP-PO4 and CCP-PO5 sources be suspended as budwood sources and that only CCP-PO6 is used as a source until more information is available. There is not a virus-free source of Clemenpons available and Shoot tip grafting should be repeated on the CFB source. The source indexed negative for Apple stem grooving virus (Tatter leaf) but the CTV-SY symptoms interfered with the Impietratura indexing.

To get more clarity, ELISA will be run on the Mexican lime and Duncan grapefruit indicators in the glasshouse. After the CTV indexing of the CFB sources have been completed, the sources will be transferred to Duncan grapefruit to assess the CTV-SY in comparison with that of LMS 6.

Figure 4.2.4.1. Duncan grapefruit seedling displaying yellow leaves after inoculation with material from Clemenpons Clementine.

APPENDIX 4.2.4.1

Practicalities in the Citrus Improvement Programme

Table 4.2.4.1.1. For indexing, the following cultivars are required:

Troyer/Carrizo citrange seedlings	For virus-free rootstocks, Tatter leaf indexing
West Indian (Mexican) lime seedlings	For CTV indexing
Rough lemon (RL) seedlings	For virus-free rootstocks and Etrog citron
861-S-1 Etrog Citron on RL	For citrus viroid indexing
Duncan or Marsh grapefruit, or Sour orange seedlings	For Impietratura indexing
Ponkan mandarin on RL	For Greening indexing
Parsons Special mandarin on RL	For Cachexia indexing
Madame Vinous seedlings or on RL	For Psorosis and Greening indexing
Nagami kumquat or Nules Clementine on Troyer	For possible Citrus Leaf Blotch indexing

Table 4.2.4.1.2. Seed and budwood sources for these are at:

Troyer/Carrizo citrange	CFB
West Indian (Mexican) lime	Leon Esselen
861-S-1 Etrog citron	VF bud source in Glasshouse (GH)
Duncan grapefruit	Leon Esselen
Ponkan mandarin	VF bud source in GH
Rough lemon	Leon Esselen
Sour orange	Leon Esselen

Parsons Special mandarin	VF bud source in GH
Madame Vinous sweet orange	VF bud source in GH, seed from Leon Esselen
Nagami kumquat or Nules Clementine	VF budwood from the Nucleus Block

Extract seed from fresh fruit, wash in sieve in 50ºC water, dry in shade, dust in fungicide, store at 4ºC till needed for planting in seed trays in GH. Keeps for a year but germination goes down.

Rootstocks in glasshouse can be held until they get too large – can be cut back a couple times. Replace rootstocks and indicators in stock annually.

Indexing

Two buds from the source are budded to each of 3 to 6 indicators.
The indicators and number for each GTD is listed in Table 4.2.4.1.3 below.
Cut indicators back directly after inoculation to force new growth and movement of the pathogens if they are present. Train new growth to single shoot.
These are monitored for symptoms.
The plants are cut back again 3 mo after inoculation (interim evaluation) for the 6 mo indexing periods and at 3, 6 and 9 mo intervals for the 12 mo indexing periods (Table 4.2.4.1.3).

Table 4.2.4.1.3. Biological and molecular indexing methods of different GTD.

DISEASE	TEST PLANT/METHOD	# PLANTS	DURATION
Tristeza	W.I. or Mex lime	3	6 mo
Tristeza	ELISA, PCR	Pool 3	2 days
Exocortis	Etrog citron	3	6 mo
Exocortis	PCR	Pool 3	2 days
Cachexia	Parsons Special mandarin	5	12 mo
Cachexia	PCR from Etrog citron	Pool 3	2 days
Psorosis	MV sweet orange	5-6	12 mo
Impietratura	Marsh/Duncan GF or Sour orange	5	12 mo
Apple Stem grooving	Troyer citrange	3	6 mo
Apple Stem grooving	PCR	Pool 3	2 days
Greening	Sweet orange or Ponkan mandarin	3	6 mo
Greening	PCR	Pool 3	2 days

DISEASES (Summary of information obtained from Roistacher, 1991)

Citrus tristeza virus
General: Endemic in South Africa. Several aphid species are vectors. Many strains exist.
Field Symptoms: Usually symptomless in sweet oranges and mandarins. Grapefruit and lime are sensitive and show decline, small fruit and stem pitting.

Bio-Indexing

Test Plant: West Indian (Mexican) lime seedlings for vein clearing and stem pitting symptoms. Grapefruit or Sour orange seedlings for Seedling Yellows component. Grapefruit will also show stem pitting.
Layout: 3 per pot, all inoculated. Positive and negative controls in separate pots.
Inoculum: 2 buds, not permitted to grow out. Cut back seedling directly after inoculation to 2 or 3 buds above inoculation.
Temperature: 24-28 / 18-22°C D/N.
Growth: Train to one shoot. Cut back at 3 mo.
Symptoms: First symptoms 3-5 weeks. Stunting may be mild, moderate or severe. Vein clearing will develop on the veins of young leaves and usually persist on the old leaves. Leaf cupping may develop when severe strains are present. Vein corking may develop, especially when the Seedling Yellows component is present. Stem pitting develops in the wood of the shoots and can be observed when the bark is peeled. It is best evaluated at the end of the indexing period. If the bark does not peal easily, steam it in an autoclave. For Seedling Yellows, the infected plants are usually mild, moderately or severely stunted. Yellow chlorotic leaves can be small to normal size. An infected plant can recover from infection and grow normally.
Terminate: After 6 mo.

Molecular Indexing

ELISA, Print, PCR.

Citrus viroids (CVd's) (Exocortis, Cachexia, Gum Pocket)

General: There are 14 CVd's known in the world. Three are important in SA. Trifoliates are very sensitive, Sweet oranges symptomless but can be dwarfed.

Field Symptoms: Exocortis will induce bark cracking on Rangpur lime, Trifoliate orange and its hybrids. The scions remain symptomless but are carriers. Cachexia cause discolouration of the phloem by gumming. Tangelo and mandarin are sensitive. Stem pitting or bumps can occur. Gum Pocket infection shows bark scaling and pitting in Trifoliate and its hybrids. Gum occurs in the bark and the cambium is discoloured. Dead "star-like" spots can occur.

Bio-Indexing

Test Plant: Sensitive 861-S-1 Etrog citron as cuttings or budded on RL rootstock. Cachexia is not easily detected on citron and should be indexed on Parson's Special mandarin as well.

Layout: Etrog citron, 3 per pot, all inoculated. Positive and negative controls in separate pots. Parson's Special mandarin, 3 per pot, all inoculated. Positive and negative controls in separate pots.

Inoculum: 2 buds, not permitted to grow out. Cut back seedling directly after inoculation to 2 or 3 buds above inoculation.

Temperature: 28-32 / 26-28°C D/N for both indicators.

Growth: Etrog - Train to one shoot. Cut back at 3 mo.
Parson's – Train to one shoot. Cut back at 3, 6 and 9 mo every time leaving 5 cm of the previous growth.

Symptoms: Etrog citron.
CEV – severe leaf epinasty and bark cracking.
Cachexia – leaf tip browning.
Gumpocket and other CVd's – petiole wrinkle, petiole browning, midvein browning, mild leaf epinasty (leaf bend).
Parson's Special mandarin.
Gum at budunion as well as at every cut-back joint.

Terminate: Etrog citron - 6 mo. Parson's – 12 mo.

Molecular Indexing

PAGE, PCR.

Citrus psorosis virus

General: Was nearly eradicated in SA. Unfortunately the law enforcing eradication was withdrawn and a couple of infected orchards remained.

Field Symptoms: Can be symptomless. Symptoms appear on trees 10 years and older.
Causes bark scaling of sweet orange, mandarin and grapefruit after 10 years in field. Wood is stained.

Bio-Indexing

Test Plant: Seedlings of sweet orange (Pineapple, Madame Vinous) can be used. Mandarin not so sensitive.

Layout: 3 per pot, all inoculated. Positive and negative controls in separate pots.

Inoculum: 2 buds, not permitted to grow out. Cut back seedling to 2 to 3 buds above inoculation point directly after inoculation.

Temperature: 24-27 / 18-21°C D/N.

Growth: Allow free growth for shock symptoms. Thereafter train to one shoot. Cut back every 3 mo to force new growth.

Symptoms: Shock symptoms on first flush at 3-4 weeks. Wilt and drying of new shoots. Not all isolates produce shock symptoms. Only some shoots may shock.
Vein flecking, leaf flecking, yellow patches on young leaves. Symptoms may disappear with age. Second flush does not shock and also shows symptoms, later flushes do not. Thus careful observation of first two flushes is important.

Terminate: After 1 year.

Molecular Indexing

RT-PCR.
Antibody tests (not available commercially) – ELISA, IEM, DTBIA.

Apple stem grooving virus (Tatter leaf) (Citrange stunt)

General: Most citrus are potential carriers as they are symptomless. Meyer lemon usually have it. Does not occur widely in SA.

Field Symptoms: Causes bud union crease, or fluting and reduced girth of the rootstock where infected scions are budded to trifoliate or hybrids (citrange). Wind can break trees at the bud-union.

Bio-Indexing

Test Plant: Troyer or Rusk citrange seedlings.

Layout: 3 per pot, all inoculated. Positive and negative controls in separate pots.

Inoculum: 2 buds, not permitted to grow out. Cut back seedling directly after inoculation to 2 or 3 buds above inoculation.
Temperature: 24-27 / 18-21°C D/N. Symptoms masked above 30°C.
Growth: Train to one shoot. Cut back at 3 mo.
Symptoms: Mild chlorotic spotting on first flush. Later flushes show intense leaf spotting, leaf deformation, "tattering" and stunting (Zig-zag growth). Symptoms persist. May get "recovery" growth.
Terminate: After 6 mo.

Molecular Indexing

RT-PCR.
Antibody tests (not available commercially) – ELISA, IEM, DTBIA.
Commercial apple stem grooving virus antibody test available from Neogen.

Impietratura disease (Means stone fruit)

General: Affects most citrus especially grapefruit and sweet orange.
Field Symptoms: Round, dark green, puffed spot on rind with gum pockets in albedo underneath. Fruit later hardens. Fruit drop. Flecking and oak leaf pattern in spring flush leaves.

Bio-Indexing

Test Plant: Marsh/Duncan grapefruit or Sour orange seedlings.
Layout: 3 per pot, all inoculated. Positive and negative controls in separate pots.
Inoculum: 2 buds, not permitted to grow out. Cut back seedling directly after inoculation to 2 or 3 buds above inoculation.
Temperature: 24-28 / 18-21°C DN.
Growth: Train to one shoot. Cut back every 3 mo.
Symptoms: Leaf flecking and oak leaf pattern in 5 to 8 weeks.
Terminate: After 1 year.

Molecular Indexing

None.

Citrus Leaf Blotch

General: Presence in SA not established.
Field Symptoms: Bud-union crease.

Bio-Indexing

Test Plants: Nagami kumquat or Nules Clementine on Troyer rootstock.
Layout: 3 per pot, all inoculated. Positive and negative controls in separate pots.
Inoculum: 2 buds onto the rootstock, not permitted to grow out. Cut back scion 5 cm above the bud union.
Temperature: 24-27 / 18-21°C D/N.
Growth: Train to one shoot. Cut back every 3 mo but not shorter than 5 cm above the bud-union.
Symptoms: Gumming at bud-union. Yellow blotches on leaves.
Terminate: After 1 year.

Molecular Indexing

RT-PCR.
Antibody tests (not available commercially) – ELISA, IEM, DTBIA.

APPENDIX 4.2.4.2

Table 4.2.4.2.1. Procedures for shoot tip grafting (STG), indexing (ID) and pre-immunisation (PI) of citrus material for the Citrus Improvement Programme.

PROCEDURES FOR THE ELIMINATION OF GRAFT TRANSMISSIBLE DISEASES OF CITRUS AND PRE-IMMUNISATION WITH A MILD *CITRUS TRISTEZA VIRUS ISOLATE* FOR THE SOUTH AFRICAN CITRUS IMPROVEMENT PROGRAMME				
PHASE	**PROCEDURE**	**ACTIONS**	**TIME SCHEDULE (DAYS)**	
			FOR PROCEDURE	**CUMULATIVE**
1	**ADMINISTRATION**	STOCKS: Should be available at all times: 1. Virus-free Troyer and rough lemon rootstocks in the glasshouse. 2. Sterile sand medium in wide mouth bottles. 3. Sterile Murashige & Skoog (MS) solid medium in test tubes. 4. Sterile MS liquid medium in test tubes. 5. Etiolated Troyer and rough lemon rootstocks on solid MS medium (can be stored at 4ºC). 6. Virus-free indicator plants for indexing in the glasshouse: Mexican lime for CTV; Etrog citron/rough lemon for CVd; Madam Vinous sweet orange for Psorosis and Greening; Troyer citrange for Tatter leaf. Marsh or Duncan grapefruit for Impietratura. 7. Pre-immunised rootstocks in the glasshouse: Mexican lime carrying LMS 6 for sweet oranges, limes, mandarins. Marsh or Duncan grapefruit carrying GFMS 12 for white grapefruit and Pummelo. Marsh or Duncan grapefruit carrying GFMS 35 for red and rosé grapefruit.	1	1
		Budwood arrives. Do documentation, assign CRI number and enter into data base. Enter the following procedures as they are used.		

PROCEDURES FOR THE ELIMINATION OF GRAFT TRANSMISSIBLE DISEASES OF CITRUS AND PRE-IMMUNISATION WITH A MILD *CITRUS TRISTEZA VIRUS ISOLATE* FOR THE SOUTH AFRICAN CITRUS IMPROVEMENT PROGRAMME				
PHASE	**PROCEDURE**	**ACTIONS**	**TIME SCHEDULE (DAYS)**	
			FOR PROCEDURE	**CUMULATIVE**
	ESTABLISH SOURCE	Two buds are budded to each of two to five (depending on condition of budwood) Troyer or rough lemon rootstocks, labelled and held in glasshouse at 28ºC (source plants). When grown out move to hot room (28-30ºC) to suppress virus. (When plants are adapted to high temperatures they can be kept at 40ºC - thermotherapy. This will make STG more successful). When the budwood is in good condition, the remaining bud sticks (6-8 cm long) are surface sterilised with 7.5% Jik and placed in bottles with sterile sand in growth room (28ºC, 16 hrs light). Bud sticks are monitored twice weekly. If buds grow out of sand cultured sticks these can go to STG. Alternatively wait until buds grow out from Troyer or rough lemon rootstocks. When growth is hardened off, defoliate and allow new shoots to develop for use.	90	90
2	**SHOOT TIP GRAFTING**	**<u>Growing Point</u>** Pick 1–10 cm tip of new shoot and place in Petri dish to prevent drying out. Break off large leaves under stereo microscope. Sterilise 5 min in 7.5% Jik. Rinse 2 X in sterile distilled water (SDW). Transfer to third rinse and can stay in this until needed on the day. To keep overnight, take out of SDW and store at 4ºC.		
		<u>Rootstock preparation</u> Remove etiolated rootstock from test tube with artificial growth medium. Cut off top square to give about 5 cm stem and remove cotyledons. Trim roots so that it will plant easily. Make horizontal cut through cortex. Cut down from above this cut to form a 'chip' 1-2 mm high. Check that it is free and replace to prevent drying out.		

PROCEDURES FOR THE ELIMINATION OF GRAFT TRANSMISSIBLE DISEASES OF CITRUS AND PRE-IMMUNISATION WITH A MILD *CITRUS TRISTEZA VIRUS ISOLATE* FOR THE SOUTH AFRICAN CITRUS IMPROVEMENT PROGRAMME				
PHASE	**PROCEDURE**	**ACTIONS**	**TIME SCHEDULE (DAYS)**	
			FOR PROCEDURE	**CUMULATIVE**
		<u>Grafting (STG)</u> Under stereo microscope, break off remaining leaves of shoot tip untill meristem dome is visible. Cut off top 0.15 mm (tiny) (shoot tip + 1-2 primordia). Transfer to the step of the rootstock on an upright position on cambium/phloem. Plant into liquid medium and label the test tube: CRI # + Xplant #. Transfer to growth room and monitor twice weekly. Remove rootstock outgrowths after 1-2 weeks (trifoliate or rough lemon if doing a trifoliate). Could be ready in 2-3 weeks, about 0.5 cm with small leaves.	30	120
3	**MICRO GRAFTING**	Remove plant from test tube with liquid medium. Cut off upper 2 cm of the plant (rootstock and shoot tip graft). Cut to form a wedge graft (two diagonal cuts). Cut corresponding strip of young bark, attached at bottom, on active growing virus-free Troyer rootstock to accommodate the wedge rootstock plus STG. Place graft on cambium layer, re-cover with bark and bind with plas-strip or parafilm. Cover with plastic bag, tie closed at bottom, label (CRI number plus Xplant number). Transfer to glasshouse at 28ºC. Open plastic bag after 5-7 days, and remove after further 5-7 days. Allow to grow.	180	300

PROCEDURES FOR THE ELIMINATION OF GRAFT TRANSMISSIBLE DISEASES OF CITRUS AND PRE-IMMUNISATION WITH A MILD *CITRUS TRISTEZA VIRUS ISOLATE* FOR THE SOUTH AFRICAN CITRUS IMPROVEMENT PROGRAMME				
PHASE	**PROCEDURE**	**ACTIONS**	**TIME SCHEDULE (DAYS)**	
			FOR PROCEDURE	**CUMULATIVE**
4	**INDEXING**	Twelve mature buds are necessary to initiate ID, therefore, the STG plant should have developed a shoot with at least 15 buds. Two buds from the STG plant are budded to each of three Mexican lime indicator plants (for CTV) held at 24-28º and three Etrog citron indicator plants (for Citrus viroids), held at 28-32º. These are monitored for symptoms: interim evaluation at 3 mo; full evaluation at 6 mo. After three months, inspect for symptoms and cut back to force new growth. When cut back, leave 2 buds (3-5 cm) of first growth after index. Checking for stem pits wil be done here at the final evaluation.		
		After six months, final inspection for symptoms. Confirm with ELISA and PCR. This is second and final screen for CTV and viroids. If negative all is OK and interim release can be done if PI plant is positive. If positive, back to STG. Destroy PI plant. Make 2 virus-free plants on virus-free Troyer for the nucleus block in the tunnel.	180	480
		After interim release continue ID for Greening, Impietratura, Apple stem grooving virus (Tatter leaf) and Psorosis virus. Imported material should also be screened for, depending on origin, any potential threats to industry. This continues a further year. If any positives, destroy foundation block material and start from beginning.	360	840

PROCEDURES FOR THE ELIMINATION OF GRAFT TRANSMISSIBLE DISEASES OF CITRUS AND PRE-IMMUNISATION WITH A MILD *CITRUS TRISTEZA VIRUS ISOLATE* FOR THE SOUTH AFRICAN CITRUS IMPROVEMENT PROGRAMME				
PHASE	**PROCEDURE**	**ACTIONS**	**TIME SCHEDULE (DAYS)**	
			FOR PROCEDURE	**CUMULATIVE**
	PRE-IMMUNISATION	When the indicators of both ID procedures are negative during the interim evaluation, two buds of the STG plant are budded to a rootstock PI with the required CTV isolate for cross-protection. This rootstock will be: Mexican lime carrying LMS 6 for sweet oranges, limes, mandarins; Marsh or Duncan grapefruit carrying GFMS 12 for white grapefruit and pummelos; Marsh or Duncan grapefruit carrying GFMS 35 for red and rosé grapefruit. Lemons and rootstocks are not PI. After 3 mo do ELISA to confirm pre-immunisation. When positive (desired isolate is present) make 2 plants on virus-free Troyer. When growth has hardened off and the final inspection of the interim ID is negative, buds are now interim released to the Citrus Foundation Block.	90 Usually no additional time. It is done during the same time as the interim ID of CTV and CVd	
	INTERIM RELEASE: **RELEASE:**	(Minimum time)		16 mo 28 mo

APPENDIX 4.2.4.3

CONFIDENTIAL

REPORT

SHOOT TIP GRAFTING, INDEXING AND PRE-IMMUNIZATION OF CITRUS MATERIAL

CLIENT NAME:

INTRODUCTION DATE OF MATERIAL: **1)**
2)
3)

REPORT DATE	CULTIVAR/SELECTION		STG # (Xplant)	STG PHASE	COMMENTS
	NAME or #	CRI #			

REPORT DATE	CULTIVAR/SELECTION		STG # (Xplant)	STG PHASE	COMMENTS
	NAME or #	CRI #			

Please note:

1) The ELISA, IEM, and PCR diagnostic methods used by CRI have been characteriSed and tested against available local viruses. The possibility must be recogniSed that there may be variants of the virus which cannot be detected in the assay.
2) The assays will detect the specified virus only above a certain threshold value. It is important to understand that the failure to detect a specific virus does not guarantee the complete absence of that virus from the sample analySed. In ANY diagnostic assay, there is a lower limit of detection, below which a virus cannot be detected. Virus levels may vary due to climatic conditions, host plant genotype, virus strain or plant tissue assayed.
3) As a consequence of points 1) and 2) no guarantee can be provided that a sample yielding a negative assay result will be completely free of the virus tested for.
4) Sampling, safe delivery and accurate labeling of the samples is the responsibility of the client.
5) Assay results refer strictly to the actual samples supplied, at the time of sampling. It should not be assumed that the same results apply to any other plants/ progeny/ predecessor of the sample, or the same sample at another time. Clients should not represent or imply that the results apply other than to the actual samples provided for assay. The client releases and indemnifies CRI in respect of any loss or liability incurred as a consequence of such representations, which remain the sole responsibility of the client.

Conclusion

During the reporting year, 22 selections were added to the virus-free source after ELISA. This brings the total to 235 virus-free selections of 14 cultivars that have successfully been established in an insect-free tunnel. Additions to the source are a continuous process. Control of insects in the tunnel is crucial.

Forty two selections were received as new additions and are in the process of STG and indexing.

Future research

Apply STG and indexing on the current contaminated sources of CRI and clients.
Receive and maintain new additions.
Maintain the virus-free source in the insect-free tunnel.
Pre-immunise virus-free sources that are required by the Citrus Foundation Block.
Introduce new information to the data-base.

Acknowledgement

Dr. B.Q. Manicom of the ARC-ITSC for the suggestion to compile the document on practicalities.

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4.2.5 **Cross-protection of Marsh and Star Ruby grapefruit using Beltsville sub-isolates of Nartia mild strain**

Experiment 679 by J.H.J. Breytenbach & S.P. van Vuuren (CRI)

Opsomming

Daar is gevind dat die Nartia isolaat (GFMS 12) wat huidiglik gebruik word vir pre-immuisering van wit pomelos en pompelmoese, gekontamineer is met 'n strawwe *Citrus tristeza virus* ras. Twintig sub-isolate van twee Nartia isolate (A=GFMS 12, C=GFMS 14) en die Mouton isolaat is in Beltsville MD, VSA, voorberei deur middel van enkel plantluis oordragings. Ses van die 20 sub-isolate wat 'n potensiaal as kruisbeskermingsagente getoon het nadat dit deur biologiese indeksering in die glashuis ge-evalueer is, is gebruik om hul beskermingsvermoëns teen strawwe rasse in die boord te bepaal. Virusvrye Star Ruby en Marsh pomelo boompies is gepreïmmuniseer met die ses Beltsville sub-isolate asook twee enkel plantluis oordraging sub-isolate van die LNR-ITSG (GFMS 12/7, GFMS 12/9), GFMS 12 en GFMS 35. Boompies is virusvry gelaat as kontrole. Preïmmunisasie is bevestig deur middel van ELISA. ELISA het uitgewys dat twee van die Beltsville sub-isolate nie voldoen aan sekere vereistes vir 'n goeie kruisbeskermingsisolaat nie deurdat hulle 'n lae persentasie oordraagbaarheid het, en stadig vermeerder en beweeg in die plant. Hierdie twee sub-isolate word nie verder ge-evalueer nie. Die Marsh boompies is uitgeplant by Riversbend in die Nkwaleni vallei en die Star Ruby is uitgeplant by Tambuti landgoed in Swaziland gedurende 2003. Die boompies se boomvolumes, stamgleufwaardes asook oesopbrengs is geneem 30 maande na plant. In die vroeë stadium is die volgende aanduidings: GFMS12 onderdruk groei en bevorder stamgleuf, GFMS35 presteer beter as GFMS12 in Marsh en Star Ruby en sub-isolaat 390/3 presteer die beste in geheel. Virusvrye kontrole bome presteer goed in die vroeë stadium maar mag verander sodra hulle geinfekteer word met wilde CTV rasse. Met tyd sal dit egter duidelik word of van die sub-isolate beter beskermers vir pomelo is as die huidige twee isolate, GFMS 12 en GFMS 35.

Introduction

Citrus tristeza virus (CTV) is the causal agent of possibly the most destructive disease of citrus in the world. The disease is spread by propagative material and various aphid species of which *Toxoptera citricida* is the most efficient. Many strains of CTV occur and may co-exist as mixtures in individual field trees and even in single buds on a tree. Symptoms produced by CTV range from mild with no noticeable effect on the host to severe stem pitting and decline resulting in uneconomic production (Marais *et al.,* 1996). The only practical means of controlling CTV disease at present is by mild strain cross-protection (van Vuuren *et al.*, 1993). A breakdown in the protection offered by the 'nartia' A (GFMS 12) isolate owing to the presence of a severe strain within the complex (van Vuuren *et al.*, 2000), motivated the separation of strains by single aphid transmission (SAT). Twenty SAT sub-isolates were obtained from two 'nartia' isolates (A=GFMS 12 and C=GFMS 14; van Vuuren *et al.*, 1993) and the Mouton isolate. In this study, the sub-isolates are being evaluated for mildness and their potential as cross-protecting isolates in the field.

Materials and methods

The 20 SAT sub-isolates of the 'nartia' A and C isolates (A=GFMS 12 and C=GFMS 14) as well as that from the Mouton isolate were prepared at the quarantine facility in Beltsville MD, USA and were imported back to South Africa. In a greenhouse experiment, they were bud-inoculated separately to CTV sensitive Mexican lime indicator plants which are differential hosts that develop symptoms characteristic of the biological activity of a sub-isolate. Growth and stem pitting were determined and the virus titer was measured by means of enzyme-linked immunosorbent assay (ELISA) for each sub-isolate 6 months after inoculation. The four mildest sub-isolates (GFMS 14 subs: B389-1, B389-4; Mouton subs: B390-3, B390-5) were bud-inoculated (pre-immunisation) to virus-free Marsh and Star Ruby grapefruit on MxT rootstocks that were

prepared under insect-free conditions in the greenhouse. They will be compared with GFMS 12 (standard for white grapefruit), GFMS 35 (standard for red grapefruit), GFMS 12/7 and GFMS12/9 (ARC-ITSC single aphid transfer sub-isolates from GFMS 12) and trees that were planted virus-free at the time of planting. Pre-immunisation has been confirmed by ELISA 6 months after inoculation. During 2003, the Star Ruby grapefruit trees were planted in Swaziland at Tambuti Estates and the Marsh grapefruit trees at Riversbend in the Nkwaleni Valley to allow field evaluation of the pre-immunised trees using the criteria tree size, production and tree health.

Results and discussion

The data of the two grapefruit cultivars cannot be compared since they are grown in different climatic conditions.

Tree size: The heights and canopy diameters of the trees were measured and the canopy volumes calculated.

Marsh grapefruit: The results of the Marsh trees are presented in Table 4.2.5.1. There are significant differences between the isolates and sub-isolates. At this early age, trees with sub-isolate B390/3 and the virus-free trees performed the best but they are not significantly better than trees with sub-isolates B389/1, GFMS 12/7, GFMS 12/9 and the GFMS 35 control isolate. Some of the sub-isolates (B389/4, B390/5) retarded growth and the trees are similar to those with GFMS 12.

Star Ruby grapefruit: The results of the Star Ruby trees are presented in Table 4.2.5.2. The Star Ruby tree sizes are more even. Only those with B389/4 were significantly larger than those with isolate GFMS 12. It is for the second year that sub-isolate B389/4 gave contradictive results where trees with this isolate performed the best in the Star Ruby trees but poorly in the Marsh trees. This, however, can be the influence of the host or due to climatic differences.

Production: The trees were harvested and the mean yield per tree calculated. The results for the Marsh and Star Ruby trees are presented in Tables 4.2.5.1 and 4.2.4.2, respectively. This was the first year that the trees bear fruit at a commercial level. Fruit sizes were not determined since fruit are usually very large and puffy at the first year. Although there are significant differences between the isolates and the sub-isolates in both the Marsh and Star Ruby trees, it is still too early to draw any conclusions from the data. The calculated yield efficiency of both cultivars show a variation but since the trees are still young, it can be attributed to excessive vegetative growth. The higher efficiency of trees in two Star Ruby treatments may be due to stress factors induced by the disease, i.e. the higher occurrence of stem pitting where GFMS 12 was used to pre-immunise.

Tree health: The Marsh and Star Ruby trees were evaluated for the occurrence of stem pitting. The results are presented in Table 4.2.5.1 and 4.2.5.2, respectively. Both the Marsh and Star Ruby trees with GFMS 12 had a significant higher occurrence of stem pitting, which confirms once again that GFMS 12 is contaminated with a severe strain. No decline was observed.

Table 4.2.5.1. Tree size (canopy volume in m^3), yield (kg/tree), yield efficiency (kg/ m^3 canopy) and stem pitting (SP) rating of Marsh grapefruit trees pre-immunised with different CTV isolates and sub-isolates, 30 months after planting at Riversbend *****.

Treatment	Canopy volume (m^3)	Production		Stem pitting rating**
		Yield (kg/tree)	Efficiency (kg/ m^3)	
B389/1	5.4 bcd	13.8 Ab	2.6	0 a
B389/4	4.9 abc	20.7 Ab	4.2	0 a
B390/3	6.8 d	28.2 B	4.2	0 a
B390/5	4.0 a	7.3 A	1.8	0 a
GFMS 12/7	5.9 bcd	23.2 ab	3.9	0 a
GFMS 12/9	5.5 bcd	21.5 ab	3.9	0 a
GFMS 12 (Marsh control)	4.5 ab	20.3 ab	4.5	1.3 b
GFMS 35 (Star Ruby control)	6.1 cd	31.0 b	5.1	0 a
Virus-free (Control)	6.9 d	28.2 b	4.1	0 a

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).
** Stem pitting rating: 1 = Smooth trunk; 2 = Mild pitting; 3 = Severe pitting.

Table 4.2.5.2. Tree size (canopy volume in m^3), yield (kg/tree), yield efficiency (kg/ m^3 canopy) and stem pitting (SP) rating of Star Ruby grapefruit trees pre-immunised with different CTV isolates and sub-isolates, 30 months after planting at Tambuti*.

Treatment	Canopy volume (m^3)	Production		Stem pitting rating**
		Yield (kg/tree)	Efficiency (kg/ m^3)	
B389/1	11.3 ab	3.5 a	0.3	0 a
B389/4	15.2 b	11.3 ab	0.7	0 a
B390/3	13.9 ab	7.6 abc	0.6	0 a
B390/5	13.4 ab	5.3 ab	0.4	0 a
GFMS 12/7	12.3 ab	4.3 ab	0.4	0.7 c
GFMS 12/9	11.6 ab	14.2 bc	1.2	0.6 bc
GFMS 12 (Marsh control)	10.5 a	17.3 c	1.7	2.2 d
GFMS 35 (Star Ruby control)	11.6 ab	6.3 ab	0.5	0 a
Virus-free (Control)	13.4 ab	2.1 a	0.2	0 a

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).
** Stem pitting rating: 1 = Smooth trunk; 2 = Mild pitting; 3 = Severe pitting.

Conclusion

The trees are still young and therefore all differences should be seen as indications:

- Some sub-isolates suppressed growth of the Marsh trees and are similar to the trees with GFMS 12, which is known to carry a severe strain;
- The Marsh (white grapefruit) trees with GFMS 35, which is the red grapefruit pre-immunising isolate, are better than trees with GFMS 12, the white grapefruit pre-immunising isolate;
- Confirmation that GFMS 12 is not a good pre-immunising isolate for Star Ruby;
- Trees with B390/3 sub-isolate performed the best overall;
- Trees that were planted virus-free are growing well and it is an indication that challenge infections of various strains by aphids have no influence yet.

Future research

Evaluate the horticultural performance of trees over a 10-year period using the following parameters:

- Growth (canopy volume);
- Yield and fruit size;
- Tree health (stem pitting and decline).

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4.2.6 Cross-protection of Marsh and Star Ruby using Beltsville sub-isolates of Nartia mild strain for the Orange River Valley

Experiment 738 by J.H.J. Breytenbach & S.P. van Vuuren (CRI)

Opsomming

As gevolg van die teenwoordigheid van 'n strawwe ras in die Nartia isolaat (GFMS 12) was dit nodig om isolate te verdeel in sub-isolate deur middel van enkel plantluis oordragings. Hierdie sub-isolate is voorberei vanaf twee Nartia isolate (A=GFMS 12, C=GFMS 14) en 'n Mouton isolaat by die kwarantyn fasiliteit in Beltsville, VSA, en ingevoer terug na Suid Afrika. Nadat die sub-isolate deur biologiese indeksering ge-evalueer is om tussen die ligte en strawwe rasse te onderskei, is gevind dat slegs vier (GFMS 14 subs: B389-1, B389-4; Mouton subs: B390-3, B390-5) potensiaal het vir verdere evaluering. Twee belowende Nartia sub-isolate afkomstig van die LNR-ITSG (GFMS 12/7, GFMS 12/9) is ingesluit in die proef asook GFMS 12 (standaard vir wit pomelos) en GFMS 35 (standaard vir rooi pomelos). Virusvrye Star Ruby boompies is in 'n glashuis voorberei en gepre-immuniseer met die isolate en sub-isolate. 'n Virusvrye behandeling is ingesluit as kontrole. Omdat *Citrus tristeza virus* deur die gasheer en klimaat beïnvloed word,

is dit nodig om isolate in die verskillende sitrus produserende streke te evalueer. Nadat pre-imunisering deur middel van ELISA bevestig is, is die boompies in die Kakamas omgewing uitgeplant gedurende September 2004, en sal jaarliks ge-evalueer word vir boomgrootte, stamgleuf, oes opbrengs en vruggrootte. Die boompies se groottes is gemeet 24 maande na uitplant. Die boompies groei heelwat stadiger as wat boompies in die ander pomelo produserende streke goei. Dit is egter nog te vroeg om afleidings te maak vanaf die vroeë resultate.

Introduction

The severe effect of *Citrus tristeza* virus on grapefruit production makes pre-immunisation with mild strains essential (Marais *et al*., 1996). A breakdown in the CTV protection offered by the GFMS 12 (Nartia A) isolate, owing to the presence of severe strains within the complex, motivated the separation of the strains in isolates by single aphid transmission (SAT). SAT from two Nartia isolates (A=GFMS 12, C=GFMS 14; van Vuuren *et al.*, 1993) and a Mouton isolate were prepared at the quarantine facility in Beltsville MD, USA. After re-importation, these sub-isolates underwent biological indexing to differentiate between the severe and mild forms. Some sub-isolates had no potential as cross protectors due to the development of unacceptable severe stem pitting, or the virus concentration and movement of the virus were poor (Breytenbach *et al.*, 2002). Four of the sub-isolates showed potential and will be evaluated as cross-protectors. Promising SAT sub-isolates of GFMS 12 (Nartia A) obtained from the ARC-ITSC will also be included in this experiment (van Vuuren *et al.*, 2000). As CTV exhibits host and geographical specificity, it is imperative that mild protecting isolates be tested in the different production areas (da Graça *et al.*, 1984).

Materials and methods

Virus-free Star Ruby budwood was budded to virus-free MxT rootstocks. When the scions had developed to approximately 5 mm thickness, they were bud inoculated with the sub-isolates of GFMS 14 and Mouton (B389-1, B389-4, B390-3, B390-5), ARC-ITSC sub-isolates (GFMS 12/7, GFMS 12/9) and compared to the two standards (GFMS 12 for white grapefruit; GFMS 35 for red grapefruit) as well as trees that were left virus-free. After pre-immunisation of the trees was confirmed by ELISA, they were planted in the Kakamas area according to a randomised block with five replications to allow field evaluation of the pre-immunised trees using the criteria tree size, production and tree health.

Results and discussion

The heights and diameters of the 12-month-old trees were measured and the canopy volumes (m^3) calculated. The results are presented in Table 4.2.6.1. Although there are significant differences in growth between trees with the different isolates and sub-isolates, it should be seen as trends since the trees are only a year old. The site of this experiment is not in a typical grapefruit area and the cooler winter climate will favour the tristeza virus.

Table 4.2.6.1. Tree size of Star Ruby trees pre-immunised with different CTV isolates and sub-isolates, 12 months after planting in the Orange River Valley.

Treatments	Tree volumes (m^3)*
B389/1	0.90 abc
B389/4	0.77 abc
B390/3	0.60 ab
B390/5	0.87 abc
GFMS 12/7	1.04 bc
GFMS 12/9	0.93 abc
GFMS 12 (Marsh control)	1.17 c
GFMS 35 (Star Ruby control)	0.77 abc
Virus-free (Control)	0.50 a

* Figures followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Conclusion

The trees are still young and no conclusions can be made at this stage.

Future research

Evaluate horticultural performance i.e. growth (tree size), yield (kg/tree and fruit size) and tree health (stem pitting and decline).

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4.2.7 Cross-protection of Marsh and Star Ruby by using the best field isolates collected in the different grapefruit production areas of southern Africa

Experiment 742 by J.H.J. Breytenbach & S.P. van Vuuren (CRI)

Opsomming

Enthout is gesny in die verskillende pomelo gebiede vanaf 108 uitstaande pomelo bome wat moontlik ligte isolate van C*itrus tristeza virus* (CTV) huisves. Die isolate is gevestig op onderstamme in die glashuis by CRI. Hierna is die verskillende isolate op Meksikaanse lemmetjie geïnokuleer (biologiese indeksering) om te bepaal of hulle wel lig is. Na die eerste biologiese indeksering het slegs 19 isolate potensiaal getoon en is gekies vir verdere evaluering. Hierdie 19 isolate is 'n tweede keer geïnokuleer op Meksikaanse lemmetjie en vergelyk met GFMS 12, GFMS 35, GFMS 12/7,GFMS 12/9 en die vier beste Beltsville sub-isolate (GFMS 14 subs: B389-1, B389-4; Mouton subs: B390-3, B390-5). Die geïnokuleerde plante is ge-evalueer vir groei en stamgleuf. Virus titer is bepaal deur ELISA. Die mees belowendste van hierdie 19 veld isolate, wat vry is van viroiede (Tabankulu 1 – versamel vanaf Star Ruby in Swaziland; New Venture 41/2 – versamel vanaf Star Ruby in die Nkwaleni Vallei; ORE 8 – versamel vanaf Marsh in die Hoedspruit gebied; Tshipise 19/5 – versamel vanaf Marsh in Tshipise), word gebruik om virusvrye Marsh en Star Ruby boompies mee te preïmmuniseer. Die isolate word vergelyk met GFMS 12 (standard vir wit pomelos), GFMS 35 (standard vir rooi pomelos), asook die vier beste Beltsville sub-isolate (B389-1, B389-4, B390-3, B390-5) en LNR sub-isolate (GFMS 12/7, GFMS 12/9). Pre-immunisering is bevestig deur middel van ELISA en sal gedurende Februarie 2007 by Bosveld Sitrus in die Letsitele omgewing geplant word.

Introduction

Shoot tip grafting is a technique to eliminate all graft-transmissible agents from citrus bud-wood sources (de Lange *et al*., 1981). In South Africa, the benefit of optimum growth and production of virus-free trees cannot be used because of the abundance of the aphid insect vector of *Citrus tristeza virus* (CTV) (Schwarz, 1965). Virus-free plantings will get infected with various strains of CTV within a few years after planting. Many strains of CTV exist and they usually occur as mixtures in a host (McClean, 1963). Factors such as the type of strain, host and environment will determine dominance of a specific strain and it may change if any of the factors change *viz*. co-infection of a new strain or extreme temperatures (da Graca *et al*., 1984; Oberholzer, 1959). The only method to overcome this problem is by cross-protection, a deliberate infection of virus-free material with a known CTV isolate. The first step in searching for mild isolates for cross-protection purposes is to look for old trees that are healthy and produce good quality fruit (Müller & Costa, 1987).

This experiment is a follow-up of the glasshouse trial (exp. 49) where 108 CTV isolates were collected in different grapefruit production areas from productive old grapefruit trees. After an initial screening in the glasshouse, 19 isolates showed potential as cross protectors. These 19 isolates were then compared to the present pre-immunising isolates. The most promising of these 19 field isolates, that are free of citrus viroids, will be evaluated as cross protecting agents in Star Ruby and Marsh grapefruit trees in the field.

Materials and methods

Virus-free Troyer citrange rootstocks were propagated and budded with virus-free Star Ruby and Marsh grapefruit budwood in a greenhouse. When the scions had developed to approximately pencil thickness,

they were inoculated with the selected CTV isolates in the scions. The following CTV isolates were used: the four most promising isolates selected from the original 108 field isolates (Tabankulu 1 – derived from Star Ruby in Swaziland; New Venture 41/2 – derived from Star Ruby in the Nkwaleni Valley; ORE 8 – derived from Marsh in the Hoedspruit area; Tshipise 19/5 – derived from Marsh in Tshipise), the four best Beltsville sub-isolates (GFMS 14 subs: B389-1, B389-4; Mouton subs: B390-3, B390-5) and two ARC-ITSC sub-isolates (GFMS 12/7,GFMS 12/9). Isolates GFMS 12 (standard for white grapefruit) and GFMS 35 (standard for red grapefruit) will be used as standards and trees will be left uninoculated as controls. Three months after inoculation, positive pre-immunisation will be confirmed by ELISA where after the trees will be planted in two grapefruit production areas according to a randomised block design with five replicates. Growth, production and tree health will be monitored.

Results and discussion

Successful pre-immunisation was confirmed by means of ELISA. Trees will be planted during February 2007 at Bosveld Sitrus in the Letsitele area.

Conclusion

The experiment is still in the early stages.

Future research

Evaluate horticultural performance i.e. growth (tree size), yield data (fruit size and kg/tree) and health (stem pitting, decline).

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4.2.8 **The response of different red grapefruit cultivars to *Citrus tristeza virus***

Experiment 785 by S.P. van Vuuren, J.H.J. Breytenbach (CRI), B.Q. Manicom (ITSC)

Opsomming

Agt-jaar oue bome van sewe rooi pomelo seleksies, nl. Star Ruby, Rio Red, Henderson, Nel Ruby, Flame, Ruben en Oran Red op Swingle citrumelo onderstam, het baie eenvormig gereageer met vier ligte *Citrus tristeza virus* (CTV) isolate (GFMS 12, GFMS 35, GFMS 67, GFMS 73) as kruisbeskermings-agente. Tussen die CTV isolate was daar nie verskille in boom grootte nie. Tussen die pomelo seleksies was Nel Ruby bome die grootste en Oran Red bome die kleinste. Dit is moontlik dat Oran Red ’n genetiese dwerg eienskap het. Daar is aanduidings van interaksies tussen sommige CTV isolate en pomelo seleksies (Rio Red met GFMS 12; Flame met GFMS 35). Die strawwe isolaat (GFSS 5) het nie die kroon volume van die Ruben bome geaffekteer nie wat ’n aanduiding is dat die seleksie tolerant is teen CTV. Die kumulatiewe produksie oor 3 jaar toon dat Star Ruby bome met GFMS 35 swakker is as bome met die ander ligte isolate. Dit is in teenstelling met verskeie vorige bevindings.

Introduction

Shoot tip grafting is a technique to eliminate all graft-transmissible agents from citrus bud-wood sources in South Africa (Fourie and van Vuuren, 1993). However, the benefit of optimum growth and production of virus-free trees cannot be attained because of the abundance of the aphid vector, *Toxoptera citricida*

(Kirkaldy) of *Citrus tristeza virus* (CTV) (Schwarz, 1965). Virus-free plantings will get infected with various strains of CTV within a few years after planting. Many strains of CTV exist and they usually occur as mixtures in a host (McClean, 1963). Factors such as the type of strain, host plant and environment will determine dominance of a specific strain and it may change if any of the factors change *viz*. co-infection of a new strain or extreme temperatures (da Graça *et al.*, 1984; Oberholzer, 1959). The only method to overcome this problem is by cross-protection, a deliberate infection of virus-free material with a known CTV isolate (Müller and Costa, 1987).

Of the commercial citrus cultivars grown in southern Africa, grapefruit is the most sensitive to the *Citrus tristeza virus* disease, which causes stem pitting, decline and production of small fruit. With the initiation of the southern African Citrus Improvement Programme (CIP), all grapefruit selections are pre-immunised with the GFMS 12 CTV isolate (von Broembsen and Lee, 1988). This isolate originated from a 50-year-old Nartia (Marsh type) grapefruit tree in the Western Cape Province. Bud-wood source trees at the Citrus Foundation Block (CFB) at Uitenhage, which is the only source for propagating certified trees, are evaluated visually each year for decline and stem pitting symptoms. In addition, each tree of every selection is biologically indexed on an annual basis to establish the CTV severity. When there are indications of severe CTV, such a tree is terminated as a bud-wood source.

In 1993, it was found that 6-year-old Star Ruby bud-wood source trees varied in stem pitting development and fruit size (unpublished data). Testing the CTV stem pitting severity revealed that severe strains were dominating in four of the five bud-wood source trees. At first it was thought that GFMS 12 did not protect against co-infection of severe strains. However, subsequent research showed the presence of a severe strain in the original isolate and that segregation of the strains, where the severe strain became dominant, may be the cause of the problem (van Vuuren *et al.*, 2000). The unsuitability of GFMS 12 as a protector for Star Ruby was also confirmed in a field trial (van Vuuren and van der Vyver, 2000). Consequently, GFMS 35 (derived from a Rosé grapefruit tree) has been approved for the pre-immunisation of all red grapefruit until more suitable isolates can be identified (Luttig *et al.*, 2002).

The first step in searching for mild isolates for cross protection purposes is to look for old trees that are healthy and produce good quality fruit (Müller and Costa, 1987). The Star Ruby grapefruit industry in South Africa started in the late 1970's and therefore no trees older than 15 years existed at the time. To overcome this problem, the best producing trees in the oldest plantings at Malelane, Mpumalanga Province, and Swaziland were selected. Isolates from these trees were evaluated in glasshouse tests and those with the best potential were evaluated in the field.

The objective of this study is to evaluate new CTV isolates in different red grapefruit selections.

Materials and methods

Seven red grapefruit selections *viz*. Star Ruby, Flame, Rio Red, Nel Ruby, Henderson, Ruben and Oran Red were budded as scions on Swingle citrumelo rootstocks. Tristeza isolates GFMS 35, GFMS 67, GFMS 71 and GFMS 73 are evaluated in each scion and compared to the standard (GFMS 12) and a severe isolate (GFSS 5). ELISA confirmed infection before they were planted in a randomised split plot with five replications at Malelane during December 1998.

Results and discussion

Tree size, yield, yield efficiency, cumulative yield and stem pitting ratings of the red grapefruit selections that were pre-immunised with different CTV isolates are presented in Table 4.2.8.1, Table 4.2.8.2, Table 4.2.8.3, Table 4.2.8.4 and Table 4.2.8.5, respectively.

Tree size: Overall, canopy volumes of trees that were pre-immunised with the different mild isolates did not differ from each other but they were significantly larger than the trees inoculated with a severe isolate. Of the selections, trees of Nel Ruby, Flame and Ruben were the largest and were significantly larger than the Henderson and Oran Red trees. The Oran Red trees were the smallest, but it is possible that Oran Red has a genetic dwarfing characteristic (Table 4.2.8.1).

The body of the table shows some interactions between selections and some of the mild isolates (Rio Red with GFMS 12; Flame with GFMS 35). It also appears that Ruben has some tolerance to CTV since the severe isolate did not affect trees size. The sizes of these trees were similar to the average sizes of trees with the mild isolates (Table 4.2.8.1).

Production: Trees with GFMS 67 had a significantly higher yield than trees with GFMS 12. With the grapefruit selections, the yield of Rio Red and Nel Ruby trees was significantly higher than those of the Henderson trees (Table 4.2.8.2). It was lower (not significantly) to that of the Oran Red trees, which had a smaller canopy size (also not significantly) (Table 4.2.8.1). This resulted in a significantly higher yield efficiency of the Oran Red trees (Table 4.2.8.3). The Star Ruby trees had significantly lower yield efficiency than the trees of all the other selections. This situation could be attributed to trees that are in a more vigorous state or trees that are in a state of decline because of disease. However, the trees were not more vigorous and also did not display decline. Disease pressure is also not reflected in the stem pitting status (Table 4.2.8.5). The yield efficiency among trees with the mild CTV isolates did not differ.

Cumulative yield: The Nel Ruby and Flame trees had significantly higher cumulative yield than Star Ruby and Oran Red (Table 4.2.8.4). The poor overall performance of the Star Ruby trees can be attributed to the low yield where GFMS 35 was used as a preimmunising agent. This is in contradiction to a number of previous cross-protection trials (van Vuuren *et al.*, 2000; van Vuuren and Manicom, 2004). The lower cumulative yield of the Oran Red trees might be attributed to genetic dwarfing characteristics (see tree size in Table 4.2.8.1).

Stem pitting: Overall the stem pitting did not differ among trees pre-immunised with the different mild CTV isolates (Table 4.2.8.5). The Ruben trees had the least stem pitting and displayed some tolerance to CTV. Generally the trunks of the trees are smooth with occasional pits.

Table 4.2.8.1. Tree size (canopy volume = m^3) of 8-year-old red grapefruit selections that were pre-immunised with different CTV isolates*.

Grapefruit selections	CTV Isolates					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	21.5 a	21.6 a	22.0 a	18.4 a	13.5 b	19.4 xy
Rio Red	18.8 b	22.7 a	20.0 ab	21.4 ab	14.3 c	19.4 xy
Henderson	18.8 a	15.1 a	18.7 a	17.2 a	16.7 a	17.3 yz
Nel Ruby	21.5 a	22.4 a	23.4 a	24.4 a	13.7 b	21.1 x
Flame	22.4 a	17.8 bc	22.1 ab	22.2 ab	16.5 c	20.2 x
Ruben	22.5 a	21.3 a	20.1 a	18.7 a	20.6 a	20.6 x
Oran Red	14.0 bc	17.0 a	16.2 ab	16.2 ab	13.0 c	15.3 z
Mean	19.9 x	19.7 x	20.4 x	19.8 x	15.5 y	

*Figures in each row in the body of the table as well as the means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.8.2. Production (kg/tree) of 8-year-old red grapefruit selections that were pre-immunised with different CTV isolates.*

Grapefruit selections	CTV isolates					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	115.8 ab	63.0 c	142.7 a	137.5 a	81.5 bc	108.1 yz
Rio Red	124.1 bc	130.7 b	126.6 bc	164.4 a	94.0 c	128.0 y
Henderson	101.1 ab	111.9 ab	124.5 a	119.6 a	77.6 b	106.9 z
Nel Ruby	118.1 a	154.0 a	139.6 a	113.6 a	111.3 a	127.3 y
Flame	121.3 a	117.3 a	130.4 a	131.0 a	103.7 a	120.7 yz
Ruben	126.2 a	116.8 a	131.2 a	109.7 a	96.6 a	116.1 yz
Oran Red	104.7 ab	134.3 a	142.1 a	142.1 a	94.6 b	123.6 yz
Mean	115.9 y	118.3 xy	133.9 x	131.2 xy	94.2 z	

* Figures in each row in the body of the table as well as the means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.8.3. Yield efficiency (kg/m^3 canopy) of 8-year-old red grapefruit selections that were pre-immunised with different CTV isolates.*

Grapefruit selections	CTV isolates					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	5.5 a	2.9 b	6.4 a	7.6 a	6.1 a	5.0 z
Rio Red	6.7 ab	5.9 b	6.3 ab	7.7 a	6.6 ab	6.5 xy
Henderson	5.5 a	7.7 a	6.8 a	7.5 a	5.3 a	6.5 xy
Nel Ruby	5.2 b	6.8 ab	5.9 b	4.9 b	8.8 a	6.3 y

Flame	5.5 a	6.9 a	5.8 a	6.0 a	6.5 a	6.3 xy
Ruben	5.7 a	5.8 a	6.5 a	6.8 a	4.9 a	7.1 wx
Oran Red	8.3 a	7.9 a	8.7 a	8.9 a	7.4 a	7.8 w
Mean	6.1 z	6.3 z	6.6 z	7.1 z	6.5 z	

* Figures in each row in the body of the table as well as the means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.8.4. Cumulative yield over a 3-year period of 8-year-old red grapefruit selections that were pre-immunised with different CTV isolates.*

Grapefruit selections	**CTV isolates**					**Mean**
	GFMS 12	**GFMS 35**	**GFMS 67**	**GFMS 73**	**GFSS 5**	
Star Ruby	354.4 a	284.6 b	380.6 a	384.6 a	268.4 b	334.5 yz
Rio Red	378.8 b	393.5 ab	372.5 b	460.6 a	261.0 c	373.3 xy
Henderson	347.4 bc	299.2 c	416.7 a	369.2 ab	298.0 c	346.1 xyz
Nel Ruby	367.4 ab	428.3 a	409.0 ab	410.4 ab	307.1 b	384.5 x
Flame	398.1 ab	338.1 b	413.2 a	404.4 a	338.4 b	378.4 x
Ruben	420.4 a	384.1 a	395.3 a	290.5 b	292.9 b	356.6 xyz
Oran Red	299.2 bc	337.7 ab	348.5 a	364.1 a	272.3 c	324.4 z
Mean	366.5 xy	352.2 y	390.8 x	383.4 x	291.2 z	

* Figures in each row in the body of the table as well as the means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.8.5. The effect of different CTV isolates on stem pitting rating** of 8-year-old red grapefruit selections.*

Grapefruit selections	**CTV Isolates**					**Mean**
	GFMS 12	**GFMS 35**	**GFMS 67**	**GFMS 73**	**GFSS 5**	
Star Ruby	2.6 a	2.6 a	2.3 a	2.0 a	3.0 a	2.5 yz
Rio Red	2.5 ab	1.9 a	2.3 ab	2.7 b	3.0 b	2.5 yz
Henderson	1.8 a	2.6 ab	3.4 b	2.9 b	2.5 ab	2.6 z
Nel Ruby	2.5 ab	2.5 ab	1.7 a	3.0 b	2.3 ab	2.4 yz
Flame	2.1 a	2.7 a	2.5 a	2.6 a	2.4 a	2.5 yz
Ruben	2.0 ab	1.7 a	2.7 b	2.2 ab	1.8 ab	2.1 y
Oran Red	2.8 b	2.3 ab	2.0 ab	1.8 a	2.2 ab	2.2 yz
Mean	2.3 x	2.3 x	2.4 x	2.5 x	2.5 x	

* Figures in each row in the body of the table as well as the means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

** Stem pitting rating: 1 = Smooth trunk; 2 = Occasional visible pits; 3 = Mild pitting; 4 = Moderate pitting; 5 = Severe pitting.

Conclusion

The reaction of the different grapefruit selections to the different CTV isolates was very similar at this stage. It appears that stem pitting development is increasing in some selection / isolate combinations. The cross-protection ability of each isolate for the selections can only be measured over time.

Future research

Measure trees, rate disease occurrence, harvest and grade fruit.

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4.2.9 **The effect of CTV pre-immunisation on the fruit size of Clementine and Satsuma**

Experiment 816 by S.P. van Vuuren (CRI), J.G. Maritz & N. Combrink (ITSC)

Opsomming

Vruggrootte is 'n groot probleem by Clementines in die Oos– en Wes Kaap. Om die invloed van pre-immunisering op vruggrootte te bepaal, word nie-gepre-immuniseerde en gepre-immuniseerde bome van sewe Clementine seleksies (Clementine late, Oronules, Esbal, Orogrande, Guillermina, Nour, Clemenpons) en een satsuma seleksie (Miho Wase) op Addo Navorsingstasie vergelyk. Die bome is nou drie jaar oud en het vir die eerste keer 'n oes aangehad. Die oes het egter nog baie gevarieer tussen bome met dieselfde behandeling. Boomvolumes is bepaal en alhoewel die bome nog te jonk is om gevolgtrekkings te maak, wil dit voorkom of al die seleksies nie dieselfde reageer op tristeza besmetting nie.

Introduction

All citrus propagation material is pre-immunised with a mild isolate of *Citrus tristeza virus* (CTV). Cross protection is specific with regard to the citrus type, i.e. the most effective protecting strain for a given citrus type is usually obtained from the same type (Müller & Costa, 1987). With the initiation of the Citrus Improvement Programme, all citrus, including mandarin types, was pre-immunised with an isolate originating from grapefruit until suitable isolates was found for the different types (von Broembsen & Lee, 1988). Subsequently, a suitable isolate, LMS 6, has been identified for lime (van Vuuren *et al*, 1993). LMS 6 contains a mild form of seedling yellows, which the grapefruit isolate does not have, and it was therefore approved to replace GFMS 12 as the pre-immunising isolate for the mandarin types. The suitability of LMS 6 as a protector for Clementines has not been confirmed and evaluations are currently being done (van Vuuren & Maritz, 2002).

Fruit size of Clementine is a major problem in the Western and Eastern Cape citrus production regions. Production costs associated with cultural practices aimed at fruit size improvement are high. Since mandarins have a lower sensitivity to CTV, it may not be essential to pre-immunise mandarin cultivars to protect them against severe strains of CTV. The prospect to improve size of fruit borne on virus-free trees was investigated in this experiment.

Materials and methods

This trial was initiated by Prof. E. Rabe and was taken over by S.P. van Vuuren when prof. Rabe left South Africa. Virus-free and LMS 6 pre-immunised trees of seven Clementine selections (Clementine late, Oronules, Esbal, Orogrande, Guillermina, Nour, Clemenpons) and one satsuma selection (Miho Wase) were prepared on Swingle citrumelo rootstock in a commercial nursery (rootstocks might have been infected with CTV prior to budding).

When the scions have developed they were planted at Addo Research Station according to a randomised block design in 2003. Since there was a variation in the number of trees available, they were split in three separate trials. Trial one consisted of the selections Clementine late, Oronules, Esbal, Orogrande, Guillermina, Nour, Clemenpons and each treatment was replicated four times. Trial two consisted of the selections Clementine late, Esbal, Orogrande, Guillermina, Nour, Clemenpons and each treatment was replicated five times. Trial three was Miho Wase satsuma and each treatment was replicated eight times.

Growth, production and fruit size will be the main criteria in the trials.

Results and discussion

The canopy volumes of the trees were determined and are presented in Table 4.2.9.1. The trials are too young to interpret the results, but there are indications that the different selections do not re-act the same to CTV infection.

The fruit were weighed and is presented in Table 4.2.9.2. It was the first meaningful production but still erratic. During the next season, the fruit will be counted and weighed to assess the average fruit size of each treatment.

Table 4.2.9.1. Canopy volumes (m^3) virus-free (VF) and pre-immunised (PI) Clementine selections and Miho Wase Satsuma 4 years after planting.

Cultivar or selection	Trial 1			Trial 2			Trial 3		
	VF	PI	SIGN[1]	VF	PI	SIGN[1]	VF	PI	SIGN[1]
Oronules	1.6	3.7	✸	-	-	-	-	-	-
Clementine late	3.8	3.3	ns	3.6	2.9	ns	-	-	-
Esbal	3.1	3.7	ns	3.6	2.9	ns	-	-	-
Orogrande	3.0	3.4	ns	3.5	2.9	ns	-	-	-
Guillermina	3.0	3.2	ns	3.2	2.9	ns	-	-	-
Nour	3.0	2.6	ns	3.1	2.9	ns	-	-	-
Clemenpons	1.8	2.8	ns	2.6	2.4	ns	-	-	-
Miho Wase	-	-		-	-		3.0	2.4	ns
MEAN	**2.7**	**3.3**	✸	**3.1**	**3.0**	**ns**	**3.0**	**2.4**	**ns**

[1] Significance: ✸Difference significant at P=0.05, ns = no significant difference (Fisher's LSD).

Table 4.2.9.2. Yield (kg) of virus-free (VF) and pre-immunised (PI) Clementine selections 4 years after planting.

Cultivar or selection	Trial 1			Trial 2		
	VF	PI	SIGN[1]	VF	PI	SIGN[1]
Oronules	5.4	7.5	ns	-	-	-
Clementine late	13.6	8.8	ns	9.2	7.5	ns
Esbal	12.0	7.3	ns	8.3	5.7	ns
Orogrande	8.1	4.6	✸	7.7	5.9	ns
Guillermina	4.4	6.9	ns	12.9	6.5	ns
Nour	5.4	7.7	ns	11.6	13.1	ns
Clemenpons	5.9	14.1	ns	10.1	15.3	ns
MEAN	**7.8**	**8.1**	**ns**	**142**	**137**	**ns**

[1] Significance: ✸Difference significant at P=0.05, ns = no significant difference (Fisher's LSD).

Conclusion

No conclusion yet.

Future research

Measure tree size, harvest, count and weigh fruit.

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4.2.10 **Evaluation of CTV isolates in navel**

Experiment 787 by S.P.van Vuuren (CRI), J.G. Maritz & N. Combrink (ITSC)

Opsomming

Verskillende CTV isolate (LMS 6, SM 36, SM 41, SM, 45, SS2) is in Palmer nawel op verskillende kommersiële onderstamme (Growweskil suurlemoen, Troyer citrange, Swingle citrumelo, C35 citrange) in die Oos Kaap geëvalueer. Bome wat met die LMS 6 CTV isolaat gepreïmmuniseer was het die beste presteer oor 'n 7-jaar tydperk. Die bome het 'n 32% hoër kumulatiewe produksie gelewer oor die laaste drie produksie jare as bome wat virusvry geplant was en 27% beter as die gemiddeld van die ander behandelings. Die doel van die proef is bereik en word gestaak.

Introduction

The failure of sour orange as a rootstock for most citrus cultivars in South Africa in 1896 is probably the earliest recorded evidence for the presence of citrus tristeza virus (CTV), although it does not necessarily mean that South Africa is the country of origin (Oberholzer, 1959; Webber, 1925). The sensitive sour orange rootstock was abandoned because of CTV quick decline, and replaced by tolerant rootstocks such as rough lemon (Marloth, 1938). This practice is no solution for sensitive scion cultivars such as grapefruit and cross protection with mild isolates is the most successful approach to reduce the effect of the disease (Müller & Costa, 1972; van Vuuren *et al*., 1993a, 1993b).

Since the establishment of the South African citrus industry on tolerant rootstocks, it was generally accepted that tristeza virus has no effect on sweet oranges and mandarins. This situation can be partly ascribed to nurserymen who unwittingly applied cross protection by selecting parent trees showing the best health and production. These trees carried virus that had the least effect on growth and production. With the implementation of shoot-tip grafting, the virus-free material is vulnerable to infection by various strains occurring in nature, which are transmitted by aphids. Evidence of the presence of severe strains that can affect sweet orange exists in foreign countries (Barkley, 1991; Roistacher, 1988) as well as in South Africa (Marais, 1994).

All citrus cultivars in the Southern African Citrus Improvement Programme are freed from viruses by shoot-tip grafting (de Lange *et al*., 1981). The abundance of the most effective aphid vector, *Toxoptera citricida* (Kirk.) will result in virus-free trees becoming naturally infected with various strains (Schwarz, 1965) including virulent strains (Barkley, 1991; Calavan *et al*., 1980; Müller *et al*., 1968). It is therefore necessary to protect the virus-freed plants from severe CTV strains by deliberately infecting them with mild strains (de Lange *et al*., 1981; von Broembsen & Lee, 1988). The interaction of mild CTV isolates with regard to cross protection is specific with regard to biological activity (Müller & Costa, 1987; Van Vuuren *et al*., 1993b) and therefore, mild CTV isolates should be identified specifically for tolerant cultivars.

The aim of this research is to obtain suitable isolates to cross-protect navel in the Eastern Cape production area.

Materials and methods

CTV isolates are evaluated in Palmer navel on four commercial rootstocks for that area *viz*. Rough lemon, Troyer citrange, Swingle citrumelo and C35 citrange (J. Miller, personal communication). Three tristeza virus isolates, with the seedling yellows component, (SM 36, SM 41, SM 45) are being evaluated and compared to trees with LMS 6 (standard), a severe isolate (SOSS 2) and trees that were left un-inoculated. The trees were prepared according to standard nursery practices in an aphid-free environment.

The trees were planted at Addo in November 1999 according to a split plot design with five replications. The effect of the CTV isolates on growth, production, fruit size and tree health were determined.

Results and discussion

Tree size, production, yield efficiency and cumulative yield over a 3-year period are presented in Table 4.2.10.1, Table 4.2.10.2, Table 4.2.10.3 and Table 4.2.10.4, respectively.

Tree size. Overall, trees with CTV isolate LMS 6 (present pre-immunising isolate) were the largest but not significantly larger than those with isolate SM 45 (Table 4.2.10.1). They were double the size of the trees pre-immunised with a known severe isolate. Trees on Swingle citrumelo rootstock were significantly larger than those on Troyer citrange and C35 citrange rootstocks. The CTV isolates affected the trees on C35 citrange rootstock the most and overall tree size were significantly smaller than those on rough lemon and Swingle citrumelo rootstocks. There are claims that C35 citrange may be a genetic dwarfing rootstock, but tree sizes were the same when comparing the trees of this rootstock with those on the vigorous rough lemon rootstock where the mild LMS 6 CTV isolate was used.

Production. Trees that were pre-immunised with CTV isolate LMS 6 had the highest yield; 30% higher than the trees that were planted virus-free. This is an indication that the CTV strains that were introduced by aphids had a detrimental effect on the trees and that isolate LMS 6 was capable of protecting the trees from the influence of these strains (Table 4.2.10.2). The second best isolate was SM 45 but production of trees with this isolate was 15% poorer than those with LMS 6. With the rootstocks, trees on Swingle citrumelo had a significantly higher production than those on Troyer citrange but not higher than the trees on rough lemon and C35 citrange rootstocks. The smaller trees on C35 citrange rootstock had a higher yield efficiency resulting in production equal to those trees on rough lemon and Swingle citrumelo rootstocks (Table 4.2.10.3). The yield efficiency of trees with the severe isolate was significantly higher than that of trees with the other CTV isolates. This may be due to stress factors induced by the severe isolate and can also be attributed to the higher overall yield efficiency of the trees on C35 citrange rootstock. The cumulative production shows that the LMS 6 CTV isolate was superior to all the other treatments (Table 4.2.10.4). Trees with this isolate produced 27% better than the average of all the other treatments. The CTV isolates had no effect on trees on rough lemon rootstock, confirming its tolerance to CTV.

Table 4.2.10.1. The effect of different CTV isolates on the canopy volumes (m^3) of 7-year-old Palmer navel on different rootstocks[1].

CTV Isolate	Rootstock[2]				Mean
	RL	Swingle	Troyer	C 35	
LMS 6	13.9 a	14.8 a	13.2 a	15.5 a	14.4 w
SM 36	10.7 ab	11.5 ab	9.6 ab	9.3 ab	10.3 y
SM 41	12.9 a	14.6 a	8.9 b	6.1 c	10.6 xy
SM 45	14.7 a	13.1 ab	12.3 ab	10.9 b	12.7 wx
SOSS 2	8.2 b	9.5 b	4.6 c	6.9 c	7.3 z
Control	12.1 ab	11.9 ab	11.8 ab	10.9 b	11.7 xy
Mean	12.1 xy	12.6 x	10.1 yz	9.9 z	

[1] Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).
[2] Rootstocks: RL = Rough lemon, Swing = Swingle citrumelo, Troyer = Troyer citrange, C35 = C35 citrange.

Table 4.2.10.2. The effect of different CTV isolates on the production (kg/tree) of 7-year-old Palmer navel on different rootstocks[1].

CTV isolate	Rootstock[2]				Mean
	RL	Swingle	Troyer	C 35	
LMS 6	86.0 a	111.4 a	89.7 a	102.0 a	97.3 x
SM 36	63.6 a	77.1 b	62.1 ab	61.5 b	66.1 z
SM 41	78.4 a	74.9 b	52.5 b	58.1 b	66.0 z
SM 45	82.5 a	86.5 b	88.1 a	72.4 b	82.4 y
SOSS 2	66.7 a	79.7 b	55.7 b	69.8 b	68.0 z
Control	66.6 a	67.0 b	71.0 ab	66.3 b	67.7 z
Mean	74.0 yz	82.8 y	69.9 z	71.7 yz	

[1] Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).
[2] Rootstocks: RL = Rough lemon, Swing = Swingle citrumelo, Troyer = Troyer citrange, C35 = C35 citrange.

Table 4.2.10.3. The effect of different CTV isolates on the yield efficiency (kg/m^3) of 7-year-old Palmer navel on different rootstocks[1].

CTV Isolate	Rootstock[2] RL	Swingle	Troyer	C 35	Mean
LMS 6	6.3 ab	8.0 ab	7.7 b	6.8 b	7.2 z
SM 36	6.1 ab	7.1 abc	6.5 b	6.6 b	6.6 z
SM 41	6.2 ab	5.3 c	5.9 b	10.0 ab	6.9 z
SM 45	5.8 b	6.7 bc	7.0 b	6.7 b	6.6 z
SOSS 2	8.3 a	9.0 a	12.2 a	12.3 a	10.5 y
Control	5.6 b	5.6 c	6.0 b	6.2 b	5.9 z
Mean	6.4 z	6.9 yz	7.6 yz	8.1 y	

[1] Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).
[2] Rootstocks: RL = Rough lemon, Swing = Swingle citrumelo, Troyer = Troyer citrange, C35 = C35 citrange.

Table 4.2.10.4. The effect of different CTV isolates on the cumulative yield over a 3-year period of 7-year-old Palmer navel on different rootstocks.*

CTV isolate	Rootstock[2] RL	Swingle	Troyer	C 35	Mean
LMS 6	172.3 a	207.2 a	170.1 ab	211.4 a	190.2 x
SM 36	126.6 a	114.2 b	123.0 ab	125.4 b	122.3 z
SM 41	167.6 a	128.6 b	94.2 b	132.5 b	130.7 z
SM 45	166.8 a	161.2 ab	181.8 a	162.1 b	168.0 xy
SOSS 2	134.9 a	139.9 b	128.5 ab	152.5 b	139.0 yz
Control	136.0 a	101.9 b	125.5 ab	154.9 b	129.6 z
Mean	150.7 z	142.2 z	137.2 z	156.5 z	

[1] Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).
[2] Rootstocks: RL = Rough lemon, Swing = Swingle citrumelo, Troyer = Troyer citrange, C35 = C35 citrange.

Conclusion

Trees pre-immunised with LMS 6, the present pre-immunising CTV isolate, performed the best over a 7-year period and had a 32% higher cumulative production over the last 3-year period than those that were planted virus-free.

Future research

The objective of this experiment has been achieved and therefore it will be terminated.

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4.2.11 **Identification of suitable *Citrus tristeza virus* isolates for pre-immunising Turkey Valencia**

Experiment 789 by J.H.J. Breytenbach & S.P. van Vuuren (CRI)

Opsomming

Daar is gevind dat Turkey Valencia meer gevoelig vir *Citrus tristeza virus* (CTV) is as ander Valencia tipes (CRI Groep Navorsings-jaarverlag, 2003). Aangesien Turkey Valencia 'n vroeë Valencia is, vorm dit 'n belangrike deel van sitrus produksie in die bedryf en daarom is dit 'n hoë prioriteit om 'n geskikte CTV isolaat te vind om Turkey Valencia te pre-immuniseer. Virusvrye Turkey Valencia op Troyer citrange onderstam word in 'n glashuis voorberei en met CTV isolate (LMS 6 (standaard), SM 45, SM 46, SM 47, SM 48, SM 49 (almal vanaf soetlemoene versamel) geïnokuleer om die beste ligte isolaat te identifiseer vir kruisbeskermingsdoeleindes. Bome wat met GFMS 12 geïnokuleer is en bome wat virusvry gelaat word dien as positiewe en negatiewe kontroles, onderskeidelik. Preïmmunisasie is bevestig deur middel van ELISA en die bome sal gedurende Februarie – Maart 2007 in die Letsitele omgewing geplant word.

Introduction

Cross protection to control *Citrus tristeza virus* (CTV) is specific with regard to the citrus type, i.e. the most effective protecting CTV isolate for a given citrus type is usually obtained from the same type (Müller & Costa, 1987). With the initiation of the Citrus Improvement Programme, all citrus, including sweet oranges, were pre-immunised with a CTV isolate originating from grapefruit until suitable isolates were found for the different types (von Broembsen & Lee, 1988). Subsequently, a suitable isolate, LMS 6, has been identified for lime (van Vuuren *et al*., 1993). LMS 6 contains a mild form of seedling yellows, which the grapefruit isolate does not have, and it was therefore approved to replace GFMS 12 as the pre-immunising isolate for sweet oranges (van Vuuren *et al*., 2000).

The suitability of LMS 6 as a protector for sweet oranges and mandarins has not been confirmed and evaluations are currently being done in Clementine, navel and Valencia. Midseasons and other sweet oranges are known to be affected by a transmissible factor causing abnormal bud-union on Rough lemon rootstock (McClean, 1974). The transmissible factor is not insect or seed transmissible and was not correlated to any known citrus disease. It has been shown that it can be removed by shoot tip grafting (Navarro *et al*., 1993).

Recently, it was found that Turkey Valencia trees on Rough lemon and Volckameriana rootstocks developed bud-union creasing symptoms (personal observation) (Beeton *et al*., 2000). Various sources of Turkey Valencia had stem pitting symptoms and it appears that this cultivar is more sensitive to CTV than other Valencia cultivars (CRI Group Annual Research Report, 2003). Since Turkey Valencia is an early cultivar, it forms an important part of citrus production, and therefore the identification of a suitable CTV isolate for cross-protection remains a high priority.

The objective of this study is to evaluate CTV isolates to identify a suitable cross protecting isolate without the bud-union crease factor for Turkey Valencia.

Materials and methods

Virus-free Turkey Valencia scions on Troyer citrange rootstocks are prepared in the greenhouse according to normal nursery practices. When the scions have developed to approximately 5 mm, they will be inoculated with different mild CTV isolates by budding two buds containing the required CTV isolate into the scions (Table 4.2.11.1). After 3 months, the trees will be tested for the presence of the CTV isolates by ELISA. When pre-immunisation is confirmed, they will be planted in the field where they will be subjected to normal CTV challenge by aphids. Each treatment will be replicated five times and uninoculated virus-free trees will serve as controls. Evaluations will be on growth, production and tree health.

Table 4.2.11.1. Treatments for Turkey Valencia on Troyer citrange rootstock to identify a suitable CTV isolate for pre-immunisation.

CTV isolates	Origin and comments
LMS 6	Mexican lime, Tzaneen. Present pre-immunising isolate for sweet orange
SM 45	Portsgate Valencia, Hoedspruit. Show promise in current cross protecting trials
SM 46	Shamouti Midseason, Messina
SM 47	Valencia, Grahamstown. Tree > 100 years old
SM 48	Midseason, Citrusdal. First planting of citrus in the area
SM 49	Valencia, Nelspruit. Induce greening tolerance
GFMS 12	Grapefruit, Nartia. Positive control
Virus-free Control	Virus-free. Negative control

Results and discussion

Successful pre-immunisation was confirmed by means of ELISA. Trees will be planted during February - March 2007 in the Letsitele area.

Conclusion

The experiment is still in the early developing stage.

Future research

Evaluate horticultural performance i.e. growth (tree size), yield data (fruit size and kg/tree) and health (stem pitting, decline).

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4.2.12 **Evaluation of CTV isolates in Valencia**

Experiment 788 by S.P. van Vuuren, J.H.J. Breytenbach (CRI) & B.Q. Manicom (ITSC)

Opsomming

Die effek van verskillende ligte *Citrus tristeza virus* (CTV) isolate (LMS 6 (standard), SM 36, SM 41, SM 45 and SM 49) word in drie Valencia bostamme (McClean, McClean Saadloos en Delta) op Troyer citrange onderstam ge-evalueer. Boomgrootte van McClean Valencia was betekenisvol kleiner as die van Delta en McClean Saadloos Valencias. Oor die algemeen was produksie weer swak met uitermatige klein vrugte. Die bome toon egter herstel na die swak toestand waarin hulle die vorige jaar was. Die data van hierdie jaar varieer egter nog baie en daar kan nie betekenisvolle onderskeid getref word tussen goed en swak nie.

Introduction

Refer to section 4.2.12. The objective of this trial is to evaluate promising CTV isolates in three Valencia scions and identify suitable cross-protecting isolates.

Materials and methods

McClean -, McClean Seedless - and Delta Valencia trees on Troyer citrange rootstock were grown according to normal nursery practices under aphid-free conditions. When the scions have developed to approximate five mm in diameter, they were inoculated with isolates derived from sweet orange and showed promise in glasshouse tests. The isolates are LMS 6 (standard), SM 36, SM 41, SM 45 and SM 49. Trees with these isolates will be compared to trees with a severe isolate (SOSS 2) as well as un-inoculated virus-free (VF) plants.

The trees were planted in 2000 according to a split plot design with five replications at Malelane.

The effect of the CTV isolates on growth, production, fruit size and tree health will be determined.

Results and discussion

Tree size. Tree sizes were measured and the canopy volumes calculated according to Burger *et al*. (1970) (Table 4.2.12.1). In general, the canopy volumes of McClean Seedless Valencia were significantly smaller than that of the Delta – and McClean Valencia trees. With the CTV isolates, trees with the SM 36 isolate and those that were planted virus-free were significantly smaller than trees with isolates SM 41 and SM 45. Some interactions between CTV isolates and the Delta scion occurred while only SM 36 performed poorer than the other treatments in the other two scions. Growth variability was observed when comparing the effects of the CTV isolates on the Delta scion, and growth of trees with mild isolate SM 41 is currently the best.

Production. The yield was generally poor and variable with the majority of fruit small (Table 4.2.12.2). This was more severe in the previous year and it appears as if the trees are recovering slowly to whatever caused the poor condition of the trees in 2005. The data show no differences among the scions or the CTV isolates. The same is reflected in the yield efficiency (Table 4.2.12.3) and cumulative yield (Table 4.2.12.4).

Table 4.2.12.1. Tree size (canopy volume = m^3) 6 years after planting of three Valencia selections that were pre-immunised with different mild CTV isolates, a severe isolate and trees that were planted virus-free.

CTV Isolate	Scion** McC	McC SL	Delta	Mean
LMS 6	19.1 a	16.6 a	18.6 c	18.1 Xy
SM 34	17.8 a	18.0 a	19.4 bc	18.4 Xy
SM 36	10.7 b	7.2 b	11.5 d	9.8 z
SM 41	19.3 a	18.6 a	22.4 a	20.1 x
SM 45	19.6 a	17.0 a	21.7 ab	19.4 x
SM 49	19.3 a	15.8 a	21.9 ab	19.0 xy
SOSS 3	18.2 a	18.4 a	19.0 bc	18.6 xy
VF	18.3 a	15.3 a	18.1 c	17.2 y
Mean	17.8 y	15.9 z	19.1 y	

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).
** Scions: McC = McClean Valencia; McC = McClean seedless Valencia; Delta = Delta Valencia.

Table 4.2.12.2. The production (kg/tree) 6 years after planting of three Valencia selections that were pre-immunised with different mild CTV isolates, a severe isolate and trees that were planted virus-free*.

CTV Isolate	Scion**			
	McC	McC SL	Delta	Mean
LMS 6	39.7 abc	31.6 bc	48.6 a	39.9 z
SM 34	28.5 bc	20.2 c	39.0 ab	29.2 z
SM 36	29.7 bc	20.8 c	38.8 ab	29.8 z
SM 41	50.3 ab	42.4 ab	39.8 ab	40.9 z
SM 45	22.8 c	60.0 a	23.3 ab	35.4 z
SM 49	37.4 abc	23.0 bc	27.8 ab	29.4 z
SOSS 3	20.0 c	42.7 ab	36.8 ab	33.2 z
VF	59.3 a	30.4 bc	17.0 b	35.5 z
Mean	36.0 z	33.9 z	32.6 z	

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).
** Scions: McC = McClean Valencia; McC = McClean seedless Valencia; Delta = Delta Valencia.

Table 4.2.12.3. Yield efficiency (kg/m^3) 6 years after planting of three Valencia selections that were pre-immunized with different mild CTV isolates, a severe isolate and trees that were planted virus-free*.

CTV Isolate	Scion**			
	McC	McC SL	Delta	Mean
LMS 6	2.0 abc	1.9 ab	2.7 ab	2.2 yz
SM 34	1.6 bc	1.1 b	2.1 abc	1.6 z
SM 36	2.8 ab	2.9 a	3.3 a	3.0 y
SM 41	2.6 ab	2.4 ab	1.4 bc	2.1 yz
SM 45	1.1 c	3.5 a	1.1 bc	1.9 z
SM 49	1.9 abc	1.9 ab	1.2 bc	1.7 z
SOSS 3	1.1 c	2.3 ab	1.9 abc	1.8 z
VF	3.3 a	2.0 ab	1.0 c	2.1 yz
Mean	2.1 z	2.3 z	1.9 z	

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).
** Scions: McC = McClean Valencia; McC = McClean seedless Valencia; Delta = Delta Valencia.

Table 4.2.12.4. Cumulative yield (kg) over a 3-year period (6 years after planting) of three Valencia selections that were pre-immunised with different mild CTV isolates, a severe isolate and trees that were planted virus-free*.

CTV Isolate	Scion**			
	McC	McC SL	Delta	Mean
LMS 6	106.9 bc	77.8 abc	122.7 a	102.5 z
SM 34	81.6 cd	68.9 abc	79.4 bc	76.6 z
SM 36	96.4 bcd	53.9 abc	125.7 a	92.0 z
SM 41	151.2 a	88.5 ab	73.1 c	104.3 z
SM 45	90.3 cd	141.8 a	56.3 c	95.9 z
SM 49	133.2 ab	65.1 bc	77.0 bc	91.8 z
SOSS 3	68.2 d	96.4 b	109.1 a	91.2 z
VF	156.0 a	80.2 abc	59.2 c	98.5 z
Mean	110.5 y	84.0 z	87.8 z	

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).
** Scions: McC = McClean Valencia; McC = McClean seedless Valencia; Delta = Delta Valencia.

Conclusion

Because of the variable production and small fruit, no conclusion can be made.

Future research

Harvest, size and weigh fruit. Determine tree size.

References cited

Refer to section 4.2.12.

4.2.13 **The effect of different CTV isolates in Valencias on different rootstock combinations for the Orange River Valley**
Experiment 739 by J.H.J. Breytenbach & S.P. van Vuuren (CRI)

Opsomming

Omdat C*itrus tristeza virus* gasheer en klimaat spesifiek is, is dit nodig om verskillende beskermende isolate in die verskillende sitrus produserende streke te evalueer. Ligte isolate wat oorspronklik uit soetlemoen bome versamel is (SM 45, SM 46, S 47, SM 48, SM 49), word gebruik om virusvrye Delta -, Midknight -, McClean -, McClean seedless - en Turkey Valencia op C 35 citrange onderstam te pre-immuniseer. Hierdie isolate sal vergelyk word met LMS 6 (standard vir soetlemoene) en boompies wat virusvry geplant word. Pre-immunisering is bevestig deur middel van ELISA, die boompies sal gedurende Maart – April 2007 in die Kakamas omgewing uitgeplant word en jaarliks ge-evalueer vir boomgrootte, vruggrootte, oes opbrengs, sowel as hul gesondheids-toestand.

Introduction

Citrus tristeza virus (CTV) is the causal agent of possibly the most destructive disease of citrus in the world. The disease is spread by propagation material and by various aphid species of which *Toxoptera citricida* is the most abundant. Symptoms induced by CTV range from mild with no noticeable effect on the host to severe stem pitting and decline resulting in uneconomic production. As CTV exhibits host and geographical specificity, it is necessary that mild protective isolates be evaluated in the different production areas. The only practical means of controlling CTV disease at present is by mild strain cross-protection. The objective of this experiment is to evaluate selected CTV isolates in four different Valencia selections on three different rootstocks in order to identify a suitable cross-protecting CTV isolate for specific rootstock/scion combinations. The experiment will give horticultural information on the use of the most suitable Valencia selection on a specific rootstock as well.

Materials and methods

Five virus-free Valencia scions (Delta, Midknight, McClean, McClean seedless, Turkey) were budded on C35 citrange rootstock. When the scions have developed sufficiently, each Valencia selection was bud-inoculated with five selected CTV isolates originating from sweet orange (SM46, SM47, SM48, SM49). These isolates will be compared to trees inoculated with LMS6 (standard) and trees planted virus-free. Successful pre-immunisation will be confirmed with ELISA where after the trees will be planted in the field and horticultural performance i.e. growth (tree size), yield data (fruit size and kg/tree) and health (stem pitting, decline) evaluated on an annual basis.

Results

Successful pre-immunisation was confirmed by means of ELISA. Trees will be planted during March – April 2007 as soon as all orchard preparation is done.

Conclusion

The experiment is still in the developing stage.

Future research

Evaluate horticultural performance i.e. growth (tree size), yield data (fruit size and kg/tree) and health (stem pitting, decline).

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4.2.14 **Screening of rootstocks for Citrus Blight tolerance**

Experiment 32 by J.H.J. Breytenbach (CRI)

Opsomming

Die inokulasie van sitrusskroei in Delta Valencia bome op 18 verskillende onderstamme induseer 'n afname in boomgrootte en produksie in vergelyking met ongeïnokuleerde bome. Serologiese analises van die 12-kd proteïen, wat slegs in sitrusskroei-besmette bome voorkom, is gebruik om bome te identifiseer wat met sitrusskroei besmet is. Uitslae bevestig die visuele simptome in die boord maar identifiseer ook die siekte in 'n vroeë stadium wanneer geen simptome nog waargeneem kan word nie. Die resultate van die boomvolumes, produksie, die water-opname toets en die voorkoms van die 12-kd protein komplimenteer mekaar nie en maak die interpretasie van die data moeilik. Onderstamme C35 citrange en Sunki mandaryn word die meeste ge-affekteer deur sitrusskroei. Bome op Sun Chu Sha en Orlando Tangelo toon die meeste toleransie teen die siekte. Indien die bome na verloop van tyd ook geinfekteer word deur sitrusskroei, beteken dit dat die onderstamme net die boom se leeftyd kan verleng en nie weerstandbiedend is nie.

Introduction

Citrus blight (CB) affects most commercially grown scion cultivars in the citrus production areas of the world where this disease occurs. CB is primarily a disease affecting the rootstock and the most sensitive rootstock cultivars appear to be Rough lemon, Volckameriana and Rangpur lime. These are followed by trifoliate orange and its citrange hybrids, Cleopatra mandarin, sweet orange and sour orange.

The symptoms of trees with CB are similar to those of a number of other declines of citrus. The finding of distinctive proteins in leaves and roots of infected trees has led to the development of serological tests that are useful in distinguishing trees with CB from those declining from other disorders. Two CB-associated proteins (35 and 12-kd) were purified by preparative electrofocusing and SDS-PAGE. Polyclonal antisera were produced to both proteins, and a monoclonal antibody was produced to the 12-kd protein. Both proteins were readily detected in crude extracts from CB trees by immuno spot and western blot assays. In several experiments, trees with symptoms of CB that were positive by water uptake tests and zinc wood analyses were also positive in the serological tests. Some bearing trees were found to contain the two proteins up to one year before CB symptoms developed. The 12-kd protein was detected in young trees three months after root-graft inoculations (Derrick, *et al.*, 1993).

Until the inception of the Citrus Improvement Programme in South Africa in 1973, practically all commercial citrus orchards were established on Rough lemon rootstock. Rough lemon remained the most popular rootstock up until 1990 and in 1991 was superseded by Volckameriana, Swingle citrumelo, Carizzo citrange and Troyer citrange. The presence of CB in South Africa has influenced the rootstock choice in affected areas. Rootstocks such as X639 (*Poncirus trifoliata* x Cleopatra mandarin), M&T (Minneola tangelo x *P. trifoliata*) and Swingle citrumelo are being used to establish new plantings. Trees on Swingle citrumelo are being used to replace trees that have succumbed to CB in existing orchards.

This investigation is to identify rootstocks that can be successfully used in CB affected areas.

Materials and methods

A rootstock experiment to evaluate the tolerance of various rootstocks to CB has been established in Letsitele at Bosveld Sitrus. The trial comprises of virus-free Delta Valencia scions on Carrizo citrange, Empress mandarin, *P. trifoliata*, Swingle citrumelo, Volckameriana and Samson tangelo that were planted in 1990. Gou Tou, Orlando tangelo, M&T, X639, Marsh grapefruit, Zhu Luan and Sweet Orange were planted in 1992. In 1995, trees on Cleopatra mandarin, C35 and Sun Chu Sha were added, and during 1996 trees on Benton citrange and Sunki mandarin were included.

Trees on the different rootstocks were planted in pairs as receptor trees equidistant from a CB infected donor tree. Three to four roots, 5-6 mm in diameter, of one of the pair of receptor trees were approach grafted to the roots of the donor tree. Six pairs of each rootstock were planted and grafted. The non-grafted trees constituted as the un-inoculated controls. The donor trees were selected using standard diagnostic techniques such as water uptake and zinc accumulation in the xylem. The donor trees were removed 3 years

after inoculation since they started to interfere with the growth of the young trees. The young trees were treated with granular formulations of Temik and Ridomil and trunk paint applications of Aliette, every 3 months to exclude the effects of *Phytophthora* and citrus nematode infections.

The following data are taken each year:

Tree sizes are measured;
Yield and fruit size are determined;
Water uptake tests;
The presence of the 12-kd protein is determined.

Results and discussion

Due to the sensitivity of Sweet orange for *Phytophthora* rootrot, the trees on this rootstock were stunted and were terminated from this experiment.

The part of the experiment where the rootstocks were planted in 1990 was terminated during 2005. The trees became too crowded, which made data taking difficult. However, the objective was reached and the results were reported in the annual report of 2005.

Since CB is a disease that develops mostly after 8 years or more after planting, the results of the canopy size and yield are presented according to their planting dates (Table 4.2.14.1 and Table 4.2.14.2). It is expected that data of inoculated and un-inoculated trees of CB tolerant rootstocks will not differ since both, the inoculated and uninoculated trees will not show any detrimental effect of the disease.

Tree size

The un-inoculated trees were generally larger than the inoculated trees, which show that CB reduces tree growth. However, there are contradictory results, i.e. 25% of inoculated trees on Gou Tou rootstock were smaller than the un-inoculated trees, which are understandable, but 26% un-inoculated trees on X639 rootstock were smaller than the inoculated trees. The tree sizes of the 1992 planting show that rootstocks Orlando Tangelo and MxT were the least affected. Results of the 1995/96 planting indicate that tree sizes of Benton citrange, Sun Chu Sha and Cleopatra mandarin rootstocks were the least affected. To summarise, at this stage trees on Orlando tangelo, MxT, Benton citrange, Sun Chu Sha and Cleopatra mandarin rootstocks show tolerance in a CB situation, while trees on C35 citrange and Sunki mandarin rootstocks were the most susceptible (Table 4.2.14.1).

Yield

Overall, the inoculated trees yielded higher and had a higher yield efficiency (kg/m^3) than the un-inoculated trees. Growth stress factors usually increase production, which results in a high percentage small fruit. However, there is virtually no difference in the % small fruit of the inoculated and un-inoculated trees. This may be due to the low overall production of the trees, i.e. the un-inoculated trees on Gou Tou rootstock had a canopy volume of 94.5 m^3 and a yield of 76.5 kg. That gives a production efficiency of less than a kg/m^3. Normal production is approximately 8 kg/m^3. Trees on X639 rootstock had the highest yield of all the plantings (Table 4.2.14.2). In the 1992 planting, 67% of the inoculated trees produced a higher yield than the un-inoculated trees, which are now 35% naturally infected according to the 12-kd protein test (Table 4.2.14.4). Comparing the production of inoculated and un-inoculated trees show that C35 citrange rootstock is the least tolerant overall.

Water uptake

Water uptake ability of CB infected trees is significantly reduced due to the presence of occlusions by amorphous plugs in the xylem. Water uptake was the quickest in the un-inoculated trees on Orlando tangelo, Sun Chu Sha and X639 rootstocks. The best uptake among the inoculated trees was those on Gou Tou, Orlando tangelo, Zhu Luan, Marsh grapefruit and Sun Chu Sha rootstocks. The poorest uptake occurred in trees on Cleopatra mandarin, C35 citrange and Sunki mandarin rootstocks (Table 4.2.14.3).

CB protein

The presence of the 12-kd protein was higher in the inoculated trees (52%) than in the un-inoculated trees (35%) (Table 4.2.14.4). The latter group of trees can get infected by natural means. None of the trees on Sun Chu Sha rootstock showed the presence of the 12-kd protein. Next best were trees on Orlando tangelo and Zhu Luan with two trees out of 12 with the 12-kd protein. The presence of the 12-kd protein was the highest in trees on C35 citrange rootstock (12/12) followed by trees on Sunki mandarin rootstock (10/12).

Visual symptoms

The trees were visually evaluated for decline symptoms associated with CB (Table 4.2.14.5). The symptoms showed a lower infection rate than the 12-kd protein test which indicates that the protein test identifies CB infection before decline symptoms can be observed. Unfortunately a direct comparison was not made.

Comparisons

To make a meaningful conclusion of the results obtained using the different criteria, the best and worst rootstocks for each criterion are summarised in Table 4.2.14.6.

Table 4.2.14.1. Comparison of tree size (canopy volume) of CB inoculated and un-inoculated Delta Valencia trees on different rootstocks.

Rootstock	Year Planted	Tree volume (m^3)		% Difference
		Un-inoculated	Inoculated	
MxT	1992	68.6	62.0	-9
X639	1992	56.5	71.3	26
Gou Tou	1992	94.5	70.7	-25
Orlando tangelo	1992	69.5	69.2	-0.4
Zhu Luan	1992	33.0	35.9	8.8
Marsh grapefruit	1992	53.4	46.6	-12
Average 1992		**62.6**	**59.3**	**-2.2**
Cleopatra mandarin	1995	42.8	44.1	3
Sun Chu Sha	1995	69.2	70.5	1.9
C35 citrange	1995	42.7	20.6	-51
Sunki mandarin	1996	34.1	21.1	-38
Benton citrange	1996	16.0	15.9	-0.6
Average 1995/96		**41.0**	**34.4**	**-17**
Mean		**51.7**	**46.8**	**-10**

Table 4.2.14.2. Comparison of yield (kg), yield efficiency (kg/m^3) and % small fruit (count <105) of un-inoculated and CB inoculated Delta Valencia trees on different rootstocks.

Rootstock	Year planted	Un-inoculated		Inoculated			
		Yield	Yield efficiency	% Small fruit	Production	Yield efficiency	% Small fruit
MxT	1992	94.2	1.4	21.1	107.5	1.7	15.8
X639	1992	104.3	1.9	16.7	163.8	2.3	14.7
Gou Tou	1992	76.5	0.8	14.7	81.7	1.2	12.5
Orlando tangelo	1992	57.8	0.8	9.0	88.5	1.3	9.1
Zhu Luan	1992	42.0	1.3	6.3	26.5	0.7	14.3
Marsh grapefruit	1992	31.0	0.6	4.9	45.6	1.0	9.2
Average 1992		**67.6**	**1.1**	**12.1**	**85.6**	**1.4**	**12.6**
Cleopatra mandarin	1995	25.7	0.6	12.1	22.0	0.5	12.2
Sun Chu Sha	1995	55.5	0.8	20.6	54.7	0.8	18.1
C35 citrange	1995	84.1	2.0	14.9	54.4	2.6	10.1
Sunki mandarin	1996	55.4	1.6	25.7	51.7	2.5	20.3
Benton citrange	1996	37.1	2.3	14.6	65.3	4.1	24.8
Average 1995/96		**45.6**	**1.5**	**17.6**	**37.6**	**2.1**	**17.1**
Mean		**56.6**	**1.3**	**14.8**	**61.6**	**1.8**	**14.9**

Table 4.2.14.3. Comparison of water-uptake (seconds/10 ml) through the trunk xylem of CB inoculated and un-inoculated Delta Valencia trees on different rootstocks.

Rootstock	Year Planted	Un-inoculated	Inoculated	% Difference
MxT	1992	64	86	34
X639	1992	56	80	43
Gou Tou	1992	64	65	2
Orlando tangelo	1992	46	61	32
Zhu Luan	1992	66	60	-9
Marsh grapefruit	1992	62	47	-24
Cleopatra mandarin	1995	67	90	34
Sun Chu Sha	1995	46	60	30
C35 citrange	1995	90	90	0
Sunki mandarin	1996	90	90	0
Benton citrange	1996	68	84	23

Table 4.2.14.4. Comparison of 12-kd protein serological tests of CB inoculated and un-inoculated Delta Valencia trees on different rootstocks.

Rootstock	Year Planted	Presence of 12-kd protein (/6)		
		Inoculated	Un-inoculated	Total
MxT	1992	5	3	8
X639	1992	5	4	9
Gou Tou	1992	1	2	3
Orlando tangelo	1992	1	1	2
Zhu Luan	1992	2	0	2
Marsh grapefruit	1992	2	2	4
Cleopatra mandarin	1995	2	1	3
Sun Chu Sha	1995	0	0	0
C35 citrange	1995	6	6	12
Sunki mandarin	1996	6	4	10
Benton citrange	1996	4	0	4

Table 4.2.14.5. Presence of decline symptoms on CB inoculated and un-inoculated Delta Valencia trees.

Rootstock	Year Planted	Un-inoculated (/6)			Inoculated (/6)		
		Visually Positive	Visually +/-	Visually Negative	Visually Positive	Visually +/-	Visually Negative
MxT	1992	0	2	4	2	1	3
X639	1992	3	1	2	1	1	4
Gou Tou	1992	1	0	5	1	0	5
Orlando tangelo	1992	0	0	6	0	0	6
Zhu Luan	1992	1	0	5	2	1	3
Marsh grapefruit	1992	0	0	6	0	0	6
Cleopatra mandarin	1995	0	1	5	2	1	3
Sun Chu Sha	1995	0	1	5	0	1	1
C35 citrange	1995	1	0	5	3	2	1
Sunki mandarin	1996	0	1	5	1	0	5
Benton citrange	1996	0	3	3	0	2	4

+/- Doubtful

Table 4.2.14.6. Summary of best (least difference between inoculated and un-inoculated) and worst rootstocks according to the CB evaluation criteria.

Criterion	Rootstock	
	Best	**Worst**
Tree size	Orlando tangelo Benton citrange Sun Chu Sha Cleopatra mandarin	C35 citrange Sunki mandarin
Yield	Sun Chu Sha Orlando tangelo	Sunki mandarin
Water uptake	Sun Chu Sha Orlando tangelo	C35 citrange Sunki mandarin
12-kd protein presence	Sun Chu Sha Orlando tangelo	C35 citrange Sunki mandarin

Conclusion

Trees on Sun Chu Sha and Orlando tangelo rootstocks appear to exhibit the most tolerance and growers should consider these rootstocks in CB areas. C35 citrange and Sunki mandarin rootstocks appear to be the most sensitive.

Future research

Continue to monitor disease development, measure canopy volumes and take yield data. A final evaluation of the 1992 and 1995/96 plantings will be made during the 2007/2008 season.

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4.2.15 Evaluation of citrus material for greening resistance

Experiment 815 by J.B. Meyer (CRI)

Opsomming

Daar word gepoog om Haunglongbing (vergroening) weerstandbiedendheid te verkry deur embrio's uit gesonde chimeras van vergroende vrugte te verwyder en op kunsmatige medium te kweek. Die plantjies wat genereer word, word op groeikragtige onderstamme deur middel van mikro-enting gevestig. Hierdie klone word op onderstamme vermeerder en aan sitrus bladvlooie, die vektor van vergroeningsiekte, blootgestel. Nadat die insekte vir 'n week op die plante gevoed het, word hulle verwyder en deur middel van PKR getoets om te bevestig dat hulle besmet was met vergroening en sodoende die plante blootgestel het aan die organisme. Na 3 maande word die plante ge-evalueer vir die voorkoms van vergroeningsimptome. Klone wat 'n hoë persentasie simptoomlose plante het word d.m.v. PKR getoets om te bepaal of hulle vry van die vergroenings-organisme is (weerstandbiedend) of die organisme huisves sonder dat simptome ontstaan (verdraagsaamheid of toleransie). Twee klone, E2 en T2, is in 2006 geïdentifiseer as simptoomloos na blootstelling aan die vektor. PKR is nog nie op die plante toegepas nie. Die klone is op onderstamme vermeerder en afsonderlik met twee *Citrus tristeza virus* isolate ge-preïmmuniseer om in die boord ge-evalueer te word. Vier nuwe klone is die afgelope jaar gegenereer. Sodra hulle groot genoeg is, moet die klone vermeerder en aan die vektor blootgestel word. Die eksperiment is ook daarop gemik om die gene wat weerstandbiedendheid of toleransie teen vergroening aan die plante verleen te identifiseer.

Introduction

Citrus greening (Huanglongbing) remains the most destructive disease in the cooler production areas of South Africa. Despite the present control measures, the disease still invades plantings and new areas become infected (Pretoriuis, 2005). The ultimate control measure will be the use of resistant plant material. Chimera's on fruit affected by greening is observed on a regular basis, some of these display "healthy looking" sectors in contrast to the infected part of the fruit. Possible resistant plants can be generated from abortive seeds in these healthy fruit sections by means of embryo rescue. Artificial challenging of generated plants with the greening bacterium, and using recent molecular techniques (Hocquellet, 1999; Planet, 1995; Villachanoux, 1992) for evaluation after challenges, may prove a rapid approach in identify truly

tolerant/resistant clones. Identifying genes that allows the plant to be resistant or tolerant to the development of Huanglongbing symptoms can provide a tool for early identification of resistant/ tolerant cultivars.

The objective of the study is to screen citrus material, recovered through embryo rescue, for genetic resistance against greening disease.

Materials and methods

Fruit with greening symptoms, displaying healthy chimera sectors, were collected in orchards at Friendenheim and Crocodile Valley Citrus Estate from greening infected branches during June 2006. Eight and seven fruits were collected from Friendenheim and Croc Valley, respectively. Each fruit was surface sterilised in the laboratory on a flow bench by dipping them for 20 min. in a 0.5% sodium hypochlorite solution containing 0.1% Tween-20. Abortive seeds were dissected aseptically from both sectors of the fruit (healthy and greening infected) and cultured on modified Murashige & Tucker (M & T) (1969) medium containing 500 mg/l malt extract and no giberillic acid (GA3). Some fruits contained more that one greening or healthy sector therefore embryos from more than one sector were sometimes removed. Albedo tissue (3 x 3 mm) pieces were also dissected aseptically from the different fruit sectors and placed on modified M & T containing 500 mg/l malt extract, 10% Orange juice and 2 µM GA3 in an attempt to generate plants. Cultures were allowed to develop for 4 weeks in continuous dark at 30ºC. Thereafter, plants were transferred to M & T medium containing 500 mg/l malt extract and 2 µM GA3.

Promising clones identified by Dr. Fanie van Vuuren in 2006 were multiplied on Rough lemon rootstocks and pre-immunised with LMS 6 and SM 49 *Citrus tristeza virus* (CTV) isolates on 12/10/2006 (Table 4.2.15.1). Five replicates of each were made, which was tested with ELISA 1 month later to confirm the pre-immunisation status (CTV reagent set, Cat nr. 78900, Agdia). Pre-immunisation with SM 49 was repeated on 23/01/2007 because of initial negative results. The clones will soon be evaluated under field conditions together with a clone from Croc Valley, containing CTV that is believed to enhance protection against greening. Midknight Valencia will serve as the control.

Results and discussion

Only a small percentage of embryos that germinated, develop into plants (Viloria, 2005). Some embryos developed shoots while some also developed roots when placed on M & T containing GA3 (Fig 14.2.15.1, top left). These were either micro-grafted, planted out into sterilised soil or both (Fig 14.2.15.1, bottom left and right, respectively). Table 4.2.15.1 indicates the embryo codes that developed into suitable plants *in vitro*. From observation, there is a tendency of the plants that were developed from the greening infected sector of the fruits to die. This may be because of the presence of the greening organism. Albedo tissue developed callus (Fig 14.2.15.1, top right), but no plants could be generated from sectors of albedo tissue thus far.

PCR has not yet been employed on the symptomless plants of 2006 or the psylla used for challenge infection. This will be done as soon as a Dot Blot system for the detection of greening infected psylla has been developed.

Results obtained with ELISA, indicating the CTV status of the preimmunised clones intended for field evaluation, are presented in Table 14.2.15.2. A follow-up ELISA is scheduled for 3 months after the pre-immunisation dates.

Table 4.2.15.1. Status of embryo codes that developed into plants suitable for propagation.

*Clone Code	Embryo Rescue date	Status	Comment
OC4/15-RA2	15/06/2006	Planted & micro-grafted	Small plants are growing well
OC4/11-RA	15/06/2006	Planted & micro-grafted	Small plants are growing well
OC4/10-RA	15/06/2006	Planted	
O/03/30-RA2	07/06/2006	Micro-grafted	Growing
O/03/30-GB	07/06/2006	Planted	

*OC = Olinda Valencia from Crocodile Valley, O = Olinda Valencia from Friendenheim, R = Healthy sector, G = Greening infected sector.

Table 4.2.15.2. Clones for field evaluation, CTV isolates and CTV status determined by ELISA 2 months after pre-immunisation.

CLONE (SCION)	PRE-IMMUNISING ISOLATE	ELISA RESULT Number tested/ Number positive
GTC E2-05	LMS 6	5 / 4
GTC E2-05	SM 49	5 / 3
GTC T2-05	LMS 6	5 / 4
GTC T2-05	SM 49	5 / 2
GTC E5-05	SM 49	5 / 3
GTC C1	Carrying original CTV source	5 / 5
Midknight (Control)	LMS 6 (Standard)	5 / 5
Midknight (Control)	SM 49	5 / 1

Figure 4.2.15.1 Different stages in the embryo rescue process. Left top: Embryos developing into plants in M&T medium showing roots and shoots. Right top: Albedo tissue showing callus development. Left bottom: Small plantlets of OC 4/11 RA growing in a sterile potting mixture. Right bottom: Micrograft of O/03/30 RA2. A piece of stem and leaves were grafted to a Troyer citrange rootstock and covered with parafilm. The picture shows the new growth developing through the parafilm.

Conclusions

A total of five embryos that were rescued developed into suitable material for micro-grafting or planting. Four of these were from the healthy sectors of chimeras while only one was derived from a greening infected sector. For clone O/03/30, a plant from the greening as well as the healthy sector developed. This provides material that may be suitable for comparison of genes. Clones from 2006 were grafted on rootstocks and pre-immunised with suitable CTV isolates. After pre-immunisation is confirmed by ELISA, they will be planted in the field.

Future research

- This project will be expanded to become a PhD study for which a bursary was allocated by the CGA.
- Do PCR on symptomless challenged plants and psylla used for challenging.

● Plant out pre-immunised plants in an environment with high disease pressure (Crocodile Valley Citrus Estate)
● Compare genes by Selective Hybridisation techniques from fruit and from plants.

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4.2.16 Eradication of citrus greening infections in existing orchards

Experiment 818 by M.C. Pretorius (CRI)

Opsomming

Huanglongbing, wat meer algemeen as sitrusvergroening in Suid Afrika bekend staan, is 'n ernstige bakteriese siekte wat alle sitrus kultivars affekteer. "*Candidatus*" Liberibacter africanum is die siekte veroorsakende spesie. Algemene siektesimptome is die vergeling van blaarnerwe en omliggende plantselle, gevolg deur die vergeling en "mottling" van die blare. Siektesimptome verskil tussen kultivars. Bekende effektiewe beheermaatreëls behels die voorkoming van beweging van plantmateriaal van besmette gebiede na onbesmette gebiede, die voorsiening van siekte-vrye plantmateriaal aan die sitrusbedryf, die effektiewe beheer van die vektor, asook die vermindering van die inokulum deur verwydering van die besmette bome en takke. Die doel van hierdie proef was om 'n nuwe benadering tot sitrusvergroeningsbeheer te evalueer deur die bakterie-inokulum in reeds besmette plantmateriaal te verminder deur 'n enkele sistemiese blaarbespuitingsproduk toe te dien. Twee potproewe en een veldproef is uitgelê. Geen resultate is tans beskikbaar nie en die finale evaluasie sal in die winter gedoen word. Gunstige finale resultate sal toekomstige beplanning van die nuwe beheerbenadering ondersteun.

Introduction

Huanglongbing, commonly called citrus greening in South Africa, is a serious bacterial disease of all citrus cultivars. The causal agent of the disease is a gram–negative phloem-limited bacteria belonging to the alpha sub-division of the Proteobacteriaceae. The bacterium was named *Candidatus* Liberibacter and has not been cultured. Species were named "*Ca.*" Liberibacter africanum, (causing the disease in Africa) and "*Ca.*" Liberibacter asiaticum (causing the disease in Asia). "*Ca.*" L. asiaticum is not known to occur in Africa. Historically, greening occurs in the northern citrus producing areas of South Africa, *viz*. the Lowveld areas of Mpumalanga, North-West and Kwa-Zulu Natal provinces. The disease had a devastating effect on citrus production in the North-West, Limpopo, Mpumalanga and KwaZulu-Natal provinces since it was first observed in 1928.

Common symptoms of the disease are yellowing of the veins and adjacent tissues, followed by yellowing or mottling of the entire leaf. The disease syndrome to some extent differs according to citrus variety. Advanced or chronically infected trees show yellowing of the entire canopy and have sparse foliage and twig die-back. Diseased trees produce small, lopsided fruit that tend to remain mostly green in colour even when mature, have undeveloped seed, and impart an objectionable bitter-salty flavour to the juice. The Asian form of greening is more aggressive than the African form. They can clearly be distinguished on the basis of temperature tolerance. With African greening, severe symptom expression was obtained in glasshouse conditions at 22°C whereas no symptoms appeared at 27-30°C. In contrast, Asian greening is pronounced at both temperatures (Schwarz, 1972).

The African species is transmitted by the citrus psyllid, *Trioza erytreae*, whereas the Asian species is transmitted by the psyllid, *Diaphorina citri*. It was demonstrated by McClean (1965) that greening was graft transmissible. There are no curative methods to control greening. The only effective control measure

presently is to prevent the trees from becoming infected. Control measures known to be effective against greening disease consist of the following: to prevent the spread of the bacteria by restricting the movement of plant material from infected regions to uninfected regions, to provide the industry with disease-free propagation material, to control the vector effectively and to eliminate the inoculum by removing infected trees and infected branches. Antibiotic control by trunk injections of tetracyclines was investigated but abandoned because of ecological reasons. Moreover, tetracycline is bacteriostatic, rather than bactericidal, and the treatment had to be repeated each year (Bové, 2006). Therefore, the objective of this experiment will be to evaluate a new approach to effectively control the greening bacteria in citrus trees in infected regions by possibly eliminating or reducing the bacterium with a single foliar application of a systemic chemical.

Materials and methods

Two pot trials were laid out at CRI to evaluate the effect of heat treatment and systemic eradicant and protectant fungicides to eliminate or reduce Liberibacter inoculum in greening infected trees. Greening infected trees at Crocodile Valley Citrus (Pty) LTD were sprayed with a systemic eradicant and protectant fungicide as a field trial to determine the effect of this product on Liberibacter inoculum in infected trees.

CRI pot trial

Eighty Delta Valencia trees in 10 l pots were used for the two trials. The first trial consisted of seven treatments that included the combination of heat treatment and foliar chemical applications. The treatments were replicated five times. Four treatments in this trial were sprayed with a systemic eradicant and protectant chemical and covered with plastic. Two treatments only were covered with plastic. The treatments and times of application are shown in Table 4.2.16.1. The potted trees were covered with handmade plastic dome structures. Control trees were left uncovered. The temperatures inside the domes and outside were logged on an hourly basis, 24 hours per day, with a Squirrel data logger (SQ 1025). The total Centigrade degree hours above 30°C inside and outside the domes was recorded.

The second trial consisted of nine treatments with five replicates per treatment. Two systemic eradicant and protectant chemicals were applied at different dosages and times during the season. The treatments and time of application are shown in Table 4.2.16.2.

Leaf samples were colleted from each tree in the trials and were sent to University of Pretoria for PCR analysis to confirm the presence of the greening organism in the trees. All the trees were treated with Imidachloprid (Confidor®) to restrict psylla damage/feeding. The trees will be visually inspected on a weekly basis to determine if any phytotoxic reaction is visible as a result of the foliar application. Leaves will be sampled in winter to determine if the greening organism is still present in the trees.

Table 4.2.16.1. The evaluation of a combination of systemic foliar applications and heat treatments in a pot experiment conducted at CRI for the control of citrus greening bacterium.

Treatment	Dosage	Period
Treatment 1- Heat treatment	Control	Full period ± 5 months
Treatment 2 – Heat treatment	Control	Half period ± 2½ months
Product A	X + heat	Full period
Product A	2X + heat	Full period
Product A	X + heat	Half period
Product A	2X + heat	Half period

Table 4.2.16.2. The evaluation of different systemic foliar applied products applied at different times and rates conducted at CRI for the control of citrus greening bacterium.

Treatment	Dosage	Period
Treatment 1	Control	Standard
Product A	X	1 application only
Product A	2X	1 application only
Product A	X	Every two weeks
Product A	X	1 x per month
Product A	X	1 x every 2 months
Product B	X	Every 2 weeks
Product B	X	1 x per month
Product B	X	1 x every 2 months

Croc Valley field trial

Seven-year-old, greening infected, Delta Valencia interplant trees at Crocodile Valley Citrus (Pty) LTD were used as an initial screening trial. A systemic eradicant and protective chemical was applied at three dosages (1X, 2X & 4X) as a single application in spring. Prior to the application, leaf samples were collected and were sent to UP for PCR analysis to confirm the presence of the greening organism. Three single trees per treatment were used in the trial. The treatments, dosages and time of application are shown in Table 4.2.16.3. The trees will be visually inspected for any phytotoxic reaction due to the foliar application. Fruit that drop to the ground will be collected and counted on a weekly basis. Leaves will be sampled in winter and the fruit of each treatment will also be inspected for symptoms to determine if the greening organism is still present in the trees.

Table 4.2.16.3. The evaluation of a single Spring application of a systemic chemical applied at three different rates on greening infected Delta Valencia trees for the control of the greening organism.

Treatment		Dosage/tree	Time of Application
1.	Untreated control	-	-
2.	Product X (x)	100 ml	Spring
3.	Product X (2x)	200 ml	Spring
4.	Product X (4x)	400 ml	Spring

Results and discussion

CRI pot trial

All the leaf samples collected in both the trials tested positive for greening. No phytotoxic reaction due to the foliar applications was observed. The trial is ongoing and no results are available yet. The final evaluation will be done during the winter and spring.

Croc Valley Citrus Estate

All the leaf samples collected from the trees tested positive for greening. No phytotoxic reaction due to the foliar applications was observed. The trial is ongoing and no results are available yet. The final evaluation will be done during the winter and spring.

Conclusion and future research

The evaluation of the systemic products to control or eradicate the greening bacterium in the plant could, if found to be effective, possibly solve greening disease in citrus worldwide. These products are safe to use with no long term residual effects and will therefore be a viable option for producers to use in greening infected orchards and regions. The trial is still ongoing and the result at the end of the season would determine the future of this project.

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4.3 **PROJECT: CITRUS BLACK SPOT**

Project Co-ordinator: J.M. Kotzé

4.3.1 **Projekopsomming**

Resultate uit die swartvlek-navorsing word met groot nut aangewend om ons vrugte tot die oorsese markte toeganklik te hou. Daar is begin met 'n oorsig-artikel wat vir 'n internasionale joernaal bedoel is om alle belangrike feite wat uit ons navorsing spruit, prominent aan te bied vir marktoegang, veral na die Europese Unie (PPL2; PPL4B; PPL6; PPL12; PPL13 en die puik werk deur Ida Paul). 'n Artikel "Failure of *Phyllosticta citricarpa* pycnidiospores to infect Eureka lemon leaf litter" het in Australasian Plant Pathology verskyn. Nog 'n artikel oor die vatbaarheid van blare vir besmetting wag om gepubliseer te word. Hierdie artikels is van groot belang om opportunistiese geskrifte van Argentinië en Brazilië se invloed op ons uitvoere te weerlê.

In hierdie jaar se navorsingsverslae, word gerapporteer dat 'n wortel-agar medium met vlugtige olies geskik gevind is om *G. citricarpa* selektief te kweek (PPL10). Voortbouend op die verslag CRI 848 het PPL17 bevind dat die empiriese "Nat-Droog" tegniek tot groot voordeel aangewend kan word om swartvlek se teenwoordigheid vas te stel. Vordering en verbetering van PCR tegnieke om *G. citricarpa* op simptoomlose vrugte en blare te bepaal (PPL 4) is gedoen. Die protokol is besonder koste effektief. Daar is nou voldoende inligting om swartvlek in kwekerye te op spoor (2006/CBS6) en die resultate moet nou in praktyk toegepas word.

QMS 2006/CBS2 beskryf pogings om askospore van *G. citricarpa in vitro* te produseer, sowel as inokulasie-studies in die veld. Dié werk moet aangemoedig word waar steriele fasiliteite bestaan.

In Eksperiment 799 is gevind dat Sporekill by standaard middels gevoeg kan word en die dosis selfs verlaag kan word. Deur die byvoeging van Sporekill by kopermiddels het stippel-vorming verminder. Dit is ook in Eksperiment 837 gevind dat 'n relatiewe ou produk, Dodine, belowende resultate gegee het en dat dit verder ondersoek moet word.

In Eksperiment 2006/CBS1 word meer gekyk na die uitwerking van besproeiing op die moontlike stimulering van inokulum. Geen betroubare resultate is nog beskikbaar nie. Eksp. 2006/CBS7 ondersoek die effek van lente-bespuitings op spoorvorming en data word steeds ingesamel.

Vir baie jare is daar onsekerheid oor die vatbaarheid van sitrusvrugte in Februarie en Maart. Dit is bewys dat onder baie gunstig toestande, daar wel 'n bietjie besmetting kan plaasvind en spuitprogramme moet daarvoor voorsiening maak. Hierdie navorsing kan nou afgesluit word (2006/CBSa).

Project summary

Results from the CBS research have successfully been used to solve some of the vital market access issues. An overview article of CBS research is being written under the guidance of the co-ordinator and Prof. Korsten. An article, "Failure of *Phyllosticta citricarpa* pycnidiospores to infect Eureka lemon leaf litter", was published in Australasian Plant Pathology (PPL2; PPL4; PPL6; PPL12; PPL17) and clearly shows that infected fruit (from South Africa) cannot spread CBS in Europe via the infected fruit to fallen leaf pathway, which was a question raised by the European Union in 2001. Another article demonstrating the susceptibility of leaves will also be published soon. These published findings by the South African scientists become crucial since superficial reports from Argentina and Brazil suggest that fruit can spread CBS to other countries.

One of the obstacles in CBS research is that no selective medium exists for the pathogen. A modified carrot agar medium encouraged the growth of *G. citricarpa*. Essential oils (Eucalyptus) and volatile chemical (Furfural) were used successfully for the selective growing of *G. citricarpa* directly from fruit and leaf samples. The impact of this work in modified atmosphere packaging is a bonus and is begging to be explored.

Kotzé reported to CRI (Experiment 848) "An empirical Wet-Dry technique to detect CBS in nurseries and production areas with low disease pressure". PPL17 progressed on this work and confirmed the usefulness of this technique. Improvements on PCR techniques for detection of *G. citricarpa* from asymptomatic leaves were also made (PPL4). Sufficient information is now available to detect CBS in nurseries, and a protocol is being researched (Experiment 2006/CBS 8).

In QMS 2006/CBS 2, experiments are described to produce ascospores of *G. citricarpa* artificially by using lemon seedlings *in vitro*. Techniques are also described to inoculate fruit and leaves *in situ*. This work should be encouraged if facilities to carry out this type of research are available.

In Experiment 799, it was found that when Sporekill is added to standard fungicides, the efficacy is improved, and dosages can even be lowered. Moreover, in the case of copper fungicides, stippling was also reduced. In Experiment 837, a relative old compound, Dodine, showed promising results and wil be investigated further.

For some time growers are uncertain about the susceptibility of fruit during February and March. In Experiment 2006/CBS 6a, it was shown that some infection can occur during these months should favourable conditions be experienced and spray programmes should be adjusted accordingly.

The effect of irrigation of CBS inoculum is still under investigation by Dr. Swart. At this early stage it seems that the level of inoculum may be influenced (Experiment 2006/CBS 1). With apple scab, which has a similar life cycle as CBS, spraying trees in autumn after the fruit has been picked has a remarkable influence on disease control. A similar strategy is under test in Experiment 2006/CBS 7. Data are still being collected.

4.3.2 **Leaf wilting to enhance detection of *Guignardia* spp. in symptomless green leaves**

Experiment PPL 17 by M Truter and L Korsten (UP)

Opsomming

Die patogeen *Guignardia citricarpa* kan latent bly in geïnfekteerde groen blare tot blaarval en afsterwing. Die sukses van tegnieke soos isolasies en PKR wat huidiglik gebruik word om die patogeen direk vanaf groen blare op te spoor, is laag. Verskillende behandelings van groen blare is in die studie vergelyk ten opsigte van tyd tot vrugliggaamvorming op blaarweefsel en opsporings sensitiwiteit met die PKR-metode spesifiek vir *G. citricarpa.* Die metode soos beskryf deur Kiely (1948) en McOnie (1967) en 'n nuwe metode ontwikkel deur JM Kotzé is vergelyk. Blootstelling van die blare aan sonlig gevolg deur afwisselende benatting en droging vorm die basis van al die tegnieke. Deur klein veranderings in die metode is vrugliggaamvorming met daaropvolgende sporulering baie verbeter. Visuele vrugliggaamvorming op blaarweefsel het voorgekom na 6 tot 14 dae, afhangend van die oorspronklike vlak van infeksie deur die patogeen. Die opsporing van die patogeen deur PKR na die verlep prosedure is met meer as 80% verbeter.

Introduction

Leaf infections by *Guignardia citricarpa* Kiely form a critical part in the pathogen's life cycle and with proper orchard sanitation where old fruit are removed from the orchard before onset of the next crop, infected leaves provide the only method of survival of the pathogen until the following crop.

The susceptibility of leaves to *G. citricarpa* was previously reported to be as short as 5 weeks (Kiely 1948; McOnie 1967), although field observations suggested the susceptibility period to be about 5 months (Kotzé 1981). Infected leaves can stay latent for up to 36 months before leaf fall and, production of pycnidio- and ascospores occurs on the leaf litter in favourable conditions. Leaf symptoms on attached green leaves were reported mainly on Eureka lemon (Kiely 1948; Wager 1952; McOnie 1967; Kotzé 1996).

Since leaf infection usually remains symptomless, and the pathogen concentration in symptomless leaves are too low for proper detection by means of DNA-based molecular techniques (L Meyer, personal communication, September 2005), a method is required to enhance the detection when using symptomless green leaves. The process of artificial wetting and drying of the detached leaves were evaluated at various temperatures to develop an efficient and consistent procedure to enhance biomass of *G. citricarpa* in naturally infected leaves.

Materials and methods

Mature green leaves of Eureka lemon and Valencia orange were collected from commercial orchards in Mooinooi (North-West province), Burgersfort (Mpumalanga province), and Nelspruit (Mpumalanga province) and from a non-commercial orchard in Pretoria (Gauteng Province). Leaves were picked randomly on all four sides of 20 trees per orchard. The leaves were placed in paper bags and kept below 20°C until used. All the leaves were used within 24 h after being collected. The leaves were washed in running tap water to remove dirt, drained to remove excess water and placed in 20 micron clear plastic bags.

The leaves were exposed to 42°C for 6 h, followed by incubation at 25°C under fluorescent and near-UV light for 18 h. These incubation conditions were repeated each day for the duration of the treatment. Leaves were wetted once a day by soaking in tap water for 30 minutes before the 42°C exposure.

The leaves were visually inspected for signs of fructification after 5 days and daily thereafter. DNA was extracted from selected leaf disks as described by Meyer *et al.* (2006) 14 days after treatment commenced.

Results and discussion

Leaves were exposed to different temperatures, humidity and light conditions to induce fruiting of *Guignardia* on the leaves. Best results were obtained with naturally infected Eureka lemon leaves, followed by Valencia orange leaves. Visual fruiting bodies (Fig. 4.3.10.1) developed on the leaves after 6 to 14 days, depending on initial infection level of the pathogen.

PCR were done on selected leaf pieces from each treatment to confirm the development and presence of *Guignardia citricarpa*. Green leaves that tested negative for *G. citricarpa* or *G. mangiferae* tested positive for *G. citricarpa* after wilting and development of fruiting structures on the brown leaves (Fig. 4.3.2.2).

The detection of the pathogen was greatly improved by the wilting treatment and the PCR technique was more sensitive than visual inspection as more samples tested positive with the PCR than with visual inspection alone (Table 4.3.2.1).

Fig. 4.3.2.1. Black fruiting bodies of *Guignardia* spp. on wilted leaves that developed after 7-10 days of wilting treatment.

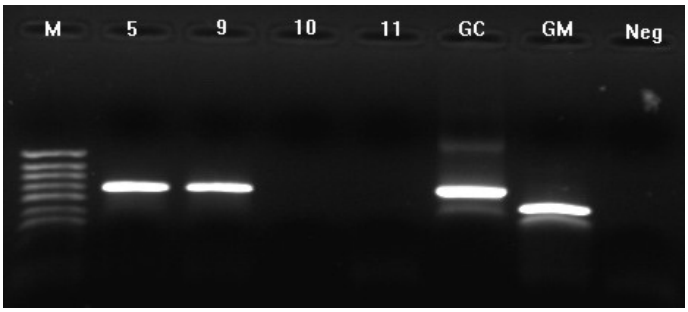

Fig. 4.3.2.2. PCR from DNA extracted from freshly detached green Eureka lemon leaves (no 10 & 11) and the same Eureka lemon leaves wilted for eight consecutive days (no 5 & 9). M = DNA marker, GC = *Guignardia citricarpa* positive control, GM = *Guignardia mangiferae* positive control, Neg = negative control with no DNA added.

Table 4.3.2.1. Detection of *Guignardia citricarpa* from naturally infected green symptomless leaves after 14 days after treatment commenced.

Area (Province)	No. of samples processed / *No. of positive samples*			
	Eureka lemon		Valencia orange	
	Visual detection	PCR detection	Visual detection	PCR detection
Mooinooi (North-West)	8 / 8	8 / 8	8 / 6	8 / 8
Burgersfort (Mpumalanga)	2 / 2	2 / 2	6 / 3	6 / 5
Nelspruit (Mpumalanga)	0	0	8 / 5	8 / 8
Pretoria (Gauteng)	6 / 1	6 / 2	6 / 0	6 / 0

Suggested protocol for artificial leaf wilting:

1. Picked mature green leaves randomly from all four sides of the tree, with about 20 leaves per tree and from at least 20 trees per orchard block.
2. Keep detached leaves cool and process within 2 hours.
3. Wash leaves in running tap water to remove dirt and drain to remove excess water.
4. Air dry leaves for 12 hours out of direct sunlight OR air dry leaves for two to four hours in direct sunlight.
5. Soak air-dried leaves in tap water for 30 minutes, drain to remove excess water and place in a 20 micron clear plastic bag. Use 20 to 50 leaves per bag, depending on size of leaves and bag.
6. Closed bag, including as much as possible air within the bag, and place bag with leaves in an incubator at 42°C for 6 h.
7. After 6 h, remove the bag from the incubator and mix leaves by shaking the bag.
8. Open the bag to allow leaves to air dry and incubate under fluorescent and near-UV light for 18 h.
9. Repeat steps 5 to 8 for at least 21 days or until ample fructification of *Guignardia* is visible on the leaf surface.

Note: It is important to monitor the moisture within the bag closely, since no fruiting bodies will develop if the leaves are to dry and the leaves will rot if it is too wet. Unfortunately the correct moisture levels are only known through experience.

Future research

No further experiments will be conducted in this project.

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Presentations made

Truter M, Kotzé JM, Meyer L & Korsten L 2006. Mysteries of *Guignardia citricarpa* – the switch from green to mean. 4th Citrus Research Symposium, Port Elizabeth, 20-23 August 2006. (Paper).

4.3.3 **Selective medium for *Guignardia citricarpa***

Experiment PPL 10 by T. Regnier, K. Zeeman and L. Korsten (UP)

Opsomming

Guignardia citricarpa wat sitrus swartvlek veroorsaak tas alle kommersiële sitruskultivars aan, veral suurlemoene en Valencia lemoene. Vinniger groeiende patogene soos *Penicllium* en *Colletotrichum* spp oorgroei dikwels die agarplate wat isolasie van *G. citricarpa* bemoeilik. Ten einde 'n rein kultuur van *G. citricarpa* te verkry, moet 'n selektiewe medium ontwikkel word. Gevolglik is verskeie kultuurmedia wat aanbeveel word vir die groei van algemene fungi ondersoek. Miseliumgroei van *G. citricarpa* op wortelagar het aansienlik toegeneem, sodanig, dat dit byna dieselfde was as groei van *Colletotrichum gloeosporioides* en *Penicillium digitatum*. Deur essensiële olies (Eucalyptus) en kommersiële vlugtige chemikalieë (furfural) te gebruik, is 'n belowende *in vitro* metode om *G. citricarpa* direk vanaf die vrug of blaar te isoleer, beskryf.

Introduction

Citrus is a major foreign exchange earner for exporting countries like South Africa. Citrus fruit pathogens such as *Guignardia citricarpa* Kiely and *Penicillium digitatum* Sacc. is a major causes of phytosanitary risks and postharvest diseases, respectively (Kotzé,1981). Although a rapid PCR method has recently been developed to detect the pathogen in suspect lesions and distinguish it from the non-pathogenic but faster growing endophyte, *G. mangiferae* (Meyer *et al*. 2005) it remains essential to develop a selective isolation medium for epidemiological studies. Currently, other faster growing pathogens such as *Penicillium* and *Colletotrichum* spp often overgrow the plate making isolation of *G. citricarpa* difficult.

Many volatile compounds such as linalool have antifungal activity against a range of fruit pathogens (Arras, 1999). It has also been demonstrated that essential oils could be used to control several postharvest diseases of fruit and vegetables (Liu *et al.*, 2002). Plaza *et al.* (2004) have tested several volatile oils *in vitro* and *in vivo* against *Penicillium* spp. However, these volatiles were not effective in reducing citrus decay *in vivo*. The last fifteen years, natural fumigants have been tested extensively in order to control pathogens in food, soil, plants and on fruit (Karr *et al.*, 1990).

The present study was undertaken to investigate the use of volatiles (essential oils and furfural) as potential chemicals to create a selective medium for *G. citricarpa* isolations.

Materials and methods

Pathogens: *Penicillium digitatum* (UPFL 745)*, Colletotrichum gloeosporioides* (UPFL 925), *Guignardia citricarpa* (GC-m155), G*uignardia mangiferae* (GC 117) and *Geotrichum citri-aurantii*, were originally isolated from symptomatic fruit, purified, preserved and identity confirmed by M. Truter (Dept. Microbiology and Plant Pathology, University of Pretoria). Isolates were maintained on half strength potato dextrose agar (PDA) (Biolab, Johannesburg, South Africa) and prepared for inoculation studies by scraping off the spores with a sterile glass rod after 6 to 14 days incubation at 23ºC.

Volatiles: The inhibitory effects of 18 essential oils and two blends (Table 4.3.3.1) were tested. All oils were purchased from health stores in Pretoria (South Africa). In addition, pure furfural (2-furfuraldehyde, Sigma, Johannesburg), the main component of Cropguard, was also tested.

Culture technique: A 5 mm diameter agar disc containing mycelia and spores of the fungus were taken from the edge of each actively growing fungal colony with the aid of a sterile cork borer.

Mediums tested: Potato dextrose agar, Malt extract Agar (MEA) and Carrot dextrose agar (CDA) were tested to determine the best mycelial growth medium for *G. citricarpa*.

Agar dilution method: Carrot dextrose agar was autoclaved at 121°C for 20 min. Each oil solution and 400 µl/l Triton X was added aseptically to the medium to give a final concentration of 100 and 1000 µl/l for the essential oils and 1, 2, 4, 6, 8 and 10 % (v:v) for furfural. The agar was poured into 90 mm Petri-dishes and a 5 mm agar disc containing mycelia and spores of the fungus, was placed onto the centre of each CDA plate after setting. In order to prevent dissemination of *P. digitatum* spores over the surface of the agar plate, the inoculation was done using 10 µl of a conidial spore suspension (10^6 spores/ml). Controls consisted of plates with CDA only. After 2, 4 and 6 days at 23°C, mycelial growth (mm) was measured with a digital caliper (Absolute Digimatic-Mitutoyo Corp. Japan). The experiment was carried out with 10 replicates per oil concentration per isolate. Data were expressed as percentage inhibition of mycelial growth according to the method described by Plaza *et al.* (2004).

Exposure to volatile oils: Glass cover slips were fixed to the inside of the Petri-dish lids using a drop of glycerol. A droplet with 10, 20 or 40 µl of pure essential oils or 2, 4, 6, 8 or 10% of furfural was placed on the cover slip. Inoculated plates, as described above, were inverted on their lids. Three replicates of each treatment combination were done and compared to five controls with water instead of oil or furfural. The Petri-dishes were sealed with Parafilm (American Natural, Chicago) and incubated upside-down at 23 °C. The growth of the pathogen was measured after 2, 4, 6, 8 and 10 days and data were expressed as percentage inhibition of mycelial growth (Plaza *et al.* 2004). The fungicidal activity of each concentration and exposure time was evaluated by transferring half of the mycelial plug onto a fresh carrot dextrose agar dish. Fungal growth was observed and measured after 6 days incubation at 23°C.

Isolation from citrus black spot lesions on fruit: Fruits with CBS lesions were surface sterilised with 70% ethanol and 50 lesions were aseptically cut with a scalpel and transferred onto the Petri-dish containing CDA just prior to the test. Fifty lesions from Valencia oranges and 50 lesions from lemons were used in this assay. Plates were exposed for 4 days to 8% furfural as volatile as previously described. In total, fifty lesions of both fruit types were also used as control and exposed to distilled water impregnated discs. The selective effect of the furfural was confirmed by aseptically transferring the lesion onto a fresh CDA agar plate and incubating it for 6 days at 23°C. Presence or absence of any CBS growth from the cut lesions was recorded and data expressed as percentage of lesion yeilding growth of the pathogen.

Isolation from leaves: Freshly picked, green Valencia leaves were surface sterilised with 70% ethanol for 2 min and discs were aseptically cut with a sterile 0.5 cm cork borer. Three hundred discs were used in total. Five discs were placed on the surface of the CDA inside the Petri-dish. Twenty of these plates were exposed for four days to 8% furfural as volatile (impregnated paper disc on the lid) and a similar number were exposed to 10% furfural. The remaining plates were used as control and distilled water was used to impregnate the discs. The presence or absence of mycelium growth from the leaf disc was recorded and data were expressed as percentage positive mycelial growth. The fungicidal effect of the furfural was evaluated by transferring the leaf discs onto a fresh CDA agar plate and incubating it for another 6 days at 23°C. Presence or absence of any further fungal growth was recorded.

Isolation from leaf litter: Citrus leaf litter, inoculated as described by Kotzé (CRI internal document), were used to confirm the efficacy of the fumigant. One hundred and fifty leaf discs were aseptically cut and transferred onto CDA plates (five discs per plate) just prior to the test. Half of the discs were exposed to furfural as volatile (8 or 10%) for 4 days as described before while the remaining fifty represented the control. The presence or absence of mycelium growth was recorded and data were expressed as percentage positive mycelial growth. The fungicidal effect of the fumigant was evaluated by transferring the disks to a fresh new PDA plate and incubating it for further 6 days at 23°C. Presence or absence of further fungal growth was recorded and expressed as percentage positive mycelial growth.

Data were analysed using the statistical program GenStat (2002). Analysis of variance was used to test for differences between values and means were separated according to Fisher's protected *t*-test for least significant difference.

Results and discussion

Current technology for maintaining the post-harvest quality of fresh produce such as citrus relies on the use of fungicides in the packline. Because natural antimicrobial activities of plant extracts in general and essential oil in particular have been demonstrated, the potential of using the plant extract as selective inhibitor could provide hope to find a method to selectively grow *Guignardia citricarpa*. Our preliminary study on growth medium for *G. citricarpa* confirms the efficacy of a carrot dextrose agar medium to significantly increase the growth of this pathogen (Table 4.3.3.1).

Table 4.3.3.1. Mycelial growth in cm of fungi after six days at 25°C.

Medium	*Penicillium digitatum*	*Colletotrichum gloeosporioides*	*Guignardia citricarpa*	*Guignardia mangiferae*
PDA	3.5	5.5	2.2	4.2
MEA	2.5	5.2	2.5	4.5
Carrot dextrose Agar	6.5	6.6	6.4	5.1

The present results indicate a selective control effect of the oil on the different pathogens at low concentration. We demonstrated and confirmed the *in vitro* antifungal activity of the oils and the furfural as biofumigant. We also demonstrated and confirmed the possible purification of *G. citricarpa* by reducing the amount of fungal contaminants *in vitro* by using an essential oil or furfural.

In order to facilitate reading and easy evaluation of the data, the results of only three of the oils are reported regarding the development of a selective medium through incorporation of the oils. The study demonstrated the potential antifungal activity of cinnamon (*Cinnamonum zeylanicum*) oil at 100 µl/l, against *G. citricarpa, G. mangiferae* and *C. gloeosporioides* (Table 4.3.3.2). At a higher concentration (1000 µl/l), the mycelial growth of all pathogens was completely inhibited with cinnamon oil, clove (*Eugenia caryophyllata*) oil and thyme (*Thymus capitatus*) oil.

Table 4.3.3.2. Percentage inhibition of mycelial growth of *Colletotrichum gloeosporioides*, *Guignardia citricarpa, Guignardia mangiferae* and *Penicillium digitatum in vitro* by three essential oils at a concentration of 100µl/l, using the agar dilution method.

Essential oil	Percentage inhibition of mycelial growth[a]			
	C. gloeosporioides	***G. citricarpa***	***G. mangiferae***	***P. digitatum***
Cinnamonum zeylanicum	100aA©	100aA©	100aA©	85.55bA
Eugenia caryophyllata	44.44D	100aA©	100aA©	22.65E
Thymus capitatus	48.39bC	100aA©	100aA©	37.66cB

[a] Average of two trials of five replicates per trial. Data were recorded after six days incubation at 25°C. Within each column or row, means followed by the same upper-case or lower-case letter, respectively, do not differ significantly at $P \leq 0.05$.
© Fungistatic activity.

The fungicidal properties of the volatile oils to all pathogens were evaluated when the pathogens were left on the same medium but with a new sterile lid without oil. Thyme and lemongrass oils were found to have a fungicidal effect on all pathogens while *Eucalyptus smithii* oil, lavender (*Lavandula augustifolia*) oil, and tea tree oil, were found to have a fungicidal effect on *C. gloeosporioides* and *G. mangiferae* but not on *G. citricarpa* and *P. digitatum*. Since we investigated the development of a selective isolation procedure for *Guignardia* spp, we found that *Eucalyptus smithii* oil had the desired effect. After 6 days exposure to the vapours of the oil, it either killed or inhibited the competitive fungi sufficiently to allow *G. citricarpa* to grow after transfer. Antifungal evaluation of the oil by vapour contact has been reported. Previous work demonstrated that the vapour activity was weaker than the activity in medium (Maruzzella *et al.* 1959). Although we didn't determine the absorption of the vapour into the mycelium and the medium, it could be possible that volatiles could be absorbed by fungus due to the nature of the structure of the mycelial wall Inouye *et al.* 1999).

This study also shows the general broad spectrum activity of furfural to control citrus fruit and leaf pathogens both *in vitro* and *in situ*. This is the first report of the selective advantage given to the pathogen *G. citricarpa* by using differential *in situ* exposure to furfural. However, this technique could not selectively inhibit the growth of the saprophyte *G. mangiferae,* which could still interfere with the selective isolation of *G. citricarpa*. Fortunately, the current PCR protocol described by Meyer *et al*. (2005) differentiate between the two organisms and can be used within 6 hours to confirm the presence of *G. citricarpa*.

The agar dilution method showed complete inhibition of mycelial growth of *P. digitatum* and *Geotrichum* spp at 1 and 2% furfural, respectively, while *G. citricarpa, C. gloeosporioides* and *G. mangiferae* showed 79% and 76% inhibition, respectively (Data not shown). When used as fumigant, furfural was able to effectively control all pathogens. However, the concentration and the time of exposure vary according the pathogen (Table 4.3.3.3).

Table 4.3.3.3. Percentage of *in vitro* mycelial growth inhibition of *Guignardia citricarpa*, *G. mangiferae*, *Colletotrichum gloeosporioides*, *Penicillium digitatum* and *Geotrichum citri-aurantii* by the furfural volatile method.

Pathogen	Concentration of furfural (%)	Exposure time (days)				
		2	**4**	**6**	**8**	**10**
	2	65.84dE	41.11eG	92.17bB	82.41cB	95.51aB
	4	41.57bH	100aA[a]	100aA[a]	100aA[a]	100aA[a]
G. citricarpa	6	91.011bD	100aA[a]	100aA[a]	100aA[a]	100aA[a]
	8	97.75bB	100aA[a]	100aA[a]	100aA[a]	100aA[a]
	10	95.51bC	100aA[a]	100aA[a]	100aA[a]	100aA[a]

Pathogen	Concentration of furfural (%)	Exposure time (days)				
		2	4	6	8	10
	2	2.18eI	69.58aE	44.88bF	21.74cG	15.79dF
	4	100aA[a]	80.99bD	81.35bD	78.07cC	69.66dC
G. mangiferae	6	100aA[a]	100aA[a]	100aA[a]	100aA[a]	100aA[a]
	8	100aA[a]	100aA[a]	100aA[a]	100aA[a]	100aA[a]
	10	100aA[a]	100aA[a]	100aA[a]	100aA[a]	100aA[a]
	2	54.22aG	49.54bF	6.56cH	0.91dH	1.25dH
	4	57.56aF	56.72bE	23.15dG	28.30cF	6.39eG
C. gloeosporioides	6	100aA[a]	90.36bC	47.72cE	45.73dE	25.15eE
	8	100aA[a]	100aA[a]	99.33aA	100aA[a]	100aA[a]
	10	100aA[a]	100aA[a]	100aA[a]	100aA[a]	100aA[a]
	2	90.80aD	93.67bB	88.14cC	57.66dD	52.01eD
	4	98.98aB	100aA[a]	100aA[a]	100aA[a]	100aA[a]
P. digitatum	6	100aA[a]	100aA[a]	100aA[a]	100aA[a]	100aA[a]
	8	98,98aB	100aA[a]	100aA[a]	100aA[a]	100aA[a]
	10	100aA[a]	100aA[a]	100aA[a]	100aA[a]	100aA[a]
	2	100aA[a]	100aA[a]	100aA[a]	100aA[a]	100aA[a]
	4	100aA[a]	100aA[a]	100a*[a]	100aA[a]	100aA[a]
G. citri-aurantii	6	100aA[a]	100aA[a]	100aA[a]	100aA[a]	100aA[a]
	8	100aA[a]	100aA[a]	100aA[a]	100aA[a]	100aA[a]
	10	100aA[a]	100aA[a]	100aA[a]	100aA[a]	100aA[a]

[a] Total control of fungal growth. Data were recorded after different exposure times (days). Within each column or row, means followed by the same upper-case or lower-case letter, respectively, do not differ significantly at $P \leq 0.05$.

After four days of exposure to Furfural, a 4% and 6% concentration was fungicidal to *P. digitatum* and *G. citri-aurantii*, respectively. *Guignardia citricarpa* showed a better resistance to the Furfural when compare to *C. gloeosporioides*. Furfural presented a fungicidal activity for *Guignardia citricarpa* only at a 10% concentration and after an exposure of 10 days while *C. gloeosporioides* was already inhibited after a shorter time of exposure.

Exposing fresh leaves to furfural as volatile at 8-10% could inhibit the growth of fungi in general. Similar results were obtained for leaves inoculated with *G. citricarpa*. For orange fruit lesions, these concentrations could also completely inhibit the growth of all fungi tested. None of the lesions exposed to 10% furfural had any fungal growth after being transferred to fresh medium. Symptomatic pieces of Lemon fruit were less affected by the fumigant, than similar lesions from Valencia. A higher percentage of *G. citricarpa* was noted growing from the lesions after transfer to the new medium (Table 4.3.3.4).

Table 4.3.3.4. Presence or absence of *in vitro* fungal growth from citrus lesions after exposure to furfural volatiles.

	Plant Material	Concentration of furfural (%)	Mycelial growth (+ or -)
At transfer*	Fresh leaf	0	+ (96%)
		8	-
		10	-
	Inoculated dry leaf	0	+ (100%)
		8	-
		10	-
	Orange CBS lesions	0	+ (98%)
		8	-
		10	-
	Lemon CBS lesions	0	+ (100%)
		8	-
		10	-

	Plant Material	Concentration of furfural (%)	Mycelial growth (+ or -)
Six days after transfer to a fresh medium	Fresh leaf	8	+ (4%)
		10	-
	Inoculated dry leaf	8	-
		10	-
	Orange CBS lesions	8	+ (10%)
		10	-
	Lemon CBS lesions	8	+ (20%)
		10	-

*Data recorded after 4 days exposure to fumigant. Positive if at least one lesion presented any fungal growth. Numbers in brackets are the percentage of leaf or fruit sections showing mycelial growth. Data are the means of three independent trials.

Conclusion

This study on the selective medium for *Guignardia citricarpa* has highlighted the unlocked potential of essential oils and furfural in the post-harvest environment. Overall, thyme oil presented a fungicidal property to all the pathogens. The volatiles released from *Eucalyptus smithii* oil appeared to be possible candidates for selective isolation of *G. citricarpa* as the volatiles showed positive fungicidal properties to *G. mangiferae*, and *C. gloeosporioides* but not to *G. citricarpa.* Moreover, tea tree oil was the most effective fungistatic oil against *P. digitatum.* This is the first report of the selective advantage given to the pathogen *G. citricarpa* by using differential *in situ* exposure to an essential oil (Eucalyptus) or furfural. An exposure of four days to the oil is the starting point of the protocol and need to be defined in order to confirm the selective isolation procedure of the pathogen

Future research

The promising results of both *in vitro* and *in vivo* studies indicate that employing essential oil or furfural as an *in vivo* fumigant during extended transport periods is worthy of further investigation. Additional studies in which the oil is used in combination with modified atmosphere packaging will be valuable.

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4.3.4 Evaluation of a protocol for detecting *Guignardia* spp. on citrus nursery trees

Experiment 2006/CBS 8 by S.H. Swart (QMS) and J.M. Kotzé

Opsomming

Twee kwekerye, een in Letsitele en een in Malelane, is gebruik om ŉ protokol te toets vir die opspoor van swartvlek geïnfekteerde sitrus kwekeryboompies. Faktore soos eksterne bronne van inokulum, tipe besproeiing, tonnel struktuur, kultivar, boom ouderdom, algemene klimaat, klimaatstoestande tydens die afharding van die bome, frekwensie van sanitasie en voorkomende spuitprogramme is geïdentifiseer wat moontlik ŉ effek op die voorkoms van swartvlek geïnfekteerde sitrus boompies kan hê. Tweehonderd en

vyftig van die oudste blare op die oudste of mees vatbare bome in die kwekerye is versamel. Van hierdie blare is 25 met verdagte letsels gebruik vir isolasies en die res is tussen twee stelle plastiek gaasvelle geplaas en in die skadu van 'n boom gelaat om te verouder. Blare is met 'n askospoor monitor geëvalueer vir teenwoordigheid van *Guignardia* spp. Die veroudering van sommige blaarmonsters is verhaas deur blare aan alternatiewelike nat en droë periodes bloot te stel. Blare is ook op die kwekery vloer versamel en met die askospoor monitor geëvalueer. In albei kwekerye is *G. citricarpa* en *G. mangiferae*. met isolasies gevind en die teenwoordigheid van *Guignardia*-tipe sakospore met die askospoor monitor bevestig. Daarom het verskille in kwekery struktuur, klimaat, tipe besproeiing, kultivar, en voorkomende spuitprogramme nie 'n bepalende rol gespeel nie. Komposterende blare, afkomstig van kwekery vloere van albei kwekerye, het askospore geproduseer, maar sulke blare was meestal nie beskikbaar nie. Tellings wat op geprosesseerde geplukte blare gekry is, het nie altyd met die van opgetelde blare gekorreleer nie. Isolasie van *Guignardia* spp. was die swakste tegniek as gevolg van baie lae herwinning van isolate. Monitering van inokulum vlakke op blare wat val in kwekerye of prosessering van geplukte blare en analise met 'n askospoor monitor, blyk die mees betroubare tegniek te wees om *Guignardia* spp. op geïnfekteerde kwekery materiaal op te spoor. Die optimale tyd vir die neem van monsters moet nog bepaal word en 'n vinnige, betroubare en ekonomiese tegniek is nodig benodig om die isolate tot op spesiesvlak te identifiseer. Toekomstige projekte behoort ook op bronne van inokulum, en die voorkoming van infeksie van kwekery materiaal te konsentreer.

Introduction

It is a well known fact that that nursery trees can be a means of introducing *Guignardia citricarpa,* and eventually citrus black spot, to previously black spot free areas (Kotzé, 1993, Swart, 2004, Van Broekhuizen and Swart, 2003, Whiteside, 1967). *G. citricarpa* and *G. mangiferae* were isolated from the leaves of citrus trees in a nursery, although the percentage positive isolations were generally very low (Van Broekhuizen and Swart, 2003). *Guignardia* spp. were mostly isolated from the oldest leaves of the oldest trees in a nursery and some cultivars seemed to be more infected than others. Swart (2004) reported that *Guignardia* spp. were difficult to detect by making isolations from leaves, irrespective of sample size, and that positive isolations were mostly associated with small, black, protruding lesions on leaves. Several factors, that could be included as control points for determining risk of *Guignardia* infection in a citrus nursery, were compiled by Kotzé (2005a). These factors included distance from external sources (nursery position with regards to commercial production of citrus), nursery structure, irrigation method, and sanitation procedures. Other factors such as general climatic conditions and citrus black spot pressure in the area, climatic conditions since grafting of the trees (rain frequency), cultivar and age and preventative spraying programs should also be taken into consideration.

The aim of this study was to determine if there were any substantial differences regarding level of *Guignardia* infections that can be detected in a nursery in Letsitele and one in Malelane. Freshly picked leaf samples for isolations and processing for analysis with an ascospore sampler were included in the survey. Fallen, semi-decomposing leaves were also gathered from the nursery floors and analysed with an ascospore sampler.

Materials and methods

Two nurseries, one in Letsitele and one in Malelane were included in this evaluation of a protocol to determine the level of citrus nursery trees infected with the citrus black spot pathogen. Three blocks of trees in the Letsitele nursery were evaluated in December 2006 and the evaluation was repeated in February 2007. These included Flame x SC (oldest trees in nursery), Eureka lemons (possibly most susceptible), and Delta Valencia x CC. The Malelane nursery was evaluated early in February 2007, which included Navels x CC and Valencia x SC (oldest trees in the nursery), as well as Limoneira x CC (also very susceptible).

Two hundred and fifty of the oldest leaves (not more than 2 per tree) from trees in the data blocks were sampled randomly. Of these, 25 leaves with conspicuous symptoms, possibly caused by *Guignardia* spp., were selected and five isolations per leaf (125 in total) were made. *Guignardia*-like isolates recovered, were cultured on oat meal agar (3% of commercial Jungle® oats, 1.5% agar), in order to distinquish *G. citricarpa* and G. mangiferae species on the basis of yellow pigment production (Baayen, 2002). The remainder of the leaves were placed between 5 sets of plastic grids (25 leaves / grid) and placed in the shade of a wild tree (not related to citrus) until leaves had deteriorated to stage 3 (QMS scale). Two grids of leaves were also placed in a mechanised structure that allows leaves to be sprayed with water, mimicking the alternative wet-dry technique described by Kotzé (2005b). Leaves were processed and analysed with an ascospore sampler. Leaves were also gathered from the nursery floor and sixty of the most deteriorated leaves were selected for evaluation in the ascospore sampler.

Results and discussion

Both nurseries have similar positioning (right next to a commercial production orchard), structures consist mostly of 40% shade netting and irrigation methods that do not promote wetting of the leaves (dripper or hosepipe). Weather conditions in the two areas differ, with Letsitele considered to be a high disease pressure area (long term rainfall average 800 mm pa) and Malelane probably medium disease pressure area (long term rainfall average 600 mm pa). Sampling of trees in the Letsitele nursery was between 6 and 27 months after grafting of the scion. Sampling of trees in the Malelane nursery was between 9 and 11 months after grafting of the scion. Therefore, a large variety of different leaf ages, exposed to different infection periods, were included in the survey. According to precipitation data, trees in the Letsitele nursery was subjected to more and longer potential infection periods compared to trees in the Malelane nursery, since trees were grafted (Table 4.3.4.1) and during the last 12 months before leaves were sampled (Figures 1a and 1b). The number of days with precipitation did not correlate with the number of isolations recovered or ascospores produced on decomposing leaves (Table 4.3.4.2).

Both nurseries use a protective spray program to prevent CBS infection. In Letsitele, mancozeb (previously also copper oxychloride) is normally applied every 21 days from October to the end of February. In Malelane, monthly applications with copper oxychloride + Sporekill (100 g + 100 ml / 100 l water) are applied from October to the end of January. In both areas the periods of applying fungicide spray programmes are similar to programs used for control of CBS in established commercial orchards, therefore approximately 6 to 7 applications in Letsitele and 4 to 5 applications in the Malelane area.

Table 4.3.4.1. Number of days with precipitation between grafting of the scion and sampling of leaves

Nursery block(s), Scion	Months after grafting	Number of days with precipitation	Precipitation (mm)
Letsitele Des 06			
A8, Delta	12	64	614
A41/42, Flame	24	133	1251
A35/39, Eureka	20	114	1222
Biological plot	-		
Letsitele Feb 07			
A9, Valencia	6	34	427
A41/42, Flame	27	> 133	> 1251
A35/39, Eureka	23	125	1236
Biological plot	-		
Malelane Feb 07			
A 36, Limoneira	9	25	422
A9, Valencia	11	28	467
D5, Navel	10	25	422

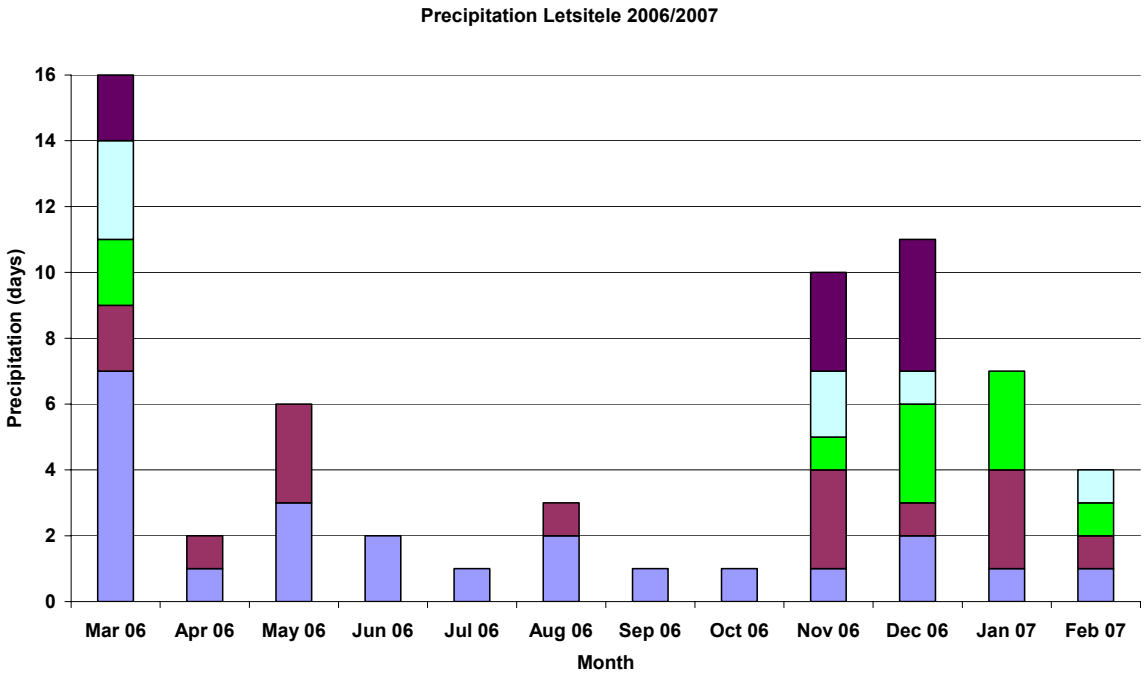

Figure 4.3.4.1a. Number of days and length of precipitation period during the last 12 months before leaf sampling on the 21st of February at the Letsitele nursery. Each colour represents consecutive days with precipitation.

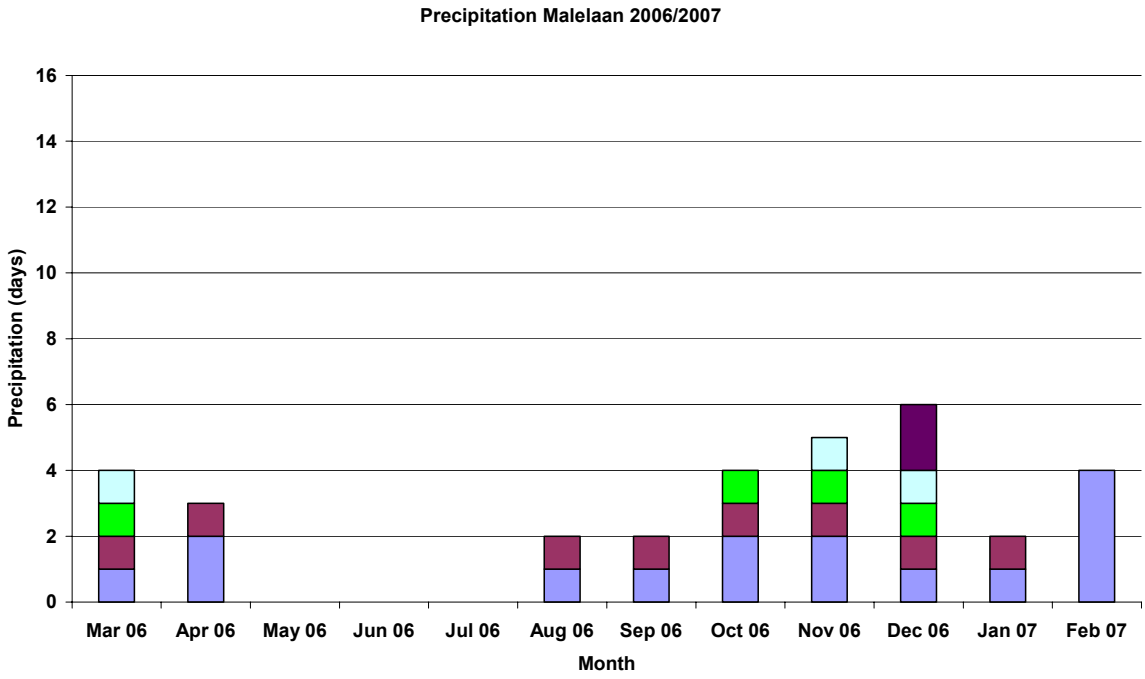

Figure 4.3.4.1b. Number of days and length of precipitation period during the last 12 months before leaf sampling on the 8th of February at the Malelane nursery. Each colour represents consecutive days with precipitation.

Two *Guignardia* isolates were recovered by isolation from the Eureka leaf samples obtained from the Letsitele nursery in December 2006 and February 2007 (Table 4.3.4.2). Eight isolates were obtained from

the Limoneira and 1 isolate from the Navel leaf samples picked in February in the Malelane nursery. Results support previous findings that both *G. citricarpa* and *G. mangiferae* can be recovered from nursery material with this technique, but only at a very low level. However, the low level of detection makes this technique not suitable to determine risk of plant material being infected (Swart, 2004).

All leaf samples obtained from the Letsitele nursery, which were placed in plastic grids and incubated under a tree at QMS Agri Science facility until February 2007 for natural aging, produced high numbers of ascospores, compared to the number of ascospores produced on leaves obtained from 42-year old, un-sprayed Navel trees, in the biological plot at Letaba Estates (Table 4.3.4.2). However, the variation between the 5 replicates of the same block was extremely large for all samples. The process was repeated in February 2007 in both Letsitele and Malelane nurseries. When leaves were evaluated with the ascospore monitor in April 2007, the number of ascospores detected was extremely low (Table 4.3.4.2). In February 2007, leaves of both nurseries were also used to evaluate the effect of accelerated aging, using the wet-dry technique described by Kotzé (2005), on the number of ascospores detected. In this evaluations higher numbers of ascospores were observed compared to natural aging, but numbers of corresponding samples did not correlate for some of the samples evaluated (Table 4.3.4.2).

No ascospores could be detected on decomposing leaves sampled in two blocks in the Letsitele nursery during December 2006. All the leaf samples that were collected on floors of nurseries at Letsitele and Malelane during February 2007, tested positive for the production of *Guignardia* ascospores. It seems that evaluation of decomposing leaves should be conducted during February, to enhance the level of detection. Unfortunately some blocks had only enough leaves for one sample while some had no leaves for this evaluation (Table 4.3.4.2).

In general, it seems that the parameters included in this study to determine risk of trees in a nursery being infected with the CBS pathogen, gave inconclusive results. Most of the nurseries in Limpopo and Mpumalanga have very similar qualities regarding position (in close vicinity of commercial citrus production), structure (shade netting that allows wetting of leaves during rain periods), method of irrigation (no overhead irrigation and general wetting of leaves), and preventative fungicide spraying programs. However, we can conclude that the general position, structure and general production practices followed in commercial nurseries do not exclude the possibility of *Guignardia* infected trees. Although vast differences in general climatic conditions exists between the 2 areas studied, especially regarding number of days with precipitation and the duration of wet periods, trees in both nurseries showed high risk of being infected with *Guignardia* spp.

In both nurseries the presence of *Guignardia* spp. was confirmed with one or more techniques used in this study. At this stage the techniques used, cannot consistently detect the presence of CBS pathogens in nursery trees and, therefore, gave erratic results. The ascospore monitor seems to be a relatively reliable means of detecting the presence of *Guignardia* ascospores in a leaf sample. However, variation between replicate samples suggests that relatively large numbers of leaves must be analysed (several replicate samples), that could have an effect on time and cost to do an analysis. Results showed the most ascospores were produced when leaves were picked in December and analysed in February after natural aging. Leaves picked in February and analysed in April had noticeably less ascospore production. The time of sampling must be optimised. The most reliable means of detecting ascopores is on dropped leaves sampled from the orchard floor, however such leaves are not always present in sufficient numbers due to regular sanitation practices, which is encouraged.

Ascospores found imbedded in Vaseline® on coated microscope slides used for trapping ascospores in the ascospore monitor did not develop into fungal colonies when plated on potato-dextrose-agar slides. Therefore, the species-identity of *Guignardia* ascospores could not be determined. This is a major drawback of the ascospore monitor at this stage that must be resolved, especially because this technique of detecting CBS pathogens seems to be the most reliable when using decomposing leaves, obtained from the nursery floor, or picked leaves allowed to decompose naturally or using techniques to accelerate the process.

Table 4.3.4.2. Nursery assessments during December 2006 and February 2007

Nursery block(s), scion	Positive isolations[a]	Ascospores[b] on picked leaves (natural aging)	Ascospores[c] on picked leaves (accelerated aging)	Ascospores[d] on decomposing leaves from nursery floor
Letsitele Des 06				
A8, Delta	0	101 ± 100	-	No leaves
A41/42, Flame	0	238 ± 159	-	0
A35/39, Eureka	1	159 ± 143	-	0
Biological plot	0	12 ± 8	-	No leaves
Letsitele Feb 07				
A9, Valencia	0	5 ± 2	0	No leaves
A41/42, Flame	0	1 ± 1	10 ± 1	65
A35/39, Eureka	1 (Gc)	1 ± 1	2 ± 1	4
Biological plot	0	-	-	105
Malelane Feb 07				
A 36, Limoneira	8 (Gm)	0	4 ± 1	103
A9, Valencia	0	1 ± 1	2 ± 1	No leaves
D5, Navel	1 (Gc)	9 ± 7	11 ± 1	323

[a] Number of *Guignardia* isolates out of 125 isolations. Gc = *G. citricarpa* and Gm = *G. mangiferae*
[b] Mean of 5 replicate samples containing 25 leaves per grid
[c] Mean of 2 replicate samples containing 25 leaves per grid
[d] Number on 1 sample containing 25 leaves per grid

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4.3.5 *In vitro* infection of plants (Preliminary report)

Experiment 2006/CBS2 by S. Serfontein and S.H. Swart (QMS)

Opsomming

Vir die kunsmatige produksie van askospore van *Guignardia citricarpa* is daar probeer om 'n tegniek te ontwikkel om suurlemoen saailinge *in vitro* te kweek en dan met piknidiospore te inokuleer sodat askospore op die blaartjies geproduseer kan word. Inokulasie met askospore van *G. mangiferae* is ook uitgevoer. *G. citricarpa* het blare van *in vitro* saailinge infekteer, maar infeksie met *G. mangiferae* was onsuksesvol. Infeksiestudies is ook op vrugte en bome gedoen. Finale gevolgtrekkings kan slegs gemaak word na die

voltooiing van die volgende projek: “The infection of citrus plants with *G. citricarpa* and *G. mangiferae*” (QMS07/SS2).

Introduction

Guignardia citricarpa (GC), the citrus pathogen, produces ascospores in nature, but not on artificial culture media. This seriously hampers infection studies. *Guignardia mangiferae,* the omnipresent endophyte produces ascospores abundantly on culture media. Very little is known about the biology of *G. mangiferae* or its interaction in the disease complex.

We tried to develop a technique to produce ascospores of *G. citricarpa* on *in vitro* seedlings. Inoculations with both *Guignardia* spp. were carried out, using pycnidiospores of *G. citricarpa* and ascospores of *G. mangiferae* to inoculate plants *in vivo* and *in vitro*. From infected leaves we tried to produce ascospores using the “wet-dry” process, as described by Kotzè (2006).

Materials and methods

In vitro inoculation. The seed coats of cv Rough Lemon seeds were removed and the seeds dipped in 70% ethanol for 30 seconds and rinsed twice in sterile water. The seeds were dried on sterile paper towels in the laminar flow cabinet. After the seeds dried, single seeds were aseptically placed on the tissue culture multiplication medium in tubs. The seedlings were grown under fluorescent lights with a 12-hour dark and 12-hour light cycle.

At 4 weeks, seedlings were inoculated with spores of strains identified by PCR as *G. citricarpa* and *G. mangiferae*. For spore production, cultures were grown on Medium 5 (Serfontein & Swart, 2005) for 21 days and the spores were harvested by flooding agar with sterile water. Spores were counted and the concentration set to 10^6 spores per mℓ. *In vitro* plants were inoculated by covering the third leaf from the growth tip with a droplet of the spore suspension, using a syringe. Plants were kept at 25°C. Twelve plants were inoculated with *G. citricarpa* and 12 with *G. mangiferae*. After 4 weeks, 15 isolations were made from the inside of the stem, after surface sterilising the surface, to determine if the infection was systemic.

In vivo inoculation. Twenty Eureka and Valencia fruit grown *in vivo* were each inoculated with spores of these two organisms by placing 100 µℓ of the spore suspension on the fruit surface and covering the drop with wet cotton wool. The wet cotton wool was secured by masking tape. The entire fruit was then covered with a plastic bag, with a ball of wet cotton wool inside, and then covered with a paper bag. The bags were secured at the fruit stem with staplers. After 48 hours, the bags and cotton wool as well as the masking tape with the cotton wool were removed and the fruit covered with a semi-transparent waxed bag. A circle was drawn around the position of the inoculation (Prof. J.M. Kotzè, personal communication). The Valencia fruit were on trees in the orchard and the Eureka trees were in pots in a shade cloth structure. The fruit was harvested when mature and kept at room temperature. They were evaluated weekly for any black spot symptoms.

Three-year-old Eureka Lemon trees in pots were inoculated (2/11/05) by spraying the entire plant with the spore suspensions and covering the trees with plastic bags. Four trees were inoculated with *G. citricarpa* and four with *G. mangiferae*. These trees were kept in a controlled environment at 25°C for 3 days. The bags were removed and the plants were then kept in a shade cloth structure. The fruit were harvested at the end of the season and evaluated weekly for symptom development. Isolations were made from symptoms on the fruit. Isolations were also made from 25 surface sterilised leaves of each plant to determine if infection took place. Leaves were also collected, put in grids and left to deteriorate. When the leaves were at stage 3 (QMS rating) grids were evaluated in an ascospore monitor (Truter *et al.,* 2004).

Results and discussion

In vitro inoculation. The *in vitro* plants inoculated with *G. citricarpa* developed chlorosis of the leaves 14 days after inoculation. The infected leaves often abscised and where it fell on the medium, a colony of *G. citricarpa* developed. In two cases, the leaves stayed attached and pycnidia developed on the small leaf (Figures 4.3.5.1 and 4.3.5.2). Five plants were still alive after 4 weeks from which isolations could be made. The following ratio *G. citricarpa* was isolated from the plants: 0/15; 7/15; 7/15; 8/15 and 11/15. The cultures had a typical *G. citricarpa* growth pattern on medium and produced pycnidiospores in culture. These plants were so small that after isolation no plant tissue was left to treat for ascospore development as described by Kotzè (2005).

Due to the high incidence of bacterial contamination in the *in vitro* cultures, inoculation of the *in vitro* plants was repeated four times. Inoculation with the *G. mangiferae* was not successful. One week after inoculation, colonies of *G. mangiferae* started to develop on the surface of the multiplication media. The plants did not develop any symptoms, but after 3 weeks they started to develop chlorosis and by then the whole surface of the medium was colonised by the fungus. The inoculated leaves did not develop any symptoms. In the fourth trial, 3 plants were still alive and without any bacterial contamination after 4 weeks, they were slightly yellow but showed no symptoms. Only one isolation from the stem tissue yielded *G. mangiferae*. As *G. mangiferae* colonised the multiplication medium in the tubs, re-inoculation as planned, of these plants with *G. citricarpa,* was not performed.

In vitro inoculation. The fruit inoculated with the spores and covered with plastic bags developed no symptoms. A third of the fruit was lost due to abscission. The surfaces of the fruit had a russet/burned appearance.

From one plant that was inoculated with *G. citricarpa*, one fruit developed a single lesion and from the other plant 3 fruit developed 2, 4 and 6 lesions respectively. Isolations from these lesions yielded *G. citricarpa* colonies. Isolations from the leaves yielded one *G. citricarpa* colony from one leaf each from the two trees which had no fruit that developed black spot symptoms. No *G. mangiferae* could be isolated from any leaves from the plants inoculated with *G. mangiferae.* No spores could be detected from any leaves, from the inoculated trees, with the spore monitor.

Conclusion

G. citricarpa was able to infect leaves that were inoculated on *in vitro* seedlings. Infection with *G. mangiferae* was not successful. Final conclusions can only be made after the completion of the following project: "The infection of citrus plants with *G. citricarpa* and *G. mangiferae*" (QMS07/SS2).

The inoculation of trees with *G. citricarpa* spore suspensions resulted in the development of a few lesions on the fruit with pycnidia. This technique can be used to test fruit age resistance to *G. citricarpa*.

We were not able to determine if *G. mangiferae* can protect trees from infection. As *G. citricarpa* colonised the multiplication medium in the tubs, re-inoculation of these plants with *G. mangiferae* was not performed. No *G. mangiferae* could be re-isolated from trees inoculated with *G. mangiferae* spores. Other inoculation techniques, for example the use of invasive methods such as colonised twigs forced into live tree tissue, can be investigated.

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Figure 4.3.5.1. *In vitro* cultured seedling with a leaf attached which developed *Guignardia citricarpa* pycnidia. Isolations from the stem tissue yielded *G. citricarpa.*

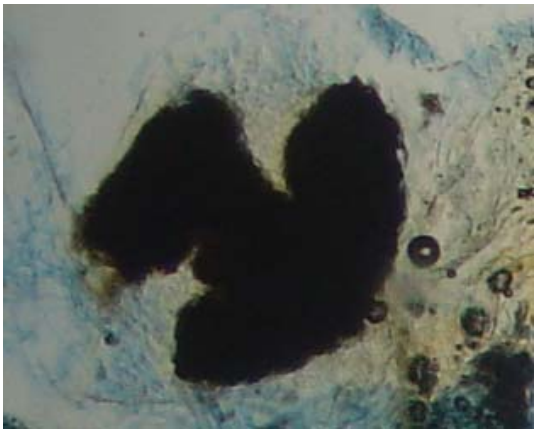

Figure 4.3.5.2. *G. citricarpa* pycnidium with pycnidio spores, which developed on a leaf of the inoculated *in vitro* plant.

4.3.6 Further developments of spray programmes consisting of registered fungicides in tank mixtures with Sporekill for the control of citrus black spot

Experiment 799 by G.C. Schutte (CRI)

Opsomming

Daar is 'n voortdurende behoefte vir die registrasie van nuwe swamdoders vir die beheer van swartvlek. Proewe die afgelope paar jaar het getoon dat Sporekill in kombinasie met kontakswamdoders uitstekend gewerk met gevolglike registrasie van Sporekill. Die doel van hierdie proewe was om verdere mengsels te beproef vir swartvlek beheer. Sporekill het in twee veldproewe getoon dat dit gebruik kan word as plaasvervanger vir minerale spuitolie in die geregistreerde tenkmengsel van Ortiva met mancozeb. Waar die dosisse van Sporekill verhoog is van 100 mℓ/hℓ water na 200 mℓ/hℓ water in kombinasie met óf mancozeb óf koper-oksichloried (teen gehalveerde dosisse van 100 g/hℓ water), het die laasgenoemde Sporekill dosis geen verhoging in die effektiwiteit vir die beheer van swartvlek tot gevolg gehad nie. Die tankmengsel van mancozeb (50 g/hℓ) met koper-oksichloried (50 g/hℓ) en met Sporekill (100 mℓ/hℓ) het nie te sleg gewerk teen swartvlek nie, maar mengprobleme is wel ondervind. 'n Nuwe DDAC het ook goeie beheer van swartvlek gegee. Geen fitotoksisiteitsprobleme of enige stippelvorming as gevolg van die opeenvolgende toedienings van koperswamdoders is in enige van die proewe ondervind of waargeneem nie.

Introduction

Results from field trials the past three seasons were very promising where a sanitising agent, Sporekill [a patented didecyldimethylammonium chloride (DDAC) formulation], was evaluated with mancozeb and copper fungicides and both fungicides were used at half their registered rates. In the two field trials conducted in the 2003/2004 and the 2004/2005 seasons, the latter combinations yielded between 95 – 99% clean exportable fruit. For registration purposes, the trials needed to be repeated at different sites and using 2x rates and combinations with mancozeb and copper oxychloride as well. The aim was also to reduce the copper and mancozeb rates even further from 100 g/hℓ water to 50 g/hℓ water and hopefully to reduce the possibility of stippling development even further if Sporekill can replace mineral spray oil in tank mixtures with mancozeb and strobilurins. A new DDAC product was also evaluated in the same orchards.

Materials and methods

Two orchards were selected in the Nelspruit region. One was at Crocodile Valley Citrus Co. and the other at Friedenheim Estates. A randomised block design with 5 and 3 single-tree plots per treatment, respectively, was used. Fungicides were applied using a trailer-mounted, high-volume, high-pressure (2,500-3,000 kPa) sprayer with two hand-held spray guns. Each treatment was replicated five and three times, respectively, in single-tree plots arranged in a randomised block design. Guard trees were assigned between plots within rows. Trees in both groves were selected for uniformity in canopy density and tree size. Spray volumes varied according to the size and canopy density of the tree but all trees were sprayed to the point of run-off. Currently, most commercial fungicide applications for CBS control in South Africa commenced in mid-October, which is based on research findings from ascospore releases and spore trap data (Kotzé, 1963; Kellerman & Kotzé, 1977). The standard registered mancozeb treatment commenced in mid-October, depending on the climatological information required for infection during the critical infection period.

Fungicides used for the trials were: Sporekill 120 g/ℓ SL DDAC from ICA International Chemicals, Ortiva 250 g/ℓ SC registered azoxystrobin from Syngenta, Mancozeb 800g/kg WP registered mancozeb from Unisun Mancozeb and Fynox 850 g/kg WP registered copper oxychloride from Agropharm.

(a) Sporekill as replacement of mineral spray oil in tank mixtures with Ortiva and mancozeb

The aim of this trial was to see if one can use the same registered rates for Ortiva and mancozeb but to use Sporekill instead of mineral spray oil.

(b) Evaluation of Sporekill at rates of 100 and 200 mℓ/hℓ in tank mixtures with reduced rates of mancozeb and copper oxychloride (100 g/hℓ)

The aim of this trial was to see if 2x rates (or 200 mℓ/hℓ water) of Sporekill is more effective than the 1x rate (or 100 mℓ/hℓ water) and to monitor any phytotoxicity.

(c) New DDAC product ("DDAC X")

The aim of this trial was to compare a new DDAC with Sporekill for the control of CBS.

At fruit maturity in July or August, CBS severity was rated on 100 fruit per tree according to a 3-point index (McOnie, 1964; Schutte, 1995) where 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. Data were analysed by ANOVA, using Fisher's LSD test ($P = 0.05$).

Results

(a) Sporekill as replacement of mineral spray oil in tank mixtures with Ortiva and mancozeb

Results from the field trial conducted at Crocodile Valley Citrus Co. show that there were no significant differences ($P > 0.05$) in clean exportable fruit (95.4 - 97.8%) between the standard registered mancozeb and copper oxychloride and all tank mixtures of Ortiva, mancozeb with either Sporekill or mineral spray oil. Concomitantly, all the treatments were significantly different from the control which resulted only in 31.2% clean exportable fruit, also showing that the disease pressure was extremely high during the test period. The same scenario was experienced with the criteria fruit with 1-3 lesions and fruit with 4 and more CBS lesions where all the treatments performed significantly better than the control. With regards to the criterion 4 and more CBS lesions, the untreated control resulted in 47.6% fruit with lesions, furthermore demonstrating the high disease pressure experienced the past season in this orchard (Table 4.3.6.1).

Results from the field trial conducted at Friedenheim Estates show that there were no significant differences ($P > 0.05$) between the standard registered mancozeb and copper oxychloride and all tank mixtures of Ortiva, mancozeb with either Sporekill or mineral spray oil with regards to the criteria clean exportable fruit (98.6 - 99.6%). Concomitantly, all the treatments were significantly different from the control which resulted only in 25.3% clean exportable fruit showing that the disease pressure was extremely high during the test period (Table 4.3.6.1). The same scenario was experienced with the criteria fruit with 1-3 lesions as well as the criteria with 4 and more CBS lesions were all the treatments performed significantly better than the control. With regards to the criterion 4 and more CBS lesions, the untreated control resulted in 61.4% fruit with lesions demonstrating the high disease pressure experienced the past season (Table 4.3.6.2)

(b) Evaluation of Sporekill at rates of 100 and 200 mℓ/hℓ in tank mixtures with reduced rates of mancozeb and copper oxychloride (100 g/hℓ)

Results from both field trials conducted at Crocodile Valley Citrus Co. and Friedenheim Estates show that there were no significant differences ($P > 0.05$) for for the criterion clean exportable fruit between either the standard registered Sporekill with mancozeb or copper oxychloride as well as the double rate of Sporekill with either mancozeb or copper oxychloride. The latter treatments were also not significantly different from the two standard treatments, mancozeb and copper oxychloride. All the treatments were significant different from the control, which resulted in 31.2% and 25.3% clean exportable fruit, respectively.

What is interesting is that the 2x rate of Sporekill (200 mℓ/hℓ) never outperformed the 1x of Sporekill (100 mℓ/hℓ) in both trials. The 1x Sporekill tank mixture with copper oxychloride resulted in 97.5% clean fruit at Crocodile Valley Citrus Co. while the 2x Sporekill rate in a tank mixture with copper oxychloride resulted in 95% clean exportable fruit. The same scenario was experienced with Sporekill and mancozeb resulting in 0.2% difference between the two treatments. At Friedenheim Estates, Sporekill and copper oxychloride

resulted in 97.6% for the 1x and 96.0% clean exportable fruit for the 2x rate, while Sporekill and mancozeb resulted in 97.6% for the 1x rate and 93.0% clean exportable fruit for the 2x rate. None of these treatments differed significantly with regards to all the criteria used for evaluation (Tables 4.3.6.3 & 4.3.6.4).

(c) New DDAC product ("DDAC X")

Results from both field trials conducted at Crocodile Valley Citrus Co. and Friedenheim Estates show that there were no significant differences ($P > 0.05$) for the criterion clean exportable fruit between the standard registered mancozeb and all tank mixtures of reduced mancozeb and copper oxychloride with Sporekill as well as with DDAC X and mancozeb. All the treatments were significantly different from the control, which resulted only in 31.2% and 25.3% clean exportable fruit, respectively.

At Crocodile Valley Citrus Co., the same scenario was experienced with the other criteria (fruit with 1-3 lesions as well as the criteria with 4 and more CBS lesions) and all the treatments performed significantly better than the control. Very high disease pressure experienced the past season as was demonstrated by 47.6% and 61.4% of the control fruit with 4 and more lesions at Crocodile Valley Citrus Co. and Friedenheim Estates, respectively. An interesting observation was that the copper oxychloride tank mixture with Sporekill at a rate of 100 g/hℓ water resulted in no stippling due the low rates of copper used (Tables 4.3.6.5 and 4.3.6.6).

Conclusion

(a) Sporekill as replacement of mineral spray oil in tank mixtures with Ortiva and mancozeb

Sporekill (100 mℓ/hℓ) was successfully mixed with Ortiva and mancozeb and two applications for the control of CBS resulted in between 95 to 99% clean exportable fruit from both trial sites. It compares favorable with the standard 4 mancozeb treatments. These are exceptional results as we export our citrus fruit mainly to the EU and Japan where there is a zero tolerance to the black spot fungus. These mixtures can be recommended for registration. No visual signs of phytotoxicity were noticed at any stage during the trial with the Ortiva, mancozeb and Sporekill tank mixture.

(b) Evaluation of Sporekill at rates of 100 and 200 mℓ/hℓ in tank mixtures with reduced rates of mancozeb and copper oxychloride (100 g/hℓ)

Sporekill at a rate of 200 mℓ/hℓ water (2x rate) did not perform better than the registered rate of 100 mℓ/hℓ water in combination with either mancozeb or copper oxychloride. The tank mixture of mancozeb (50 g/hℓ) plus copper oxychloride (50 g/hℓ) plus Sporekill (100 mℓ/hℓ) in 100 ℓ water was effective against CBS, but did show some mixing problems. If this can be solved, then it will be a new combination for future use. No phytotoxicity was observed with any of the tank mixtures of mancozeb or copper oxychloride with Sporekill.

(c) New DDAC product ("DDAC X")

DDAC X at a rate of 100 mℓ was successfully mixed with reduced rates of mancozeb (100 g/hℓ) for the control of CBS at both Crocodile Valley Citrus Co. and Friedenheim Estates resulting in 100% and 95.4% clean exportable fruit. No visual signs of phytotoxicity were noticed at any stage during the trial. Mancozeb + Sporekill and copper oxychloride + Sporekill resulted in 97.6% and 97.3% clean exportable fruit, respectively, which is also exceptional as we export our citrus fruit mainly to the EU and Japan where there is a zero tolerance to the black spot fungus. These mixtures can be recommended for registration.

Future research

More trial work is planned to look at other DDAC formulations for CBS control. A new formulation comprising of a combination of copper oxychloride and mancozeb is planned.

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Table 4.3.6.1. Evaluation of Sporekill in tank mixtures with Ortiva and mancozeb applied from October and January during 2005 and 2006 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Estates, Nelspruit, South Africa.

Treatment	Rate / hℓ water	Lesions/fruit [x]		
		Clean exportable fruit[x]	Fruit with 1-3 CBS lesions[x]	Fruit with 4+ CBS lesions[x]
Copper oxychloride [y]	200 g	97.8 a	1.4 a	0.8 a
Mancozeb [y]	200 g	97.8 a	0.6 a	1.6 a
Mineral spray oil + Ortiva + mancozeb [z]	250 mℓ + 20 mℓ + 150 g	96.0 a	1.4 a	2.6 a
Sporekill + Ortiva + mancozeb [z]	100 mℓ + 20 mℓ + 150 g	95.4 a	2.0 a	2.6 a
Control		31.2 b	21.2 b	47.6 b

[x] Means in each column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.
[y] Spray dates were 10 October 2005, 7 November 2005, 5 December 2005 and 3 January 2006.
[z] Spray dates were 7 November 2005 and 3 January 2006.

Table 4.3.6.2. Evaluation of Sporekill in tank mixtures with Ortiva and mancozeb applied from October and January during 2005 and 2006 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Friedenheim Estates, Nelspruit, South Africa.

Treatment	Rate / hℓ water	Lesions/fruit [x]		
		Clean exportable fruit[x]	Fruit with 1-3 CBS lesions[x]	Fruit with 4+ CBS lesions[x]
Copper oxychloride [y]	200 g	99.6 a	0.0 a	0.4 a
Sporekill + Ortiva + mancozeb [z]	100 mℓ + 20 mℓ + 150 g	99.0 a	0.3 a	0.7 a
Mancozeb [y]	200 g	99.0 a	0.3 a	0.7 a
Mineral spray oil + Ortiva + mancozeb [z]	250 mℓ + 20 mℓ + 150 g	98.6 a	0.0 a	1.4 a
Control		25.3 b	13.3 b	61.4 b

[x] Means in a column, based on 3 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.
[y] Spray dates were 11 October 2005, 8 November 2005, 6 December 2005 and 4 January 2006.
[z] Spray dates were 8 November 2005 and 4 January 2006.

Table 4.3.6.3. Evaluation of Sporekill at rates of 100 and 200 mℓ/hℓ in tank mixtures with copper oxychloride and mancozeb applied from October and January during 2005 and 2006 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Treatment[y]	Rate / hℓ water	Lesions/fruit		
		Clean exportable fruit[x]	Fruit with 1-3 CBS lesions[x]	Fruit with 4+ CBS lesions[x]
Copper oxychloride	200 g	97.8 a	1.4 ab	0.8 a
Mancozeb	200 g	97.8 a	0.6 a	1.6 a
Sporekill + copper oxy- chloride	100 mℓ + 100 g	97.6 a	2.4 ab	0.0 a
Sporekill + mancozeb	100 mℓ + 100 g	97.6 a	0.8 ab	2.6 a
Sporekill + mancozeb	200 mℓ + 100 g	97.4 a	1.2 ab	1.6 a
Sporekill + copper oxy-chloride	200 mℓ + 100 g	95.0 a	2.4 ab	2.6 a
Sporekill + mancozeb + copper oxychloride	100 mℓ + 50 g + 50 g	92.8 a	4.0 b	3.2 a
Control		31.2 b	21.2 c	47.6 b

[x] Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.
[y] Spray dates were 10 October 2005, 7 November 2005, 5 December 2005 and 3 January 2006

Table 4.3.6.4. Evaluation of Sporekill (didecyldimethylammonium chloride) at rates of 100 and 200 mℓ/hℓ in tank mixtures with and mancozeb applied from October and January during 2005 and 2006 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Friedenheim Estates, Nelspruit, South Africa.

Treatment[y]	Rate / hℓ water	Lesions/fruit		
		Clean exportable fruit[x]	Fruit with 1-3 CBS lesions[x]	Fruit with 4+ CBS lesions[x]
Mancozeb	200 g	99.6 a	0.4 a	0.0 a
Copper oxychloride	200 g	99.0 a	0.4 a	0.6 a
Sporekill + mancozeb	100 mℓ + 100 g	97.6 a	0.0 a	2.4 a
Sporekill + copper oxy- chloride	100 mℓ + 100 g	97.6 a	0.6 a	1.8 a
Sporekill + copper oxy-chloride	200 mℓ + 100 g	96.0 a	1.3 a	2.7 a
Sporekill + mancozeb + copper oxychloride	100 mℓ + 50 g + 50 g	96.0 a	1.0 a	3.2 a
Sporekill + mancozeb	200 mℓ + 100 g	93.0 a	1.6 a	5.4 a
Control		25.3 b	13.3 b	61.4 b

[x] Means in a column, based on 3 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.
[y] Spray dates were 11 October 2005, 8 November 2005, 6 December 2005 and 4 January 2006.

Table 4.3.6.5. Evaluation of DDAC X in tank mixtures with mancozeb applied from October and January during 2005 and 2006 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Estates, Nelspruit, South Africa.

Treatment[y]	Rate / hℓ water	% Lesions/fruit		
		Clean exportable fruit[x]	Fruit with 1-3 CBS lesions[x]	Fruit with 4+ CBS lesions[x]
Sporekill + copper oxychloride	100 mℓ + 100 g	98.4 a	1.4 a	0.2 a
Mancozeb	200 g	97.8 a	1.4 a	0.8 a
Sporekill + mancozeb	100 mℓ + 100 g	97.6 a	0.8 a	1.6 a
DDAC X + mancozeb	100 mℓ + 100 g	95.4 a	2.0 a	2.6 a
Control		31.2 b	21.2 b	47.6 b

[x] Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.
[y] Spray dates were 10 October 2005, 7 November 2005, 5 December 2005, 3 January 2006

Table 4.3.6.6. Evaluation of DDAC X in tank mixtures with mancozeb applied from October and January during 2005 and 2006 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Friedenheim Estates, Nelspruit, South Africa.

Treatment[y]	Rate / hℓ water	% Lesions/fruit		
		Clean exportable fruit[x]	Fruit with 1-3 CBS lesions[x]	Fruit with 4+ CBS lesions[x]
DDAC X + mancozeb	100 mℓ + 100 g	100.0 a	0.0 a	0.0 a
Mancozeb	200 g	99.0 a	0.3 a	0.7 a
Sporekill + mancozeb	100 mℓ + 100 g	97.6 a	0.0 a	2.4 a
Sporekill + copper oxychloride	100 mℓ + 100 g	97.3 a	0.6 a	2.1 a
Control		25.3 b	13.3 b	61.4 b

[x] Means in a column, based on 3 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.
[y] Spray dates were 11 October 2005, 8 November 2005, 6 December 2005, 4 January 2006.

4.3.7 **Evaluation of a guanidine fungicide, new copper and mancozeb formulations and a surfactant for the control of citrus black spot on Valencias**

Experiment 837 by G.C. Schutte (CRI)

Opsomming

Daar is 'n voortdurende behoefte vir die registrasie van nuwe swamdoders vir die beheer van swartvlek. Swamdoders wat effektief was teen soortgelyke siektes soos swartvlek wat registrasie op ander gewasse gekry het, is beproef. Proewe die afgelope paar jaar het getoon dat vier bespuitings van Dodine teen dosisse van 80 mℓ/hℓ water en 200 mℓ/hℓ water van die 400 en 200 SL formulasies het goeie beheer van swartvlek gegee. 'n Nuwe koperhidroksied formulasie van Du Pont teen dosisse tussen 88 tot 263 g/hℓ water het goeie beheer van swartvlek gegee. Koper kan as plaasvervanger vir mancozeb gebruik in tenkmengsels met Cabrio, tensy dit maandeliks afgewissel word met mancozeb om sodoende stippelvorming te voorkom. Nuwe BP en BG mancozeb formulasies van Dow Agrosciences het ook goeie beheer van swartvlek gegee sonder enige newe-effekte. Wetcit, 'n nuwe benatter, kan ook in plaas van minerale spuitolie aangewend word vir swartvlekbeheer. Twee nuwe koperhidroksied (C30 en C40) as BG formulasies en 'n nuwe 420 SK formulasie van mancozeb het teen sekere eksperimentele dosisse ook goeie beheer van swartvlek gegee.

Introduction

There is an urgent need for new chemicals to control citrus black spot. The registration of new fungicidal groups are extremely difficult and we therefore need to look at new formulations of copper and mancozeb and how they can fit in with existing spray programmes without causing phytotoxic effects such as copper

stippling. Traditionally, mineral oil was added to mixtures for full cover sprays. However, due to cost and phytotoxical issues, alternatives to mineral oils need to be evaluated. The aims of this project was to evaluate a guanidine fungicide, new copper and mancozeb formulations and a surfactant for the control of citrus black spot on Valencias.

Materials and methods

Two orchards were selected in the Nelspruit region. One was at Crocodile Valley Citrus Co. and one at Friedenheim Estates. A randomised block design with 5 and 3 single-tree plots per treatment, respectively, was used. Fungicides were applied using a trailer-mounted, high-volume, high-pressure (2,500-3,000 kPa) sprayer with two hand-held spray guns. Each treatment was replicated five and three times, respectively, in single-tree plots arranged in a randomised block design. Guard trees were assigned between plots within rows. Trees in both groves were selected for uniformity in canopy density and tree size. Spray volumes varied according to the size and canopy density of the tree but all trees were sprayed to the point of run-off.

Currently, most commercial fungicide applications for CBS control in South Africa commence in mid-October, based on research findings from ascospore releases and spore trap data (Kotzé, 1963; Kellerman & Kotzé, 1977). The standard registered mancozeb treatment commenced in mid-October, depending on the climatological information required for infection during the critical infection period.

The fungicides to tested include mancozeb (Sancozeb 80% WP; Dow AgroSciences), guanidine (Dodine 100 & 400 SL; ICA Chemicals), copper hydroxide (524 g/kg WG; new test product from Du Pont), copper hydroxide (Copstar 180 g/ℓ SC; Agchem), copper hydroxide (Kocide 2000, 538 g/kg WG; Du Pont), copper oxychloride (Fynox 850 g/kg; Agropharm), pyraclostrobin (Cabrio 250 g/ℓ EC; BASF), mancozeb (Mancozeb 800 g/kg WP; Unisun) and two mancozeb formulations (Dithane 750 g/kg WG and 800 g/kg WP; Dow AgroSciences (SA)), a new mancozeb formulation (Pennfluid 420 g/ℓ SC; Total), two new copper hydroxide WG formulations (C30 and C40; Total) and the mineral spray oil, Citrex. Wetcit, a surfactant from Oro-Agri comprising of borax (10 g/kg) and orange oil (50 g/kg), was also included. The spray programmes evaluated are specified below.

At fruit maturity in July or August, CBS severity was rated on 100 fruit per tree according to a 3-point index (McOnie, 1964; Schutte, 1995) where 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. Data were analysed by ANOVA, using Fisher's LSD test (*P* = 0.05).

Results

a) Dodine

Results at Friedenheim Estates showed that there was no significant difference in the percentage clean exportable fruit between any of the treatments (Table 4.3.7.1). All these treatments were significantly different from the control that had only 25.33% clean exportable fruit. Apart from the control, treatment with the lowest rate of Dodine 100 evaluated (100 mℓ/hℓ) yielded the most fruit with 1-3 CBS lesions compared with the other treatments. This difference was significant for all treatments, except for Dodine 100 at 150 mℓ/hℓ. The latter treatment yielded the most fruit with four and more CBS lesions (7.66%), although the difference between fungicide treatments was not significant. Dodine 400 evaluated at a rate of 80 mℓ/hℓ water (or 3.2 g a.i.) was the best treatment of all the Dodine treatments tested.

Results from the trial site at Crocodile Valley Citrus Co. showed that the standard treatments (mancozeb and copper oxychloride) yielded markedly higher percentages clean exportable fruit compared with the Dodine 100 and 400 treatments (Table 4.3.7.1). These differences were significant for the Dodine 400 treatment at 100 and 60 mℓ/hℓ, and the Dodine 100 treatment at 150 and 100 mℓ/hℓ. However, all fungicide treatments were significantly different from the control that resulted in 31.20% clean exportable fruit.

If results from both trials are taken into account, Dodine 400 at 80 mℓ/hℓ water (or 3.2 g a.i.) was the best Dodine treatment. Dodine 100 at 200 mℓ/hℓ water (or 2 g a.i.) was the second best.

b) New copper formulation from Du Pont

Results from the field trial conducted at Crocodile Valley Citrus Co. (Table 4.3.7.2) show that there were no significant differences ($P < 0.05$) between the standard registered Copstar treatment, Kocide 2000, and all five rates of copper hydroxide tested. For the criterion clean exportable fruit, all the treatments were significant different from the control. Although not significant different from each other, there was a 5.6% difference in the amount of clean exportable fruit between the best copper hydroxide treatment applied at a rate of 263 g/hℓ water and the lowest rate of the same fungicide tested at a rate of 88 g/hℓ water. This aspect

is an important consideration for export to a zero tolerance market. No statistical significant differences were observed for the other two criteria between any of the fungicide treatments, but they were significantly different from the untreated control. No stippling was observed but some degree of accentuated wind blemishes did occur (Fig. 4.3.7.1).

c) Copper oxychloride as replacement for mancozeb in tank mixtures with Cabrio and mineral spray oil
Results from the field trial conducted at Crocodile Valley Citrus Co. show that there were no significant differences ($P < 0.05$) between the standard registered mancozeb and copper oxychloride treatments and spray programmes consisting of either mancozeb / Cabrio+mancozeb+oil / Cabrio+mancozeb+oil / mancozeb (4 applications) or Fynox / Cabrio+mancozeb+oil / Cabrio+Fynox+oil / mancozeb (4 applications) or Cabrio+Fynox+oil / Cabrio+mancozeb+oil / Cabrio+Fynox+oil (3 applications) for all the criteria used for evaluation. All treatments were sprayed during the susceptible period from October to January for CBS. All treatments differed significantly from the untreated control (Table 4.3.7.3).

d) DOW mancozeb

Results from the field trial conducted at Crocodile Valley Citrus Co. show that, for all the criteria used for evaluation, there were no significant differences ($P < 0.05$) between the standard registered mancozeb (4 applications) and the two mancozeb formulations (WP and WG) from Dow Agrosciences. No side-effects or phytotoxicity was observed with any of these formulations (Table 4.3.7.4).

e) New surfactant (Wetcit) as replacement for mineral spray oil
Results from the field trial conducted at Crocodile Valley Citrus Co. show that, for all the criteria used for evaluation, there were no significant differences ($P < 0.05$) between the standard registered mancozeb (4 applications) and spray programmes consisting of Ortiva+mancozeb+oil / Ortiva+mancozeb+oil (2 applications) and spray programmes that consisted included Wetcit at 100 or 25 mℓ/hℓ viz. Ortiva+mancozeb+Wetcit / Ortiva+mancozeb+Wetcit (2 applications). All these treatments were sprayed during the susceptible period from October to January for CBS. These treatments were, however, significantly different from the untreated control. Although there was no significant differences between the the treatments and the Ortiva+mancozeb+Wetcit programme at 25 mℓ/hℓ, the latter treatment yielded 10.2% fruit with CBS lesions, which is not acceptable for export markets that have a zero tolerance towards CBS (Table 4.3.7.5).

f) New copper hydroxide (WG) and mancozeb (SC) formulations
Results from the field trial conducted at Crocodile Valley Citrus Co. show that there were no significant differences ($P < 0.05$) between the standard registered mancozeb and copper oxychloride treatments and the C30 and C40 (copper hydroxide) treatments except for the C40 rate of 50 g/hℓ water. C40 (200 g/hℓ water) and C30 (400 mℓ/hℓ water) gave the highest percentage clean exportable fruit of 98.8% and 97.8%, respectively. These two treatments also resulted in the least fruit with four and more CBS lesions. There was a dosage response with all the C40 treatments except for the C40 rate of 50 g in a tank mixture with mineral spray oil. The mineral spray oil with 50 g of C40, resulted in 7.6% more clean exportable fruit, showing that mineral spray oil serves as a good spreader-sticker for this copper formulation. Although not significant different, C30 tested at the highest rate of 400 mℓ/hℓ water performed better than the lower rates of 100 g, 150 g and 200 g/hℓ water. This was also evident in the other criteria where C30 at the lowest rate of 100 mℓ/hℓ water resulted in the most fruit with four and more lesions. Pennfluid tested at a rate of 200 mℓ/hℓ water resulted in 96.6% clean exportable fruit, which was significant different from the lower rate of Pennfluid (100 mℓ/hℓ water) that resulted in only 88.8% clean exportable fruit. Similar observations were made for the criterion 4 and more lesions per fruit (Table 4.3.7.6).

Discussion

Dodine is an old guanidine compound developed by American Cyanamid Company in 1954 and was used as an eradicative and preventative foliar fungicide. The formulation can be used for protective control of apple scab, leaf spot, blossom brown rot and leaf blight in fruit cultivation. As a scab fungicide, Dodine has an excellent curative effect, but there is only a minor translocation to new growth in apple seedlings sprayed with ^{14}C-dodine. Dodine is one of the few multi-site action fungicides against which fungicide resistance developed in the field. Therefore, its combined or alternated use with other fungicides is recommended. Among the other control options for Dodine is that of the control of *Xanthomonas campestris,* the causal agent for bacterial canker of citrus (Lyr, 1995). If the latter do occur in South Africa in future, then this treatment will be effective for the control of both CBS and citrus canker. We therefore hope that we can get a set MRL for Dodine in future to be used for CBS control.

The new copper hydroxide formulation from Du Pont evaluated at rates of 88 g/hℓ water and higher was effective in controlling CBS and can be recommended for registration. No phytotoxicity in the form of stippling or concentric rings was evident on any fruit in any of the treatments but there was, however, darkening of wind blemishes which are normal with all copper treatments.

Copper oxychloride (Fynox) in tank mixtures with Cabrio and mineral spray oil were effective in a spray programme for the control of CBS where copper oxychloride (Fynox) alone at a rate of 200 g/hℓ water (October) was alternated with Cabrio + mancozeb + oil (November) and again with Cabrio + copper oxychloride (Fynox) + oil (December) and mancozeb. No phytotoxicity in the form of copper stippling or concentric rings was evident on any fruit in any of the treatments but darkening of wind blemishes was again observed.

After the blue Dithane fiasco at Letaba Estates during 2006, Dow AgroSciences (SA) wanted to prove that their two yellow formulations did not pose any problems on citrus for the control of CBS. It was indeed so and none of either the WP or the WG formulations had any side-effects on citrus trees and both controlled the disease very well.

The surfactant, Wetcit, in tank mixtures with Ortiva and mancozeb was effective in a spray programme for the control of CBS if sprayed at a rate of 100 mℓ/hℓ water. Where the Wetcit rate was lowered to 25 mℓ also in a tank mixture with Ortiva and mancozeb, the amount of infected fruit was too high and is not recommended as an effective rate for CBS control. No phytotoxicity was evident on any fruit in any of the treatments.

C40 at rates of 100 g/hℓ water and higher can be registered for CBS control. No phytotoxicity was observed on the fruit of C40, tested at the highest rate of 200 g/hℓ water (Fig. 4.3.7.2), nor C40 at a rate of 50 g/hℓ water with mineral spray oil. Concomitantly, no phytotoxicity was observed on the C30 treatments tested at its highest rate of 400 g/hℓ water either (Fig. 4.3.7.3). On the other hand, a lower rate of C40 (50 g/hℓ water) can also be recommended, but only in a tank mixture with mineral spray oil. C30 at a rate of 400 mℓ/hℓ water can also be recommended for registration against CBS. Pennfluid at a rate of 200 mℓ/hℓ water can also be recommended for registration for the control of citrus black spot on citrus.

Future research

More trial work is planned since it is possible that Dodine can perform better if it is used in tank mixtures with either copper or mancozeb. Further work with the new copper hydroxide formulation from Du Pont is planned while the copper tank mixture with Cabrio was handed in for registration.

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Table 4.3.7.1. Evaluation of two formulations of guanidine (Dodine 100 SC and 400 SC), mancozeb (Sancozeb) and copper oxychloride (Fynox) for citrus black spot (CBS) control on Valencia oranges at Friedenheim Estates and Crocodile Valley Citrus Co. during the susceptible period from October to January 2005 and 2006.

Treatments	Rate / hℓ water	Friedenheim Estates, Nelspruit[y]			Crocodile Valley Citrus Co., Nelspruit[z]		
		% Clean exportable fruit[w]	% Fruit with 1-3 CBS lesions[w]	% Fruit with 4+ CBS lesions[w]	%Clean exportable fruit[x]	% Fruit with 1-3 CBS lesions[x]	% Fruit with 4+ CBS lesions[x]
Copper oxychloride	200 g	100.00 a	0.00 a	0.00 a	97.80 a	0.60 a	1.60 ab
Mancozeb	200 g	97.00 a	0.66 a	2.34 a	97.80 a	1.40 a	0.80 a
Dodine 400	100mℓ	97.66 a	0.66 a	1.68 a	80.60 b	4.80 ab	14.60 bc
Dodine 400	80 mℓ	100.00 a	0.00 a	0.00 a	85.20 ab	5.80 ab	9.00 abc
Dodine 400	60 mℓ	96.34 a	0.66 a	3.00 a	74.40 b	9.00 b	16.60 c
Dodine 100	200 mℓ	97.00 a	0.66 a	2.34 a	84.60 ab	7.40 b	8.00 abc
Dodine 100	150 mℓ	90.00 a	2.34 ab	7.66 a	79.00 b	4.40 ab	16.60 c
Dodine 100	100 mℓ	93.33 a	4.00 b	2.67 a	74.60 b	7.40 b	18.00 c
Control		25.33 b	13.34 c	60.33 b	31.20 c	21.20 c	47.60 d

[w] Means in a column, based on 3 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.
[x] Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.
[y] Spray dates were 12 October 2005, 9 November 2005, 7 December 2005, 4 January 2006
[z] Spray dates were 10 October 2005, 7 November 2005, 5 December 2005, 2 January 2006

Table 4.3.7.2. Evaluation of a new copper hydroxide formulation (Du Pont) applied from October to January during 2005 and 2006 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Treatment [y]	Rate / hℓ water	% fruit		
		Clean exportable fruit[x]	Fruit with 1-3 CBS lesions[x]	% Fruit with 4+ CBS lesions[x]
Copstar	350 mℓ	97.4 a	0.8 a	1.8 a
Kocide 2000	150 g	96.8 a	1.8 a	1.4 a
Copper oxychloride	200 g	97.8 a	0.6 a	1.6 a
Copper hydroxide	263 g	99.0 a	0.8 a	0.2 a
Copper hydroxide	219 g	97.0 a	1.4 a	1.6 a
Copper hydroxide	131 g	96.2 a	2.6 a	1.2 a
Copper hydroxide	175 g	94.4 a	3.4 a	2.2 a
Copper hydroxide	88 g	93.4 a	2.2 a	4.4 a
Control		31.2 b	21.2 b	47.6 b

[x] Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.
[y] Spray dates were 10 October 2005, 7 November 2005, 5 December 2005, 2 January 2006.

Table 4.3.7.3. Evaluation of copper oxychloride in tank mixtures with Cabrio and mineral spray oil applied from October to January during 2005 and 2006 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Treatment	% fruit		
	Clean exportable fruit[w]	Fruit with 1-3 CBS lesions[w]	% Fruit with 4+ CBS lesions[x]
Mancozeb [x] (200 g)	97.8 a	1.2 a	1.0 a
Fynox [x] (200 g)	97.8 a	0.6 a	1.6 a
Mz / Cabrio+Mz+oil / Cabrio+Mz+oil / Mz [z] (200g / 10 mℓ + 150g + 250 mℓ / 10 mℓ + 150 g + 250 mℓ / 200 g)	99.6 a	0.4 a	0.0 a
Mz / Cabrio+Cu+oil / Cabrio+Mz+oil / Cu[z] (200g / 10 mℓ + 150g + 250 mℓ / 10 mℓ + 150 g + 250 mℓ / 200 g)	96.4 a	1.8 a	1.8 a
Cabrio+Cu+oil / Cabrio+Mz+oil / Cabrio+Cu+oil [y] (10 mℓ + 150g + 250 mℓ x3)	97.8 a	1.4 a	0.8 a
Control	31.2 b	21.2 b	47.6 b

[w] Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.
[x] Spray dates were 10 October 2005, 7 November 2005, 5 December 2005, 3 January 2006.
[y] Spray dates were 10 October 2005, 21 November 2005 and 3 January 2006
[z] Spray dates were 10 October 2005, 7 November 2005, 19 December 2005 and 3 January 2006

Table 4.3.7.4. Evaluation of mancozeb 750 WG and mancozeb 800 WP applied from October to January during 2005 and 2006 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Treatment [y]	Rate / hℓ water	% fruit [x]		
		Clean exportable fruit[x]	Fruit with 1-3 CBS lesions[x]	% Fruit with 4+ CBS lesions[x]
Mancozeb (Standard)	200 g	97.8 a	1.4 a	0.8 a
Mancozeb 800 WP	200 g	97.2 a	2.2 a	0.6 a
Mancozeb 750 WG	200 g	95.2 a	2.0 a	2.8 a
Control		31.2 b	21.2 b	47.6 b

[x] Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.
[y] Spray dates were 10 October 2005, 7 November 2005, 5 December 2005, 3 January 2006.

Table 4.3.7.5. Evaluation of Wetcit in tank mixtures with Ortiva and mancozeb applied from October and January during 2005 and 2006 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Treatment	% fruit		
	Clean exportable fruit[x]	Fruit with 1-3 CBS lesions[x]	% Fruit with 4+ CBS lesions[x]
Macozeb (200 g) [y]	97.8 a	1.4 a	0.8 a
Ortiva+mancozeb+mineral spray oil (10 mℓ + 150g + 250 mℓ)[z]	96.0 a	1.4 a	2.6 a
Ortiva+mancozeb+Wetcit (10 mℓ + 150g + 100mℓ)[z]	98.8 a	0.8 a	0.4 a
Ortiva+mancozeb+Wetcit (10 mℓ + 150g + 25 mℓ) [z]	89.8 a	3.2 a	7.0 a
Control	31.2 b	21.2 b	47.6 b

[x] Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.
[x] Spray dates were 10 October 2005, 7 November 2005, 5 December 2005, 3 January 2006.
[z] Spray dates were 7 November 2005 and 3 January 2006.

Table 4.3.7.6. Evaluation of C30, C40 and Pennfluid from Total applied from October and January during 2005 and 2006 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., orchard Brits 1, Nelspruit, South Africa.

Treatment [y]	Rate / hℓ water	% fruit [x]		
		Clean exportable fruit[x]	Fruit with 1-3 CBS lesions[x]	% Fruit with 4+ CBS lesions[x]
Mancozeb	200 g	97.8 ab	1.4 ab	0.8 a
Copper oxychloride	200 g	97.8 ab	0.6 a	1.6 a
Pennfluid	200 mℓ	96.6 ab	2.4 abc	1.0 a
Pennfluid	100 mℓ	88.8 c	4.0 abc	7.2 b
C40	200 g	98.8 a	0.8 a	0.4 a
C40	100 g	96.4 ab	2.6 abc	1.0 a
C40	75 g	95.8 ab	3.2 abc	1.0 a
C40	50 g	88.6 c	4.4 bc	7.0 b
C40 + mineral spray oil	50 g + 250 mℓ	96.2 ab	1.2 ab	2.6 ab
C30	400 g	97.8 ab	1.4 ab	0.8 a
C30	200 g	91.6 bc	5.8 c	2.6 ab
C30	150 g	94.8 abc	3.4 abc	1.8 ab
C30	100 g	92.6 abc	2.6 abc	4.8 ab
Control		31.2 d	21.2 d	47.6 c

[x] Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

[y] Spray dates were 10 October 2005, 7 November 2005, 5 December 2005, 3 January 2006.

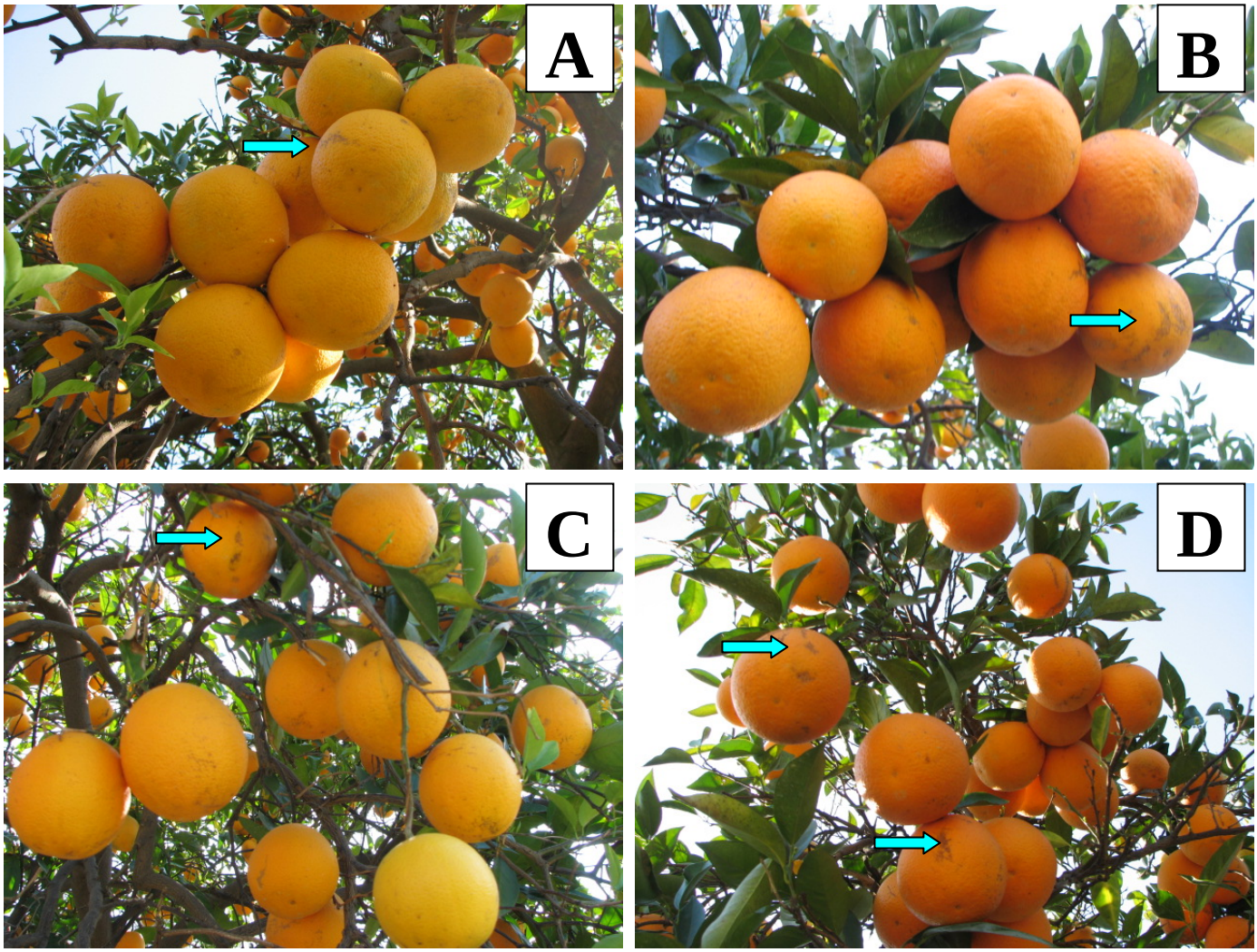

Fig 4.3.7.1. New formulation of copper hydroxide applied at different rates (A = 88 g/hℓ water; B = 175 g/hℓ water; C = 219 g/hℓ water; D = 263 g/hℓ water) showing good CBS control, no stippling but some degree of accentuating of wind blemishes (blue arrows).

Fig. 4.3.7.2. No phytotoxicity was observed on the stylar-end side of 'Valencia' oranges of the C40 treatment at the highest rate of 200 g/ hℓ water. No copper residues are visible on the fruit.

Fig. 4.3.7.3. No phytotoxicity was observed on the stylar-end side of 'Valencia' oranges of the C30 treatment tested at the highest rate of 400 g/hl water while copper residues are still visible on the fruit (see white arrows).

4.3.8 **Determining when fruit becomes resistant to black spot infection with increasing maturity**
Experiment 2006/CBS 6a by S.H. Swart, S. Serfontein and V. Phalanndwa (QMS)

Opsomming

Om vas te stel wanneer vrugte bestand raak teen swartvlekinfeksie, is verskeie proewe op Letaba Landgoed, naby Tzaneen, in die Limpopo provinsie, uitgevoer. Die een proef is gebaseer op infeksie met piknidiospore van *Guignardia citricarpa* gedurende Februarie en Maart 2006. In 'n tweede proef is gedeeltelik ontbinde sitrus blare, wat askospore en piknidiospore van *Guignardia* spp. bevat het, as inokulum gebruik vroeg en laat in Februarie en weer in Maart 2006. Die persentasie vrugte met swartvlek simptome was laag tydens beide evaluasies, en resultate kon nie aandui of vrugte wel meer bestand teen infeksie geraak het met toename in ouderdom nie. Resultate het wel gewys dat *Guignardia* spp. sitrus vrugte kon besmet in Maart in die Limpopo provinsie. In die derde proef is staatgemaak op natuurlike infeksie, waar vrugte vir spesifieke periodes onbeskermd gelaat is deur weglaat van sekere spuit toedienings met 'n voorkomende, kontak swamdoder. Resultate het getoon dat natuurlike infeksies vir 3% vrugte met letsels verantwoordelik was indien vrugte nie beskerm was vroeg in Maart 2006 nie. Ongeveer 1% vrugte met letsels is waargeneem indien vrugte nie laat gedurende Maart beskerm was nie. Dus, in hoë siektedruk areas, soos Letsitele en Letaba, is dit belangrik om boorde met 'n geskiedenis van swartvlek tot die einde Februarie, en soms selfs tot in Maart te beskerm, veral as laat vrugte na sensitiewe markte uitgevoer gaan word. In sekere gevalle sal 'n sesde rondte en selfs 'n sewende rondte swamdoder met beskermende eienskappe nodig wees. Dit hou egter groot ekonomiese implikasies in en daarom is dit belangrik om na ander meganismes te ondersoek om laat infeksies te beheer. Die vermindering van inokulum in boorde kan 'n belangrike effek op siektedruk in die algemeen hê. Bestuursprogramme moet ook deur epidemiologiese geskiedenis en spesifieke klimatoriese data vir die onderskeie produksie areas gebaseer en ondersteun word.

Introduction

Kotzé (1981) reported that ascospores, produced on dead leaves on the orchard floor, are the primary source of citrus black spot infection. The control of citrus black spot is mainly aimed at preventing fruit from getting infected. According to published data, citrus fruits are highly susceptible to infection by *Guignardia citricarpa* from fruit set, but become more resistant to infection as fruits mature (Kotzé, 1981; 1988, 1996). Popular belief is that fruits only need to be protected from October until the end of January in the Limpopo province. In the Letaba, Letsitele and Hoedspruit areas, ascospore discharges can occur throughout the year and normally peak between November and March, and even April (Swart, 2005; Swart *et al*., 2005). During the 2002/2003 and 2003/2004 seasons, high numbers of ascopores were discharged between December and March and climatic conditions were extremely favourable for infection during March of both seasons according to data recorded by QMS Agri Science. In these seasons, late hanging fruits (September and October) in several orchards in the Letsitele area showed high incidences of citrus black spot symptoms in spite of spray programmes that ensured protection from early October until the end of February. This raised some questions regarding current management strategies that are based on ontogenic resistance with increasing fruit maturity.

A trial was conducted during the 2004/2005 season in the Letsitele area to study the effect of omitting specific spray rounds during October 2004 and March 2006 on the occurrence of citrus black spot. The omission of late applications in February and March 2005 was included in the trial to investigate the status of acquired fruit resistance with increased maturity (Swart, *et al.,* 2005). There was only one infection period that was rated high during this season. The omission of an application scheduled for the 6th of December 2004 resulted in 9% infected fruit in this treatment. Very little disease was observed due to the omission of other spray rounds during this season because climatic conditions did not favour any early or late infections. Therefore, results obtained during this season were inconclusive regarding ontogenic resistance. Results did show that this is a useful and practical method to evaluate the effect of fruit age on resistance to infection by *G. citricarpa.*

The aim of this study was to determine if fruits do become more resistant to infection by *G. citricarpa* with increasing maturity by means of artificial infection with pycnidiospores. However, since ascospores are the major source of infection, an additional trial was conducted, using infected leaves containing mature ascospores. We also repeating the trial based on the omission of spray rounds under natural infection conditions.

Materials and methods

Several trials were conducted at Letaba Estates and in the Letsitele area, near Tzaneen, Limpopo Province. The one trial entailed artificial infection of citrus fruits with pycnidiospores of *G. citricarpa.* Fruits on 20-year-old Delta Valencia trees in an orchard at Letaba Estates were closed with paper bags on the 4th of January 2006. Inoculum of *G. citricarpa* was prepared on a medium containing 0.5% malt extract, 1.5% agar and pH set on 5.6, which was found to be conducive for production of pycnidiospores (Serfontein and Swart, 2005). Pycnidiospores were collected by flooding agar surfaces with sterile distilled water. The spore concentration was set on 5000 pycnidiospores / ml water, but it also contained spermagonia (micro conidia) and mycelium fragments that could not be counted. Due to problems with inoculum preparation, the January inoculation could not be performed in time, however, inoculations were done on the 8th of February and again on the 17th of March 2006. Fruits were inoculated with 120 µl (approximately 600 pycnidiospores) inside a 2 cm diameter circle, drawn with a permanent marker on each fruit. Droplets were covered with wet cotton wool, and kept in place with masking tape. Fruits were placed inside plastic bags to ensure high humidity and also covered with paper bags to reduce temperature build-up. Treatments consisted of 30 fruits for each infection period, replicated four times. Seven days after each inoculation, bags and cotton wool were removed. The paper bags were replaced to prohibit any further natural infections. All paper bags were removed in May 2006 when inoculum sources normally are low and climatic conditions are usually less favourable for infection. Fruits were harvested on the 10th of July 2006 when some black spot lesions became visible. Fruits were incubated at 25±1ºC in an ethylene-rich environment (5 ppm) with the RH maintained at 85%, in order to enhance ripening and citrus black spot lesion development. Inspection of fruit was done at weekly intervals for 4 weeks.

In the second trial, fruits were covered with paper bags on the 5th of January 2006. Partly decayed leaves (stage 3 according to QMS ratings) that tested positive for the presence of mature ascospores belonging to *Guignardia* spp., were collected on the floor of a naturally infected, unsprayed orchard at Letaba Estates. Leaves were placed between two plastic grids to cover an area of approximately 15 cm diameter (167 cm^2). Paper bags were removed from fruits and grids were placed above fruit in selected trees. Treatments consisted of 30 fruits per infection period, replicated four times. Rainy conditions were simulated with an overhead irrigation system for a period of 7 days, where after fruits were covered again with paper bags. Inoculations were done on the 6th of February, 21st of February and 22nd of March 2006. Fruits were uncovered on the 15th of May and picked on the 10th of July 2006. Fruits were incubated and inspected as described previously.

During the 2005/2006 season a trial similar to the omission-trial, conducted at Mahela, Letsitele during the 2004/2005 season (Swart *et al.*, 2005), was conducted at Letaba Estates. Fruits were protected every 23 days with a contact fungicide (mancozeb, applied at 200 g / 100 l water) from the middle of October 2005 to late in March 2006. Every 23 days a set of trees was left unprotected. Fruits were picked in September 2006 and the percentage infected fruit determined for each program.

Results and discussion

Four replications of 30 fruits each were artificially infected for each infection period. Between 90 and 100% of the fruit was recovered from the respective replicates for citrus black spot evaluations. Development of symptoms was slow in spite of artificial ripening with ethylene. The number of lesions that developed on fruits after artificial ripening and 4 weeks of incubation was used for analysis. At the time of evaluation, 16% of fruits inoculated in February 2006, had developed black spot lesions. Eleven percent of the fruit inoculated in March developed citrus black spot symptoms (Fig. 4.3.8.1). In spite of the fact that most fruits were recovered for evaluation purposes, the percentage fruit that developed black spot symptoms was very low. Artificial inoculation with pycnidiospores has been used successfully in other trials, and it is uncertain why the technique had limited success in this trial. The low infection rate might be associated with adverse conditions inside the plastic bags during the post-inoculation period, or inside the paper bags when fruit remained covered until May. Conditions might have been non-conducive to pathogen survival, infection, and/or disease manifestation. High temperatures often occur during January, February and March in the Letaba/Letsitele areas. Unfortunately we did not record of temperature fluctuations on covered fruit, but in future studies this factor should be included.

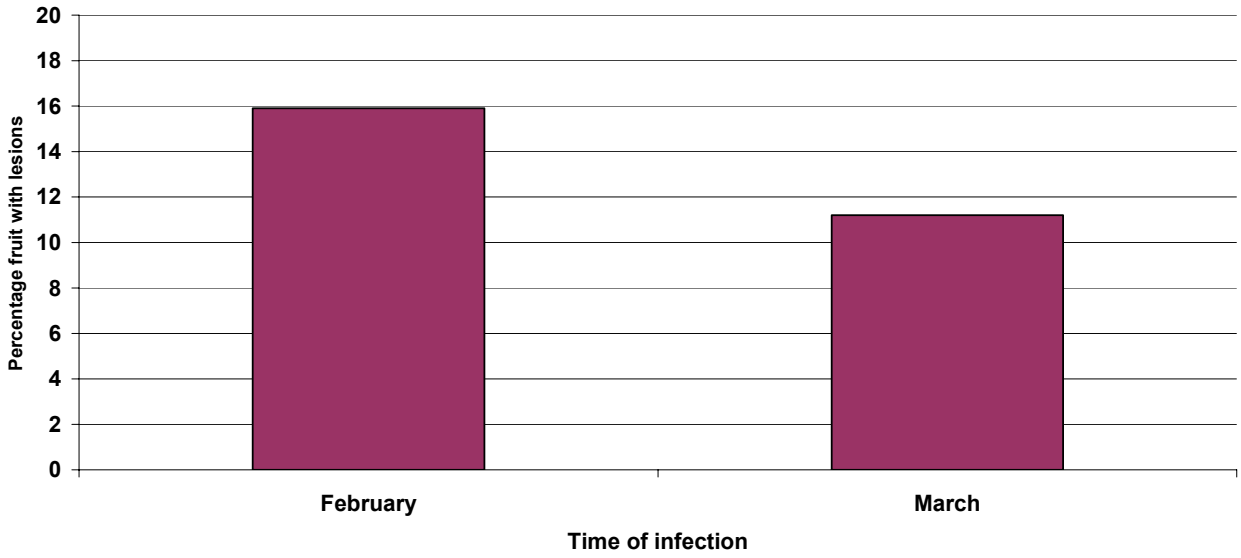

Figure 4.3.8.1. Percentage fruit with citrus black spot lesions after artificial infection with pycnidiospores

Results suggested that mature Valencia fruits can be infected artificially with pycnidiospores in February and March, and that the percentage successful infections tend to be lower during March. These results might be an indication that fruits do become more resistant to infection by pycnidiospores with increasing maturity. Several aspects such as the source of inoculum and low success rate of symptom development cloud the interpretation of results. In order to make sound recommendations regarding spray programmes, more elaborate trials are needed to determine the status of ontogenic resistance.

Artificial infection of fruits by using naturally infected decaying leaves included both pycnidiospores and ascospores as inoculum source. Analysis of leaf samples in an ascospore sampler, showed that leaf sources contained variable numbers of mature ascospores. Two sub-samples of grids containing leaves, used for the February infection process, had 22 and 398 spores per grid, respectively. Two sub-samples of grids with leaves, used for the March infection process, produced 14 and 340 ascospores, respectively. We can therefore assume that large variation existed in inoculum levels produced on the leaves that were used for artificial inoculation of fruits at different maturity stages. This variation also existed amongst replicates within the same treatments. Naturally infected leaves also contained other inoculum types, such as pycnidiospores and spermagonia (micro conidia). Natural infection of fruit, due to airborne ascospores during the 7 days of simulated rainy conditions, could also have contributed to some lesion development. However, in spite of several sources of inoculum, few fruits developed citrus black spot symptoms due to artificial infection during January and February 2006.

Results showed that fruits exposed to inoculum and infection conditions during March 2006 developed more black spot disease symptoms than those exposed early and later in February (Fig. 4.3.8.2). Natural infection probably did not have a significant effect on results in this trial because extremely conducive climatic conditions and large numbers of airborne ascospores have been recorded during all three infection periods (Fig. 4.3.8.3). There was no statistical difference regarding the percentage of fruit with black spot symptoms for the different infection periods, probably due to low levels of successful infections and variation within treatments. Nonetheless, results do suggest that Valencia fruits were still sensitive to infection by *Guignardia* spp. in March in the Limpopo province. It is, however, not possible to make sound detailed conclusions regarding ontogenic resistance. This question can only be answered if a method can be developed to produce pure cultures of ascospores of *G. citricarpa* that can be used for artificial inoculation of citrus fruits and leaves.

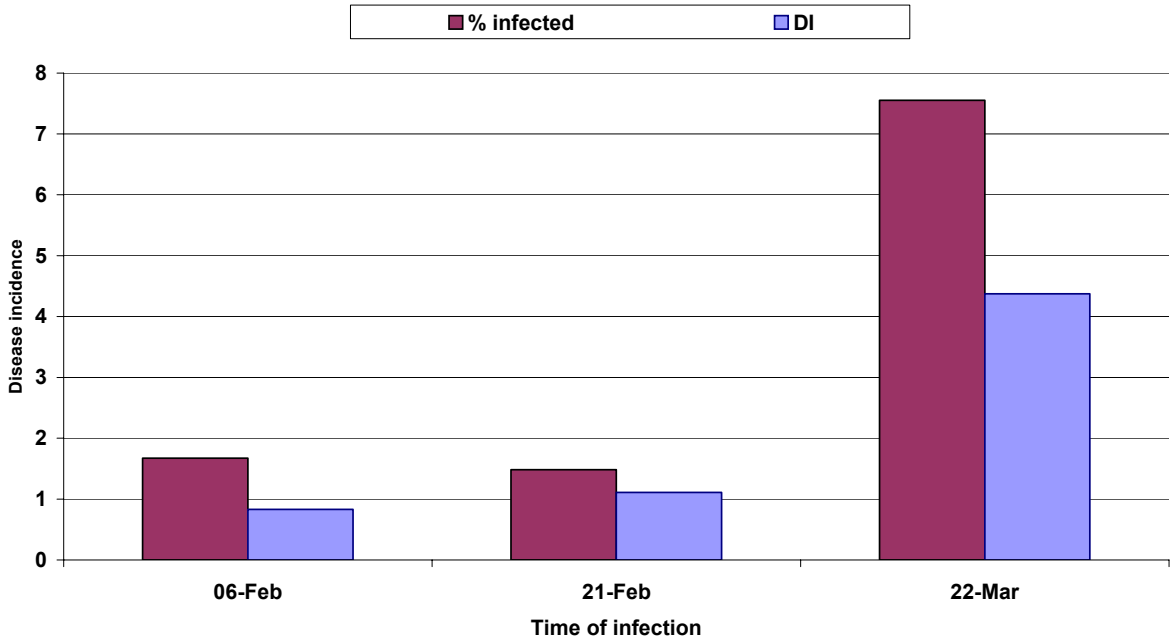

Figure 4.3.8.2. The percentage fruit with citrus black spot lesions after artificial infection using decaying leaves that tested positive for the ability to support ascospore production.

In the third trial, specific spray applications were omitted in order to determine the most important infection periods during the season and whether fruit do become more resistant with increasing maturity. Several infection periods were recorded during the 2005/2006 growing season (Fig. 4.3.8.3). The first part of the season was not very conducive to infection due to relatively dry conditions and low numbers of airborne ascospores. Results showed that the omission of a spray application on the 1st of December, just prior to the first extremely conducive infection period of the season (between 8 and 10 December 2005), resulted in more than 5% fruit with black spot lesions at harvest (Fig. 4.3.8.4). A second extremely conducive infection period was experienced between 6 and 12 January 2006. A spray application omitted on the 22nd of December 2006 resulted in more than 7% fruit with lesions. In spite of increasing infection potential with extremely conducive conditions from 5 to 12 February, 26 February to 7 March and again on 27 to 30 March, the effect of omitted spray applications reduced. The application omitted on the 7th of February only resulted in just more than 3% fruit with black spot lesions. These fruit was unprotected during three infection periods where climatic conditions were extremely conducive to infection while large numbers of airborne ascospores were trapped in orchards. Similarly, the application omitted on the 8th of March only resulted in just more than 1% fruit with lesions in spite of the fact that the previous spray interval was more than 30 days and an extremely conducive infection period was recorded between 27th and 30th of March 2006. Therefore, it can be concluded that in spite of increased disease pressure, the susceptibility of Valencia fruit decreased with increasing maturity. Fruit did not acquired full resistance but extremely conducive conditions during late February and early March did not cause more than 3% fruit with citrus black spot lesions at harvest. Closer to the end of March, only 1% of unprotected fruit developed black spot lesions at harvest.

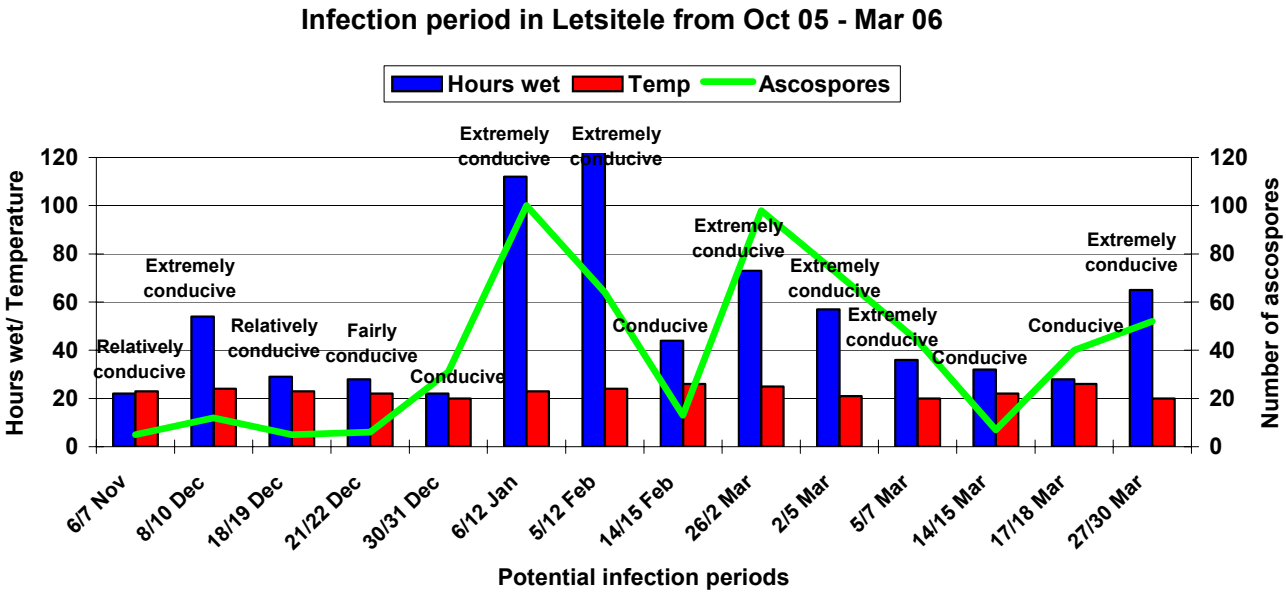

Figure 4.3.8.3. Infection periods in the Letsitele area based on climatic conditions and ascospore numbers during conducive periods as determined by the QMS citrus black spot monitoring protocol

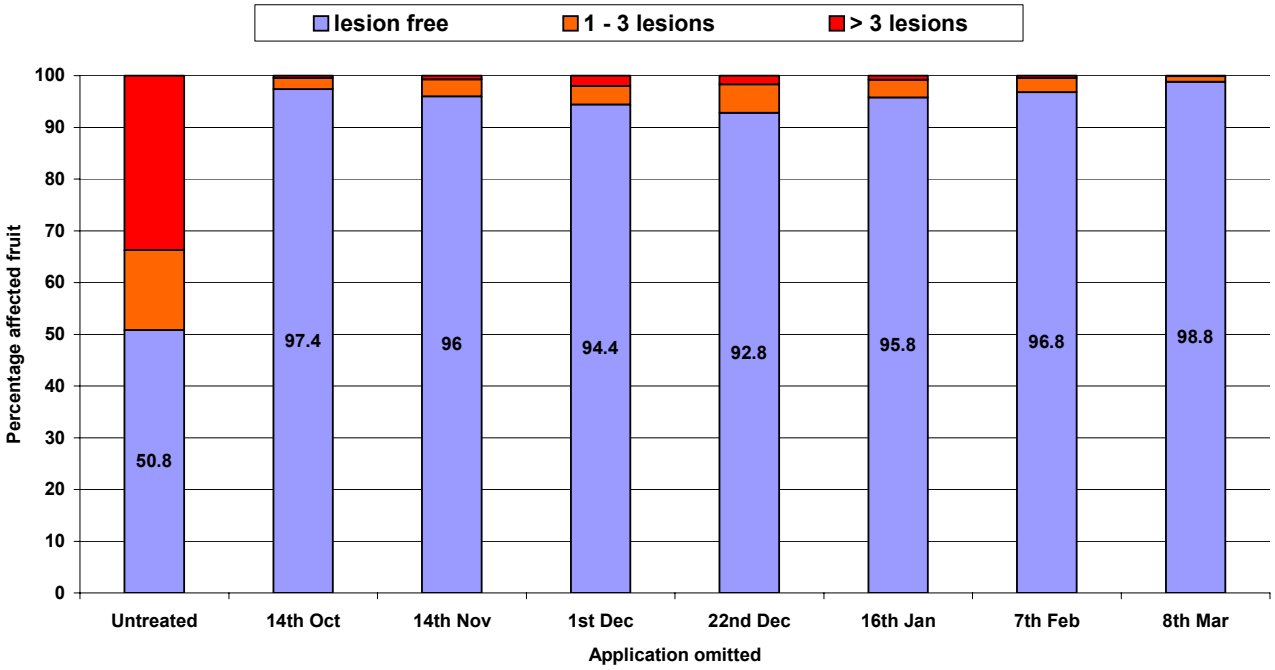

Figure 4.3.8.4. Percentage fruit with black spot lesions due to the omission of spray applications at specific dates

Conclusions

There are several shortcomings in artificial inoculation techniques with *Guignardia* spp. that can affect results. Pycnidiospores of *G. citricarpa* can be produced artificially, but the presence and role of spermagonia (micro conidia) and mycelium fragments is not known. Ascopores are the major source of inoculum, but have never been produced in abundance on artificial media. When infected leaves are used, the level of ascospore inoculum is variable and the presence of other sources of inoculum and *Guignardia* spp. is unknown. Both ascospores and pycnidiospores can be present and inoculum levels cannot be quantified for individual inoculation sites. As a consequence the results from the first two trials were not conclusive. It was nonetheless obvious that mature fruit were not completely resistant to infection in March.

Data obtained by omitting spray applications at specific times and utilising infection data based on climatic conditions and ascospore releases supported these findings as between 3% and 1% of fruit exhibited CBS lesions when fruit was not protected during early and late March. Ascospores are often recorded in high numbers during March and even April in citrus producing areas such as Letsitele, Letaba and Hoedspruit (Swart, 2005). Extreme weather conditions occasionally occur that can be conducive to infection during March. Therefore, in high disease pressure areas, such as Letsitele and Letaba, it is important to protect orchards that are prone to black spot disease development at least until the end of February, and sometimes until the middle of March, especially if late hanging fruit are destined for sensitive markets. In some cases a sixth and even a seventh round of protective fungicides would be necessary to protect fruit effectively for such a lengthy period. Certain markets have restrictions regarding the use of mancozeb after January and February, and other means of reducing late infections should be investigated. The reduction of inoculum pressure in orchards can possibly play an important role in order to reduce overall disease pressure. For this we need epidemiological history and specific data for different production areas. We also need a method to produce ascospores of *G. citricarpa* in order to do artificial infection studies to determine critical factors involved in infection, disease manifestation, symptom development and management of this disease.

Future research

- The effect of climatic conditions conducive to black spot infection on lemon fruit, where small and mature fruit can be simultaneously on trees during February.
- Evaluating the effect of spray programmes, aimed at reducing late infections during February and March, on citrus black spot levels.
- Developing spray programs for areas with low, medium and high disease pressure.
- Improving and expanding citrus black spot monitoring systems.
- The artificial production of *G. citricarpa* ascospores.

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4.3.9 Evaluation of leaf litter inoculum potential on the orchard floor as affected by the irrigation system (Preliminary report)

Experiment 2006/CBS 1 by S.H. Swart and V. Phalanndwa (QMS)

Opsomming

Die doel van die studie, wat in September 2006 in aanvang geneem is, was om te bepaal of besproeiingsisteme (drup vs. mikro) 'n effek op inokulum produksie deur *Guignardia* spp. kan hê met inagneming van spesifieke posisies onder die boom. Die studie is in twee boorde op Letaba Oranje, Letsitele, uitgevoer. Volwasse blare is vanaf 42-jarige Nawel bome, met 'n geskiedenis van *Giugnardia* besmetting, op Letaba Landgoed in September 2006 versamel. Blare is goed vermeng en tussen plastiek gaasvelle geplaas. Gaasvelle is ewekansig uitgekies en op spesifieke posisies in onderskeie boorde uitgeplaas (30 / boord): rondom die besproeiingspunt, halfpad tussen die stam en die drupkring in die rigting van die pad tussen rye, en weg van die besproeiing, waar 'n bome verwyder is. Elke posisie is vyf keer herhaal in elke boord en stelle gaasvelle is na 3 maande (Desember 2006), en na 5 maande (Februarie 2007), versamel. Blare is ook in November 2006 versamel en op dieselfde wyse hanteer as die wat in September versamel is. Die eerste versameling van gaasvelle met blare in hierdie groep het in Januarie 2007 plaasgevind en die volgende versameling sal in Maart 2007 wees om die effek van moontlike klimaatsverskille in te sluit. Blare is met 'n askospoormonitor ondersoek om die teenwoordigheid van askospore van *Guignardia* spp. te bevestig. Voorlopige resultate dui daarop dat daar groot verskille in die askospoorproduksievermoë van blare mag bestaan in mikro-klimate wat in boorde as gevolg van

verskillende besproeiingsstelsels ontstaan. Die mees ooglopende verskil tussen blare uit die verskillende boorde was die graad van afbraak wanneer blare uit boorde verwyder is. Blare uit boorde met mikrobesproeiing was baie verder verweer as blare uit boorde met drupbesproeiing. Indien verdere analises bogenoemde resultate ondersteun, behoort die metode van besproeiing 'n bepalende rol te speel in toekomstige strategieë om inokulumpotensiaal in boorde te bestuur.

Introduction

Citrus black spot is traditionally controlled by protecting fruits with fungicide applications from early October until the end of February. This is a labour intensive and costly process that cannot ensure 100% lesion free fruit, especially under high disease pressure conditions. Fungicide applications are very effective, however, occasionally some orchards produce fruits with lesions in spite of sound spraying programmes. With the current phytosanitary status of citrus black spot in EU, USA and Japan, we need to assess alternative methods that can contribute to disease control and that can possibly play an important role in a systems approach for disease management strategies.

The reduction of inoculum by targeting the inoculum source is a relatively new concept in citrus black spot control strategies that can be incorporated in a systems approach strategy. Results of the project where ascospore inoculum potential was studied over time, indicated that the macro-climate in an area, but also the micro-climate in an orchard can have an effect on the rate at which leaf litter deteriorate and therefore on production and availability of ascospore inoculum (Swart, 2005).

The aim of this project was to study the effect of microclimate under tree canopies, as influenced by the type of irrigation system that are used, on the production and availability of ascospore inoculum. The trials were designed to evaluate the effect of micro and drip irrigation systems and to determine the areas under the canopy where the majority of ascospore inoculum is produced.

Materials and methods

Mature leaves, from 42-year-old, unsprayed Navel trees at Letaba Estates, with a history of high levels of *Guignardia* infection, were sampled in September 2006. Leaves were mixed thoroughly and placed between two plastic grids. Grids were randomly selected and placed in orchards (30 / orchard) at set positions under the tree canopy. Grids were placed next to the irrigation point in the row, halfway between the trunk and the drip zone between rows, and in full sun at a distance from the irrigation source (where a tree has been removed). Each position was replicated five times in each orchard. The first set of grids was retrieved after 3 months (December 2006) and the last set after 5 months (February 2007).

Leaves were also sampled in November 2006, and treated in a similar manner to the leaves sampled in September. The first retrieval of grids for leaves sampled in November 2006 was in January 2007 and the last will be in March 2007 in order investigate the effect of different climatic conditions on ascospore development. Leaves were screened with an ascospore monitor for the presence of *Guignardia* ascospores.

Results and discussion

Preliminary results obtained from leaves picked in September and retrieved in December 2006 showed that in an orchard with micro irrigation, no ascospores could be found on leaves positioned next to the micro irrigation, 35 ascospores when positioned halfway between trunk and the drip line towards the road, and 108 ascospores if positioned in some sunlight where a tree has been removed (no irrigation). In a drip irrigation orchard, very high numbers of ascospore were produced on leaves positioned at the dripper (2000 to more than 10000 spores / slide). Similar numbers were observed halfway between the trunk and the drip line towards the road, while very low levels (1 ascospore) were found at a distance from the irrigation system where a tree was removed. Grids with leaves sampled in September 2006 and November 2006 must still be analysed.

These preliminary results suggest significant differences with regards to the potential to develop ascospores in the microclimate created under the canopies of trees with drip and micro irrigation systems. The most obvious difference between leaves retrieved from the two different irrigation systems was the level of leaf deterioration that occurred. Leaves obtained from orchards with micro irrigation systems were mostly in an advanced stage of decay, while those from drip irrigation orchards were still relatively intact.

Conclusions

Should results support this preliminary data, the means of irrigation might play a significant role regarding the choice of irrigation systems especially regarding black spot inoculum management strategies.

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Swart, S.H. 2005. The correlation between leaf drop and production of ascospore inoculum in citrus orchards. CRI Annual report 2005: 256 – 266.

4.3.10 **Reducing the inoculum potential in leaves by applying fungicides to citrus trees in the orchard (Preliminary Report)**

Experiment 2006/CBS 7 by S.H. Swart, W. van der Pypekamp and v. Phalanndwa (QMS)

Opsomming

Die doel van die projek was om die effek van chemikalieë met sistemiese of translaminêre werking, wat aan lewende sitrusblare op die boom toegedien is, op askospoor produksie deur *Guignardia* spp. op blare op die boordvloer te bepaal. Verskeie chemiese middels is voor vrugset op 31-jarige Valencia bome toegedien. Veertien dae na behandeling (vroeg in September), en weer in November en Desember, is ongeveer 300 blare van die binnekant van die vier middelste bome versamel. Met al drie geleenthede, is blare van 'n spesifieke behandeling goed vermeng en vier groepe plastiek gaasvelle, met ongeveer 25 blare tussen elke twee velle gemonteer, voorberei. Gaasvelle met blare is ewekansig gekies en in die boord onder sitrus bome uitgeplaas. Na twee maande is gaasvelle op 'n maandelikse basis vir 4 opeenvolgende maande uit die boord verwyder, waarna dit met die askospoor monitor ontleed is. Voorlopige resultate wys groot variasie tussen verskillende behandelings en herhalings van dieselfde behandeling, maar data word steeds ingesamel. Sodra alle data ontleed is, sal afleidings rakende die effek van chemiese behandelings op askospoorproduksie gemaak word.

Introduction

Standard spray programs for control of citrus black spot have been developed and tested over many years. Good results have been obtained and in some areas and black spot free fruit are exported without any problems. However, in certain areas citrus black spot is a major problem and elaborate spray programmes have to be applied each year, sometimes 6 to 7 applications per season. Therefore, controlling citrus black spot with fungicide spray programs is a major expenditure for the citrus industry.

Citrus black spot is traditionally controlled by protecting fruits with fungicide applications from early October until the end of February. This is a labour intensive and costly process that cannot ensure 100% lesion free fruit, especially under high disease pressure conditions. With the current phytosanitary status of citrus black spot in EU, USA and Japan, we need to assess alternative methods that can contribute to disease control and that can possibly play an important role in a systems approach for disease management strategies.

According to literature, the major source of inoculum is ascospores produced on infected leaves on the orchard floor. In order to break the life cycle of citrus black spot, or to reduce inoculum levels, leaves as the source of inoculum, should be targeted. The reduction of inoculum by targeting the inoculum source is a relatively new concept in citrus black spot control strategies that can be incorporated in a systems approach strategy. The aim of this project was to screen different fungicides with systemic or translaminar modes of action, for their ability to inhibit ascospore production when leaves have dropped to the orchard floor.

Material and methods

Several chemicals were applied on 17 August 2006 (before fruit set) to 8-tree-blocks randomly selected in a 31-year old Valencia orchard (Table 4.3.10.1). Each treatment was replicated twice. Fourteen days after treatment (early September 2006), approximately 300 mature leaves were picked from the middle four trees of each treatment block. Sampling of leaves was repeated in November and December. On each occasion, leaves from a specific treatment were thoroughly mixed and 4 grids per replicate were prepared by fixing approximately 25 leaves between two plastic grids. Grids were placed on the orchard floor and one grid per replicate was retrieved from the orchard on a monthly basis for 4 consecutive months, starting 2 months after being placed in the orchard.

Treatment no	Treatment description	Active ingredient	Formulation	Dosage (g or ml / 100 l)
1	Benlate	Benomyl + oil	500 g / kg, WP	50 + 300
2	Ortiva	Azoxystrobin + oil	250 g / l, SC	20 + 300
3	Rambo	Triadimefon/carbendazim	165/200 g / l, SC	35
4	Tilt	Propiconazole	250 g / l, EC	20
5	Ureum	Ureum		1010
6	Phytex	Potassium phosphonate	200 g / l, SL	1000
7	Mancozeb	Mancozeb	800 g / kg, WP	200
8	Cabrio	Pyraclostrobin + oil	250 g / l, EC	10 + 300
9	Flint	Trifloxystrobin + oil	250 g / l, WG	10 + 300
10	Control	-	-	-

Results and discussion

Results from leaves picked in September (14 days after treatment) showed that none of the treatments could reduce ascospore production on leaves analysed in November (2 months after being picked). The total number of spores for the two replicates of each treatment varied between 80 and 2160 ascospores. The least number of ascospores was produced on untreated leaves during this analysis (only 2 ascospores were counted). On grids picked up in December (3 months after being placed in the orchard), a slightly different pattern was observed with an increase in the number of ascospores produced on untreated leaves (86). The number of spores observed for treated leaves varied between 30 and 1057. On grids picked up in January some difference between treatments was also observed. Untreated leaves produced 218 spores in total, while the majority of leaves treated with strobilurin fungicides, only produced between 10 and 15 spores. Leaves treated with mancozeb and phosphonates produced the most ascospores with 1682 and 2187 spores, respectively. Leaves treated with propiconazole had no ascospores during this evaluation.

Grids with leaves picked in September 2006 that should be analysed in February 2007 must still be analysed, as well as grids with leaves picked in October and November 2006. The last sets of leaf samples will be retrieved from the orchard during March 2007. Once all data have been analysed, conclusions regarding the effect of chemical treatment of intact citrus leaves on ascospore inoculum production can be made.

4.3.11 Development of a reliable Citrus Black Spot (CBS) disease forecasting model for the South African citrus producer

Experiment CBS Forecasting PPL 15 by C.M. van Ginkel (UP)

Opsomming

Die waarskynlikheid dat sitrus-swartvlek (*Guignardia citricarpa* Kiely) infeksies sal plaasvind hang af van die beskikbaarheid van ryp askospore; die plotselinge styging in relatiewe humiditeit, temperatuur; die beskikbaarheid van vogtigheid en die periode wat hierdie vogtige kondisies voortduur op die oppervlak van die gasheer. Suksesvolle infeksie sal ook slegs plaasvind indien die oppervlak van die sitrusgasheer waarop hierdie askospoor land nie beskerm word deur ’n stof wat die askospoor dood of inhibeer nie.

Omgewingsinligting is *in situ* oor die afgelope vyf seisoene (2002-2007) in die Marble Hall sitrus-distrik ingesamel en in verband gebring met werklike spoorvrystellings en daaropvolgende effektiewe infeksies. Die beskikbaarheid van ryp inokulum vroeg in die seisoen is met behulp van die Kotzé Inokulum Monitor (KIM) bepaal.

In die ontwikkeling van die voorspellingsmodel word hierdie omgewingsinligting, tesame met die groeistadium van die gewas, en die beskerming deur chemiese agente verleen gekoppel aan ’n weervoorspellingsdiens gelewer deur die Nederlandse ***Dacom*** **maatskappy** via die **Plant-PLUS** rekenaarprogram.

Introduction

This study was initially started in the District of Marble Hall because the resistance of *Guignardia citricarpa* Kiely (CBS) towards the chemical active ingredient *benomyl* was still negligible in the area. By careful timing

and the elimination of unnecessary spraying the active life of this chemical (and others) could be prolonged. A cost saving would be an additional spin-off. Through the initiative of Mr. Anton Bredell (Lowveld Agrochem, Marble Hall) and the *Vallei-adviesdiens* a weather station network was established in order to facilitate the development of a Disease Forecasting Model for CBS. It was anticipated to extend this model to other citrus producing areas, once developed.

The possibility for CBS to successfully infect citrus fruit or -leaves is a function of the availability of ripe ascospores; the sudden rise in relative humidity; minimum temperature; moisture; and the duration of these conditions on the surface of host tissue. Furthermore, infection will only occur if the surface of the citrus host on which the ascospore lands is not protected by a fungicide or an inhibiting agent.

Environmental data was collected *in situ* over the past five seasons (2002-2007). The study focused on the citrus district of Marble Hall. This was correlated to real-time spore-trapping and the eventual development of disease symptoms on fruit and leaves. The availability of the first ripe inoculum early in the season was confirmed by the use of the Kotzé Inoculum Monitor (KIM).

In developing the disease forecasting model the following were integrated:

- The environmental data (as measured *in situ*);
- The growth stage of the citrus crop;
- Spore-trapping as measured in tandem with the environmental data;
- Protection rendered by applied chemical agents;
- A weather forecast service by the Dutch ***Dacom*** Company via the **Plant-PLUS** computer program.

Citrus producers in the district of Marble Hall that acted according to the guidelines presented over the past four seasons encountered few, if any, CBS blemishes on the packing lines for export. It was also possible for these producers to control the epidemic by two chemical sprays compared to three and even four preventative sprays that used to be the norm. The ultimate aim of the Disease Forecasting Model would be to accredit partaking orchards as being “Managed free of CBS”.

Materials and methods

The principal study area is the District of Marble Hall with a radius of approximately 90 km. This incorporates the areas of Loskopdam, Groblersdal, Marble Hall, Toitskraal and Nutfield. This District was covered by five ADCON Meso-climatic weather stations as well as five crop specific Disease stations; the latter incorporating Austrian leaf-wetness sensors. All stations transmit “raw” environmental data by radio telemetry to a central hub connected to a computer in Marble Hall, once every 15 minutes. Raw data include date, time, air temperature, precipitation, relative humidity, wind speed, wind direction, and solar radiation. In addition to these the government weather stations at Marble Hall and at Groblersdal serve as back-up.

This *in situ* data can be studied as a multiple climatological graph on the computer program ***Addvantage.*** This real-time data can be compared to actual *Guignardia citricarpa* Kiely ascospores trapped *in situ* by the Quest volumetric spore trap to an accuracy of 15-30 minutes linked events. Two Quest Volumetric Spore Traps are employed. Either a two- or an eight day (spray-vaseline covered) sampling disk is used. This disc interfaces with a standard light microscope (Nikon YS 100) for identifying and counting of the sample. Air sampling takes place at 20 litres of air per minute. The orchard chosen as benchmark for ascospore trapping is central to the District, has an established history of CBS and is therefore ideally suited as an early warning site for CBS ascospore releases. The GPS (Garmin 5) established co-ordinates for the disease station and spore-trap is: S 25° 3’ 38.9” and E 29° 16’ 55.7” at an elevation of 937m.

Photographic records of actual spores trapped, as well as observable infection symptoms on fruit are kept. Infection development time on fruit artificially inoculated by pycnidiospores will be compared to natural field infected symptom development.

Weather data from ***Addvantage*** is transmitted every 90 minutes by modem to the Dacom program, ***Plant-PLUS*** to generate an individualistic five day weather prediction for all five Meso-climatic weather stations. This weather prediction is supported by a United States of America government owned weather satellite and updates can be obtained by modem at 180 minute intervals. **It is essential to link this weather prediction to the Disease Forecast Model in order to have any meaningful timely decision support.** The five day forecast is simultaneously an indication of expected spraying conditions, -evapo-transpiration, and -irrigation requirement.

A KIM (Kotzé Inoculum Monitor) is utilized to confirm the presence of early inoculum in collected leaf litter as well as in chemically treated leaf samples. The possibility of integrating the parameters of the developed model into the existing Plant-PLUS disease forecasting software arsenal is being investigated.

Results and discussion

As this is a progress report, focus will be on some fresh observations that emerged from the elaborate amount of direct data as well as from related experiments that were undertaken to fill in blanks over these four seasons. The main events of the past four CBS seasons are attached as Figs. 4.3.11.1 to 4 at the end.

The presence of inoculum

The presence of ripe CBS perithecia on decomposing leaf litter in an orchard equals an orchard with a CBS epidemic. The KIM offered a reliable and quick way to test this. In a separate trial in an orchard 3 km downwind, CBS was completely removed as detectable disease within three years of thorough leaf-litter removal in September-October (Mr. Johan Meyer, Vaalfontein, PPL 12 experiment) and no CBS chemical spraying is necessary at present. Likewise it was found that a hail event in October, enhancing the available leaf-litter inoculum, will influence the inoculum availability twelve months later. The very early availability of ascospores in 2005 (16/09/2005 detected by KIM) could be attributed to this. The first natural ascospore release for 2005/6 was recorded on 19/10/2005. This is more in tune with the historical norm, albeit a week early.

Triggering ascospore release

The environmental conditions found to be associated with an ascospore release amounts to this:

- Temperature: Above 18°C. The minimum temperature for the day was never found to be below 17°C. This correlation is evident from Figs. 4.3.11.1-4. The highest temperature at which spores were trapped was 32°C.
- Relative Humidity: A sudden rise in relative humidity (typically 10% or more) triggered the actual spore release. This sudden *rise* is more important than a very high relative humidity. This could mean spore release before the advent of the actual rain event. This would enable the ascospores to utilize the typical pre-precipitation turbulent winds for dispersal. This sequence was confirmed by careful interpretation of the spore disk, linked to environmental conditions measured in real time.

The strength of an ascospore release

The reliability of a high ascospore release recording on the Quest volumetric spore trap is open to debate. However, a large amount of air is sampled by active suction (20 liters/minute) through the spore trapping apparatus. From data collected it was evident that:

- Spore releases at the start of the season were typically low or medium in strength.
- High releases could be correlated to high minimum temperatures for the day.
- At the end of the season spore release strengths would diminish as leaf-litter inoculum is decomposing. Environmental conditions are evidently influencing this.
- At the end of the season, spore catches would fade away and end as minimum day temperatures fall below 17°C. This would also signal the end of leaf infection (future inoculum) via ascospores, for the season.

The actual infection event

Specific detail is still being investigated. The time-span for infection (formation of penetration peg from appressorium) to occur is expected to be very similar to those found for infection by pycnidiospores (Shaw *et. al.* 1998). This work indicates a period of ideal conditions for infection lasting from 18-24 hours. I found this ideal infection conditions to occur more often than initially expected within the many niches that exist in an orchard citrus tree. The fact that the pycnidiospores, and most probably the ascospores too, require dryness on the substrate (a hydrophobic substrate) to bond effectively is very significant. Germination will not occur readily, if this bonding is jeopardized by either too much moisture or an anti-penetrant fungicide (e.g. *Tricyclazole*).

The Guignardia citricarpa (CBS Disease) model for Citrus

1. Susceptible part of the crop
    (i) Fruit (20 October – 31 January) = Four months

(ii) Leaf infection (October – April) = Seven months
(iii) Degradation and Wear-off of Chemicals
(iv) Ascospore availability: 20 October until 30 April according to inoculum and climate

2. Infection events of the disease
   (i) Formation of ascospore producing perithecia
   (ii) Spore release: Ejection & Dispersal
   (iii) Infection conditions
3. Treatment Recommendations
   (i) Combining Susceptible Parts data (1) with Infection events of the disease (2)
4. INPUTS: Crop data
   (i) Crop emergence = Always recorded as 100% (Crop (Fruit infection: 20th October to 31st January)
   (ii) Tree Density 1-5 years/ 10 l/tree: value 3
   6-10 years/ 20l/tree: value 6
   10-25 years/35l/tree: value 10
   (iii) Crop Protection: Enter Chemical Treatments
   (iv) Fruit Growth: Flowering to set- 1-3, Intermediate growth- 4-8, Near maturity- 9-10

5. Observed disease symptoms: ID the Disease (region and/or local on site), downwind distance from disease.
6. Epidemiology: Light infection in region=1, Moderate in region=2, Heavy in region=3, Light local spot=4, Medium local spot=5, Heavy local spot=6, Light local extended=7, Medium local extended=8, Heavy local extended=9, Heavy local extended and region=10.
7. Additional Data: Hail events (before October of the present season), Orchard history of CBS, History of Chemical Resistance, Cultural Practices- Inoculum removal, -treatment, -covering, -testing.

The data thus far collected links events from the CBS life cycle (sporulation, spore release, conditions for infection and first appearance of symptoms) very closely to climatic conditions at the exact time the event took place. Further information concerning the precise effect of temperature, relative humidity and the longevity of inoculum under different climatic regimes became apparent over these four seasons.

Knowledge thus obtained was used over four seasons to predict critical periods and to base management decisions thereon. These decisions concern chemical intervention, inoculum management and the management of infection conditions. Identified bench-marks were thus validated for reliability and usefulness at the same time over several orchards in the Marble Hall district.

The primary handicap of forecasting in general is vested in the weather forecast being only fairly reliable for 5 days into the future.

The value of this model in the protection of the citrus crop is based upon improved timing for spraying, the choice (and protection) of appropriate chemical agent, and in the reduction of the available inoculum for the next season.

Progress during 2006/7 season

Benchmarks were transferred to the ***Plant-PLUS*** framework of ***Dacom***. Due to this modelling framework's five to eight day weather forecasting capability and previous experience with the system and the company, it became the framework of choice. Monitoring continued in order to correct any previous erroneous observations and/or to add any omissions. No further changes were necessary and the "new" model with the inputs generated performed apparently well. Scrutiny in the packing facilities during the 2007 packing season is commencing now. This is done in collaboration with **PPECB**. The field work on model development for a decision support system (**DSS**) for citrus black spot is practically completed. After final evaluation this project will form part of a PhD thesis at the University of Pretoria.

An artificial inoculation experiment for comparison to natural infection was designed but this will not be part of this project.

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4.4 PROJECT: SOILBORNE DISEASES

Project Co-ordinator: M.C. Pretorius (CRI)

4.4.1 Project summary

Two contract trials for different companies were evaluated during the 2006 season. A new biological control product was evaluated for BASF in a 12-year-old Delta Valencia orchard with ± 6000 ♀/10 g roots at Friedenheim Citrus Estate. A final report was sent to BASF for the work conducted during the 2006 season, and BASF indicated that the company is not prepared to continue with this product (4.4.2). The second contract trial was conducted for Illovo Sugar to evaluate Crop Guard, a non-organophosphate nematicide, for its efficacy in controlling the citrus nematode, *Tylenchulus semipenetrans.* The results of the registration trials with recommendations were handed to Illovo Sugar company in a final report. If this product is registered on citrus, the producers will have an alternative product for the control of the citrus nematode (4.4.3).

At present, none of the registered post-plant nematicides have an effect on the eggs of the citrus nematode. The eggs can survive for up to 9 years in the soil and during favourable conditions the eggs hatch and the life cycle continues. It is therefore essential to develop a new strategy to possibly synchronise the egg hatching process by applying potentially egg stimulating products. The potentially stimulating products identified were evaluated in the laboratory and the initial results were not very promising. In certain treatments the eggs were stimulated but at a very low percentage. It was also clear that these products did not move easily through the soil profile and that as a result of the possible volatile nature of these products the means of application should be investigated further. A new experimental ready-to-use formulation will be evaluated and might accommodate the above-mentioned difficulties experienced with the different stimulating products (4.4.4).

Armillaria root rot is caused by various species of the fungal pathogen *Armillaria.* The genus Armillaria contains about 40 species of important wood-rot fungi which are widely distributed across the world. All

species of shrubs, vines, fruit, amenity, forest trees and some herbaceous plants are affected. Armillaria infects roots of healthy trees by rhyzomorph contact from diseased tissue, or by direct mycelial contact from diseased tissues. Species such as *A. mellea, A. ostoyae, A. novae–zelandiae* and *A. luteobubalina* are highly virulent pathogens. Armillaria root rot is commonly associated with drought-stressed trees, excess soil moisture may be as stressful as drought. All commonly grown citrus cultivars are susceptible to this disease. The fungus damages the root system either affecting single trees or a group of trees. Above-ground symptoms are generally not visible until the disease is well established in the root system and trunk of the tree. The root infection induces a slow decline that involves the whole canopy when a major part of the root system is infected. Initially leaf and twig dieback may be observed, progressing to the death of major branches of the tree and eventually death of the entire tree. The general approach of avoidance seems to be the best approach to prevent Armillaria infections. Therefore an integrated control management approach would be the most effective approach for the control of Armillaria root rot and it includes cultural, biological and chemical control actions (4.4.5).

Phytophthora nicotianae var. *parasitica* (Dastar) Waterhouse is an aggressive root and fruit rot pathogen in citrus. The pathogen has a wide range of host species and can be isolated from soils in most of the older citrus orchards in southern Africa as well as in young nursery trees. Potassium phosphonates are used worldwide for the control of *Phytophthora nicotianae*, the causal agent of citrus brown rot, collar rot and root rot. Foliar application of this product has proved itself to be the most effective control method in citrus. Phytoxicity as a result of phosphonate sprays is the only aspect that negatively influences the use of these products on citrus trees. Different application options were evaluated with the aim of reducing the risk of phytotoxicity. Different adjuvants were added to phosphonate mixtures and the stability and pH readings of these mixtures were determined in the laboratory. Potted trees were sprayed with the mixtures to determine if these mixtures will enhance phytotoxicity. The results indicate that most of the adjuvants +phosphonate mixtures, had a pH reading of 6 and lower and a phytotoxic reaction on the potted trees leaves was visible. It was concluded that no unnecessary products should be added to spray mixtures when using phosphonates (4.4.6).

Phytophthora root rot is an ongoing problem in citrus nurseries. Metalaxyl-M is the primary fungicide used for curative treatment of this disease in South African citrus nurseries. The status of metalaxyl sensitivity of *Phytophthora* in South African citrus nurseries is unknown. A total of 40 isolates obtained from different nurseries and a few from orchards has been selected for the study. Isolates are mainly from two nurseries in the Limpopo Province while nursery isolates from the Western and Eastern Cape and some field trials have also been included. The growth of isolates on PDA containing 1 µg/ml, 10 µg/ml en 100 µg/ml metalaxyl-M was compared to growth on ADA. The results showed differences in Metalaxyl-M sensitivity between isolates with only one isolate showing a high level of resistance. This finding puts a limitation of the sole use of metalaxyl-M for the control of *P. nicotianae* in citrus nurseries and emphasises the urgency to evaluate and register alternative systemic fungicides to control the disease (4.4.7). In a separate experiment, the use ofpotassium phosphonate against *Phytophthora* root rot in citrus orchards was evaluated. This product is the most widely used product to induce resistance in the plant against the pathogen and is registered as foliar application or stem paint. This is, however, labour intensive and can, in adverse conditions, be phytotoxic. Applying the product through the irrigation can reduce the cost of application, and may be less phytotoxic to trees under extreme conditions. The aim of the trial was firstly to determine the optimal dosages for irrigation application, and the effect of different types of irrigation systems. Secondly, to determine the effect of soil texture and dosages on efficacy, and whether the product can become unavailable in certain soil types. Finally the effect of different application methods on efficacy and curative action on infected plants (4.4.8).

Projekopsomming

Twee kontrakproewe is gedurende die 2006 seisoen vir twee verskillende maatskappye uitgevoer. 'n Registrasieproef vir BASF is uitgevoer op 'n kontrak basis om 'n nuwe biologiesebeheer produk te evalueer vir die beheer van die sitrusaalwurm. 'n Finale verslag is aan BASF gestuur maar die maatskappy het aangedui dat hul nie belangstel om voor te gaan met ontwikkelings werk met die produk nie (4.4.2). CRI is deur Illovo Suiker genader om Crop Guard, 'n chemiese byproduk van suiker, se doeltreffendheid teen die sitrusaalwurm op 'n kontrakbasis te evalueer. 'n Registrasie proef is uitgelê en CRI het aangedui dat Illovo ondersteun word met die registrasie van Crop Guard as 'n alternatiewe aalwurmdoder vir gebruik aan die Suid Afrikaanse sitrus industrie. 'n Finale verslag is aan hulle gestuur en die inhoud word as konfidensieel beskou totdat Illovo aandui dat die data bekend gemaak kan word (4.4.3).

Geen geregistreerde aalwurmdoder het 'n effek op die sitrusaalwurmeiers nie en daarom is die projek ge-inisieer om die nuwe konsep van die effek van eierstimulante te evalueer. Die effek van eierstimulante op hule eie en in kombinasie met mekaar asook met ander chemiese en nie-chemiese produkte is in die laboratorium ge-evalueer. Die vermoë van die produkte om deur die grongprofiel te beweeg is ook ge-

evalueer. In die laboratorium stimulasie proef het slegs een produk die vermoë gehad om eiers te laat uitbroei. Die logingsproef se resultate was ook wisselvalling en die resultaat het getoon dat die produkte moontlik vlugtig van aard is of moeilik loog deur die grondprofiel. 'n Nuwe korrel formulasie van 'n eierstimulant is as 'n eksperimentele formulasie aan CRI voorsien na onderhandelinge met 'n maatskappy. Die produk wat moontlik beskerm sal word teen ultra violet, uitermatige omgewingstoestande en wat as 'n korrel formulasie maklik toegedien kan word hou moontlik heelwat potensiaal in vir die nuwe konsep in aalwurmbeheer. Veldproewe is uitgelê om die dosis en effektiwiteit van die produk te bepaal. (4.4.4).

Armillaria wortelvrot word wêreld-wyd veroorsaak deur 40 verskillende Armillaria swamspesies. Armillaria kan as primêre patogeen, sekondêre patogeen en as 'n saprofiet in die grond voorkom. Die patogeniese spesies wat uiters aggresiewe wortel- en stamverrotting veroorsaak is *A. mellea, A. ostoyae, A. novae–zelandiae* en *A. luteobubalina.* Die patogeen word algemeen geassosieer met spanningstoestande wat veral droogte en oormaat nat en vogtige toestande insluit. Alle algemeen-bekende sitrus kultivars is vatbaar vir die patogeen. Voorkoming van die siekte is die beste verweer maar indien die patogeen reeds gevestig is in boorde moet 'n geintegreërde beheerprogram gevolg word wat fisiese-, verbouings-, biologiese- asook chemiese beheer maatreëls insluit (4.4.5).

Phytophthora nicotianae var. *parasitica* (Dastar) Waterhouse is 'n uiters aggressiewe grondgedraagde patogeen wat wortel-, kraag- en bruinvrot op sitrus kultivars veroorsaak Die fosfonate is tans die mees effektiewe produkte beskikbaar vir die beheer van *Phytophthora* spp. op sitrus en word tans met groot sukses in die bedryf gebruik. Die doel van hierdie proef was om fitotoksisiteit wat soms op vrugte voorkom te verminder indien fosfonate in tenkmengsels met sekere byprodukte gemeng word. Die pH van die verskillende byvoegmiddel + fosfonaat mengsels wat ge-evalueer is, het gewissel van 6 tot so laag as 4.7. In die potproef waar jong boompies met die mengsel gespuit is, is chemiese brand simptome op die blaarpunte waargeneem, wat die gebruik van so 'n mengsel nie moontlik maak nie. Die resultate toon duidelik dat geen onnodige bymiddels by die fosfonate gevoeg behoort te word nie, aangesien dit moontlike fitotoksiese reaksies kan bevorder. Hierdie resultate ondersteun verlede jaar se proef resultate waar 'n buffer by die fosfonaat oplossing gevoeg is en die pH van die mengsel dramaties verlaag is (4.4.6).

Phytophthora wortelvrot is 'n wesenlike probleem in sitruskwekerye. Metalaxyl-M is die primêre swamdoder wat vir kuratiewe beheer van die siekte in Suid-Afrikaanse sitruskwekerye gebruik word. Die status van metlaxyl-M sensitiwiteit van *Phytophthora* in sitruskwekerye in Suid-Afrika is onbekend. 'n Totaal van 40 isolate vanaf verskillende kwekerye en enkele isolate uit boorde is geselekteer vir die studie. Isolate uit die Limpopo Provinsie en isolate uit die Wes-Kaap en Oos-Kaap is geevalueer. Uit die resultate is dit duidelik dat daar wel verskille in metalaxyl-M toleransie is met 'n enkele isolaat wat 'n hoë mate van weerstand toon. Hierdie bevinding plaas 'n beperking op die uitsluitlike gebruik van metalaxyl-M vir die beheer van *P. nicotianae* in sitruskwekerye en beklemtoon die noodsaaklikheid om ander sistemiese swamdoders vir die beheer van die siekte te evalueer en te registreer (4.4.7). In 'n ander eksperiment, is die gebruik van fosfonate teen *Phytophthora* wortelvrot in sitrusboorde ge-evalueer. Hierdie produk word algemeen gebruik in die bedryf gebruik om weerstand teen die patogeen in die plant op te bou. Die produk is as blaarbespuiting en stamverf geregistreer, maar hierdie metodes is egter baie arbeidsintensief en kan in uitsonderlike omstandighede fitotoksies vir die plant wees. Deur die produk toe te dien deur die besproeiing, kan kostes tot 'n groot mate verminder, en kan dit ook minder fitotoksies vir die plant maak. Die doel van die projek was eerstens om te bepaal wat die optimale dosis is vir toediening deur die besproeiingstelsel, en die effek van verskillende besproeiingstelsels. Tweedens, is die invloed van grondtekstuur en dosis op effektiwiteit bepaal, en of die produk onbeskikbaar raak in sekere grondtipes. Laastens, is die kuratiewe effek van die verskillende toedieningsmetodes bepaal. Positiewe resultate wat die boomtoestand verbeter is 'n aanduiding dat die proef vir nog 'n seisoen geevalueer behoort te word (4.4.8).

4.4.2 **Evaluation of a new biological control product for the control of the citrus nematode**
Experiment 832 by M.C. Pretorius (CRI)

Opsomming

'n Registrasieproef vir BASF is uitgevoer op 'n kontrakbasis om 'n nuwe biologiese beheer produk te evalueer vir die beheer van die sitrusaalwurm. Die proef is op Friedenheim Sitrus Landgoed uitgelê in 'n 12 jaar oue Delta Valencia boord. 'n Finale verslag is aan BASF gestuur maar die maatskappy het aangedui dat hul nie belangstel om voort te gaan met ontwikkelingswerk met die produk nie.

Summary

BASF approached CRI to conduct a trial to establish the efficacy of a biological control product for the control of the citrus nematode on a contract basis. The trial was laid out at Friedenheim Citrus Estate. The trial site

was selected on 12-year-old Delta Valencia trees with ± 6000 ♀/10 g roots. The product was applied and the site was sampled three times during the season. A final report was sent to BASF for the work conducted during the 2006 season, and BASF indicated that the company is not prepared to continue development of this product.

4.4.3 Evaluation of Crop Guard against the citrus nematode, *Tylenchulus semipenetrans*

Experiment 675 by M.C. Pretorius (CRI)

Opsomming

CRI is deur Illovo Suiker genader om Crop Guard, 'n chemiese byproduk van suiker, se doeltreffendheid teen die sitrusaalwurm op 'n kontrakbasis te evalueer. 'n Registrasie proef is uitgelê en CRI het aangedui dat Illovo ondersteun word met die registrasie van Crop Guard as 'n alternatiewe aalwurmdoder vir gebruik aan die Suid Afrikaanse sitrus industrie. 'n Finale verslag is aan hulle gestuur.

Summary

Illovo Sugar approached CRI to evaluate Crop Guard, a non-organophosphate nematicide, to determine its efficacy of the product in controlling the citrus nematode, *Tylenchulus semipenetrans*, on a contract basis. Crop Guard is registered as a nematicide on peanuts and potatoes. The registration trials were concluded and a recommendation was made to Illovo to support the registration of Crop Guard as a nematicide on citrus, and as an alternative product to be utilised by the citrus producers. A final report was handed to Illovo Sugar and on their request the contents of the report is confidential until the product is registered.

4.4.4 Stimulation of egg hatching of *Tylenchulus semipenetrans* eggs

Experiment 860 by M.C. Pretorius (CRI)

Opsomming

Geen geregistreerde aalwurmdoder het 'n effek op die sitrusaalwurmeiers nie en daarom is die projek ge-inisieer om die nuwe konsep van die effek van eierstimulante te evalueer. Die effek van eierstimulante op hule eie en in kombinasie met mekaar asook met ander chemiese en nie-chemiese produkte is in die laboratorium ge-evalueer. Die vermoë van die produkte om deur die grongprofiel te beweeg is ook ge-evalueer. In die laboratorium stimulasie proef het slegs een produk die vermoë gehad om eiers te laat uitbroei. Die logingsproef se resultate was ook wisselvalling en die resultaat het getoon dat die produkte moontlik vlugtig van aard is of moeilik loog deur die grondprofiel. 'n Nuwe korrel formulasie van 'n eierstimulant is as 'n eksperimentele formulasie aan CRI voorsien na onderhandelinge met 'n maatskappy. Die produk wat moontlik beskerm sal word teen ultra violet, uitermatige omgewingstoestande en wat as 'n korrel formulasie maklik toegedien kan word hou moontlik heelwat potensiaal in vir die nuwe konsep in aalwurmbeheer. Veldproewe is uitgelê om die dosis en effektiwiteit van die produk te bepaal.

Introduction

Although they are aquatic animals, plant parasitic nematodes have evolved protective structures and metabolic adaptations that allow them to survive and flourish in what is often a harsh and competitive soil environment. The body of the nematode is protected by a multi-layered, proteinaceous cuticle, which functions as a flexible skeleton and as a barrier to undesirable elements in the environment. The cuticle is freely permeable to water but differentially permeable to various ions and other chemicals, thus providing nematodes with a selective barrier, which can prevent the entry of some chemicals (Bird, 1971). It is also a relatively resistant structure and is not readily destroyed by chemical or biological agents.

The high reproductive capacity of most plant parasitic nematodes is one of the features that makes them such significant pests, and it also makes them difficult to control. The life cycle of many of the most important species takes only a few weeks at optimum temperatures and each female has the capacity to produce hundreds, and in some cases thousands, of progeny resulting in yield losses of thousands of Rands per annum to the growers. On a susceptible crop under ideal conditions for the nematode, populations that are virtually non-detectable at planting can increase to damaging levels in less than 3 months. This tremendous capacity for multiplication tends to negate the effects of antagonists as high levels of parasitism and predation may do little to diminish final nematode numbers (Stirling, 1990).

In addition to the structural features, which provide protection against antagonism, the physiological capacity of many plant parasitic nematodes to survive adverse conditions (Cooper & Van Gundy, 1971) may give them an advantage over some of their parasites and predators. For example, nematodes are the most

successful anhydrobiotic animals (Womersly, 1987) and are less likely to be affected by dry conditions than many of the organisms that prey on them. Also, the behavioural modifications that occur in the anhydrobiotic state (e.g. coiling) possibly reduce the susceptibility of nematodes to parasitism and predation. However, it is important to recognise that the capacity of nematodes to survive adverse conditions does not give them an advantage over all their antagonists.

None of the registered post-plant nematicides have an effect on the eggs of the citrus nematode, *Tylenchulus semipenetrans*. These eggs can survive for up to 9 years in the soil and during favourable conditions the eggs hatch and the life cycle continues. It is therefore essential to follow an integrated nematode control strategy to assist producers in obtaining an economically viable control strategy for effective citrus nematode control.

Initial trial results with potential egg hatching products appear to be very promising, but the variability of the results in the follow up trials was not acceptable and requires more research. The purpose of this trial was to determine if the egg stimulating concept is an effective option for the producers to implement as part of an Integrated Pest Management approach to effectively control the citrus nematode in citrus orchards in South Africa.

Materials and methods

A laboratory trial was laid out to screen and evaluate the potential of different combinations of egg stimulating products, which were previously tested and known to be effective in stimulating citrus nematode eggs, the combination with other chemical products to enhance the efficacy of these products, as well as to determine the movement of these products through the soil profile. Three laboratory trials were laid out at CRI's Diagnostic Centre. The products and rates evaluated are presented in Tables 4.4.4.1, 4.4.4.2 & 4.4.4.3.

Trial 1. One plastic bag per treatment was filled with 2ℓ dampened soil, infected with citrus nematode larvae and eggs. 100 mλ of each mixture was applied per bag. Three days later 100 mℓ of municipal tap water was applied to each bag to keep the soil moist. The second stage juvenile populations were extracted and determined from the soil five days after the applications were done according to the Baerman funnel method of Whitehead and Hemming (1965).

Table 4.4.4.1. The evaluation of several citrus nematode egg stimulating products that were applied on their own and in combination with other chemical products at different dosages in plastic bags filled with 2 ℓ soil, infected with citrus nematode eggs and larvae.

Treatments	Dosage
Untreated Control	-
Furfural	2.5 mℓ / ℓ water
Furfural + Sporekill	2.5 mℓ/ℓ + 2 mℓ/ℓ
Product A	5 mℓ/ℓ
Product A + Sporekill	5 mℓ/ℓ + 2 mℓ/ℓ
Product B	5 mℓ/ℓ
Product B + Sporekill	5 mℓ/ℓ + 2 mℓ/ℓ
Product C	50 mℓ/ℓ
Salcylic acid	0.5 mℓ/ℓ
Product D	0.25 mℓ/ℓ
Poduct A + Product C	5 mℓ/ℓ + 50 mℓ/ℓ
Product E	50 mℓ/ℓ

Trial 2. PVC piping with a 150 mm diameter and a height of 500 mm was used for evaluating the different products. Two holes were drilled into the cylinders one at 25 mm from the top and one at 200 mm from the top to determine the movement of the products through the soil profile. Each cylinder was filled to the top with dampened soil with citrus nematode larvae and eggs. The treatments were applied with 200 mℓ of each treatments mixture. Three hours after the first application, each cylinder was watered with 50 mℓ of municipal tap water to wash the products into the soil profiles. One day after the first application the cylinders were watered again with 100 mℓ municipal tap water. Three days after the first application the cylinders were watered with 100 mℓ municipal tap water. One week after the initial applications, the second stage

juvenile populations were extracted and determined from the soil according to the Baerman funnel method of Whitehead and Hemming (1965).

Table 4.4.4.2. The evaluation of several citrus nematode egg stimulating products that were applied on their own and in combination with other chemical products at different dosages in round plastic cylinders filled with soil infected with citrus nematode eggs and larvae.

Treatments	Dosage
Furfural	2.5 mℓ / ℓ water
Furfural + Sporekill	2.5 mℓ + 2 mℓ / ℓ water
Product A	50 mℓ / ℓ water
Product A + Sporekill	2.5 mℓ + 2 mℓ/ ℓ water
Product B	50 mℓ / ℓ water
Product B + Sporekill	50 mℓ + 2 mℓ/ ℓ water
Product C	100 mℓ / ℓ water
Salcylic acid	5 mℓ / ℓ water
Product D	2.5 mℓ / ℓ water
Product A + Product C	50 mℓ + 30 mℓ / ℓ water
Product E	100 mℓ / ℓ water
Temik	0.5 g / cylinder
Citrofresh	50 mℓ / ℓ water
Sporekill	2 mℓ / ℓ water
Untreated Control	-

Trial 3. PVC piping with a 150 mm diameter and a height of 500 mm were used for evaluating the different products. Two holes were drilled into the cylinders, one at 25 mm from the top and one at 200 mm from the top, to determine the movement of the products through the soil profile. Each cylinder was filled to the top with dampened citrus nematode larvae and eggs. The treatments were applied with 200 mℓ of each treatment's mixture. Three hours after the first application each cylinder was watered with 50 mℓ of municipal tap water to wash the products into the soil profiles. This process was repeated three more times at three day intervals. Twenty-one days after the initial application two samples per treatment were collected, one at 25 mm from the top and the other at 200 mm from the top. The second stage juvenile populations were extracted and determined from the soil according to the Baerman funnel method of Whitehead and Hemming (1965).

Table 4.4.4.3. The evaluation of several citrus nematode egg stimulating products that were applied on their own and in combination with other chemical products at different dosages in round plastic cylinders filled with soil infected with citrus nematode eggs and larvae.

Treatments	Dosage
Furfural	2.5 mℓ / ℓ water
Furfural + Salcylic acid	2.5 mℓ + 5 mℓ / ℓ water
Product A	50 mℓ / ℓ water
Product A + Salcylic acid	50 mℓ + 5 mℓ/ ℓ water
Product B	50 mℓ / ℓ water
Product B + Product C	50 mℓ + 30 mℓ / ℓ water
Product B + Salcylic acid	50 mℓ + 5 mℓ / ℓ water
Product C	100 mℓ / ℓ water
Salcylic Acid	5 mℓ / ℓ water
Product D	2.5 mℓ / ℓ water
Product A + Product C	2.5 mℓ + 30 mℓ / ℓ water
Product A + Furfural	50 mℓ + 25 mℓ / ℓ water
Product E	100 mℓ / ℓ water
Rugby	0.5 mℓ/ ℓ water
Citrofresh	100 mℓ / ℓ water
Product F	1 mℓ / ℓ water
Untreated Control	-

Trial 4 - New formulation field trial

The next approach was to negotiate with several chemical and private companies to formulate a new formulation of one of the promising egg stimulating products. The new formulation should be a ready to use, slow release granular product that will not be affected by environmental conditions such as high soil temperatures and harmful ultra-violet rays from the sun. A laboratory trial to determine the efficacy of the new formulation will be evaluated at the Diagnostic Centre in Nelspruit. A field trial to determine an effective rate will be laid out at Crocodile Valley Citrus Co.

Results and discussion

The purpose of these trials was to investigate the egg stimulating concept by evaluating different combinations of various products to improve their possible abilities to stimulate the hatching of citrus nematode eggs. The results obtained in Trial 1 (Table 4.4.4.4) indicate that some of the nematode juvenile population numbers were increased as a result of the application of the different products. Product X was able to increase the juvenile counts by 47%. The Furfural on its own and in combination with Sporekill had the second highest increase of juvenile population numbers, 30 and 31% respectively. The Salcylic Acid treatment on its own and the combination of Product A and Product C resulted in a -44% decrease of juvenile population counts, which indicate that this product has got the potential to kill both the nematode eggs and larvae.

Table 4.4.4.4. The effect of the different nematode egg stimulating products applied on their own and in combination with other products on citrus nematode larvae population counts.

Treatments	Citrus nematode larvae/250 cc soil
Untreated Control	3200 (-)*
Furfural	4200 (31)
Furfural + Sporekill	4150 (30)
Product A	3850 (20)
Product A + Sporekill	2750 (-14)
Product B	3600 (13)
Product B + Sporekill	3150 (-2)
Product C	4700 (47)
Salcylic acid	1800 (-44)
Product D	3250 (2)
Poduct A + Product C	1800 (-44)
Product E	2900 (-9)

*% Percentage increase/decrease of citrus juvenile populations compared to the untreated control treatment.

The juvenile population counts in Trial 2 (Table 4.4.4.5) were not increased at the 25 mm depth. All the counts were reduced when compared to the untreated control. It is clear that some of the products killed the nematode juveniles and eggs. Juvenile population counts at the 200 mm depth were increased only in the Salcylic Acid, Product D and the combination of product A and C treatments. In all the other treatments the juvenile counts were less than the untreated control treatment. The result of the standard chemical treatment, Temik, clearly indicates that the current registered nematicides do not have any affect on the nematode eggs.

Table 4.4.4.5. The effect of the different nematode egg stimulating products that were applied on their own and in combination with other products on citrus nematode larvae population counts collected at two different depths in PVC piping.

Treatments	Citrus nematode larvae/ 250 cc soil	
	25 mm depth	200 mm depth
Furfural	0	200
Furfural + Sporekill	0	200
Product A	400	800
Product A + Sporekill	1200	300
Product B	200	500
Product B + Sporekill	500	900
Product C	0	300
Salcylic acid	300	1700
Product D	400	1300

Treatments	Citrus nematode larvae/ 250 cc soil	
	25 mm depth	200 mm depth
Product A + Product C	0	700
Product E	200	1400
Temik	200	1000
Citrofresh	500	200
Sporekill	800	500
Untreated Control	1200	

Product A with Salcylic acid and the Product E treatment were able to increase the juvenile population counts at the 25 mm depth when compared to the untreated control treatment (Table 4.4.4.6). These treatments increased the juvenile population number to 2200 and 1800 juveniles/250 cc soil, respectively. Treatments with the zero juvenile counts were possibly eliminating both the juveniles and the eggs stages in the soil. Product B, the combination of Product B and C and Product D were the only three treatments capable of increasing the nematode numbers when compared to the untreated control at the 200 mm depth. In most of the other treatments the juvenile counts were the same as the untreated control, or less that the control treatment.

Table 4.4.4.6. The effect of the different nematode egg stimulating products that were applied on their own and in combination with other products on citrus nematode larvae population counts collected at two different depths in PVC piping.

Treatments	Citrus nematode larvae/ 250 cc soil	
	25 mm depth	200 mm depth
Furfural	0	0
Furfural + Salcylic acid	0	0
Product A	200	0
Product A + Salcylic acid	2200	700
Product B	0	1600
Product B + Product C	0	1800
Product B + Salcylic acid	100	500
Product C	500	300
Salcylic Acid	900	700
Product D	1300	1500
Product A + Product C	0	400
Product A + Furfural	0	100
Product E	1800	200
Rugby	1300	1300
Citrofresh	1300	500
Product F	700	300
Untreated Control	1300	

It is clear from the results obtained in Trial 1 that Product C was the only treatment able to increase the juvenile counts in the plastic bags. The results in Trials 2 and 3 were disappointing because, although some of the treatments increased the nematode juvenile population counts, it was not a dramatic increase. This is a clear indication that the product did not stimulate the nematode eggs satisfactorily. The low counts at the 25 mm depth might be due to the possible volatile characteristic nature of some of these stimulating products and that most of the product's mode of action is lost due to its possible volatile nature. The low juvenile counts at the 200 mm depth could also be as a result of the volatile nature of these products as well as their possible inability to move through the soil profile and therefore lose their anticipated effectivity.

One company indicated their interest in developing an experimental formulation of a previously evaluated stimulant that was able to stimulate citrus nematode eggs to hatch. CRI received an experimental granular formulation of this stimulant. Pilot trials will be laid out to determine the required rates necessary and to evaluate the efficacy of these products.

Conclusion

The results of the laboratory trials clearly indicate that the products evaluated were not successful in stimulating nematode eggs to hatch. It was also clear that the characteristic nature of some of these products require a different approach regarding the way in which they are applied. Therefore CRI is very optimistic that the new experimental formulation will possibly, according to the manufacturing company, be

able not only to stimulate the eggs to hatch but also be protected against the environmental conditions and have a slow-release ability.

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4.4.5 To determine the most effective control measures for *Armillaria* die-back of citrus

Experiment 814 by MC Pretorius (CRI)

Opsomming

Armillaria wortelvrot word veroorsaak deur verskeie Armillaria swamspesies. Armillaria kan as 'n primêre patogeen, 'n sekondêre patogeen en as 'n saprofiet in die grond voorkom. Die patogeniese spesies wat wêreldwyd uiters aggresiewe wortel-en stamverrotting veroorsaak, is *A. mellea, A. ostoyae, A. novae–zeelandiae* en *A. luteobubalina.* Die patogeen word algemeen geassosieer met spanningstoestande wat veral droogte en oormaat nat en vogtige toestande insluit. Gesonde weefsel word hoofsaaklik deur Rhizomorf kontak vanaf besmette plante besmet, hoofsaaklik vanaf wortels. Die siekte kom gewoonlik in ou bosbou gebiede voor wat ontbos is en voorberei word vir die aanplant van ander landbou gewasse oa. sitrus. Alle bekende sitruskultivars is vatbaar vir Armillaria infeksies. Enkele sitrusbome of 'n paar in 'n ry kan geaffekteer word deur die patogeen. Bogrondse simptome is nie algemeen sigbaar nie aangesien die wortelstelsel die hoofbron van infeksie is. Namate die wortelvrot vererger weens die toename in die patogeen se aktiwiteit sal 'n stadige agteruitgang van die boom waargeneem kan word en namate die wortelsiekte toeneem kan die boom vrek. Voorkoming van die siekte is die beste verweer maar indien die patogeen reeds in boorde gevestig is, moet 'n geintegreerde beheerprogram gevolg word wat fisiese-, kulturele-, biologiese- asook chemiese beheer insluit.

Introduction

Armillaria root rot is caused by various species of the fungal pathogen *Armillaria.* The genus *Armillaria* contains about 40 species of important wood-rot fungi, which are widely distributed across the world (Shaw, 1991). All species of shrubs, vines, fruit, amenity, forest trees and some herbaceous plants in temperate and tropical regions are affected. Armillaria can act as a primary pathogen, a stress–induced secondary invader, and as a saprophyte. Many of the species that have been described are virtually harmless saprophytes. Species such as *A. mellea, A. ostoyae, A. novae–zeelandiae* and *A. luteobubalina* are highly virulent pathogens. Robert Hartig, considered as the father of forest pathology, described *Agaricus melleus*, which is currently known as *A. mellea*, in 1874. *Armillaria* is commonly referred to as the honey fungus or as oak root fungus (Fox 2003) and is commonly associated with drought-stressed trees (excess soil moisture may be as stressful as drought). Excess soil moisture and increased severity of Armillaria root rot have been observed in oak and chestnut species in Germany and Austria (Bassinger 1956)

The major species include *A. mellea* and *A. ostoyae* in Europe and North America, and *A. luteobubalina* in Australasia. Distinctions can be made in terms of the characteristic host ranges of the different species. *A. mellea*, for example, is mainly a pathogen of broadleaved trees in ornamental parklands, natural woodlands, fruit orchards, etc, but it can infect and kill young coniferous trees (pines and spruce) planted in sites where the broadleaved species were felled. In contrast, *A. ostoyae* seems to be a more important pathogen of coniferous trees, causing major damage in even second and third rotation stands of conifers. Thus, the genus *Armillaria* seems to have diversified into a number of forms with different degrees of pathogenic virulence and host preference (Shaw 1991).

Disease development and spread

Armillaria infects roots of healthy trees by rhyzomorph contact from diseased tissue, or by direct mycelial contact from diseased tissues. Hyphae penetrate the outer bark and challenge the inner bark which, if stressed, becomes invaded and dies. In many cases, the spread is achieved by the rhizomorphs that can grow along the root surfaces, under the bark of dead trees, or can spread several meters through soil from an established food base. The disease is sometimes referred to as the "bootlace or shoe-string" fungus because the spread through the soil or under the bark produce rhizomorphs that resemble bootlaces. The pathogenic species have astonishingly wide host ranges which extend far beyond forest trees. Armillaria can cause serious damage to orchard crops whenever these are planted in sites where indigenous woodland or shrubland infected with Armillaria was cleared. The fungus will infect these crops from stumps or major root tissues that were left after clearing (West, 2002).

Symptoms and damage on citrus cultivars

All commonly grown citrus cultivars are susceptible to this disease. The fungus damages the root system either affecting single trees or a group of trees. The disease will often move along the tree row, progressing from tree to tree as the infected roots of one tree come in contact with the next. Above ground symptoms are generally not visible until the disease is well established in the root system and trunk of the tree. The root infection induces a slow decline that involves the whole canopy when a major part of the root system is infected. Initially leaf and twig dieback may be observed, progressing to the death of major branches of the tree and eventually death of the entire tree (Munnecke, 1976). Characteristic white fan-shaped mycelial mats can be seen growing on the wood when bark is peeled off. The wood of infected roots is rotted by the fungus, becoming either white and powdery or wet and jelly-like with black bands. Black bootlace-like strands, rhizomorphs, can occur on the root surface partially embedded in the bark. These rhizomorphs may occasionally grow on the surface of roots ahead of the infection area or into the surrounding soil. Clusters of mushrooms (fruiting bodies of the fungus) grow from the infected roots at the base of the tree during humid moist conditions in autumn. The mushrooms are brown or honey-coloured on top and creamy-white underneath. The incidence of the disease is higher in unusually wet seasons due to the increase in activity of the fungus. Citrus trees under stress as a result of drought may be more vulnerable to Armillaria infection (Barkley, 2004).

Current control methods

A major impediment in the chemical and biological control approach for the control of Armillaria is the inability of the control agents to reach the site of inoculum inside the plant. Therefore, an integrated management approach would be the most effective approach for the control of Armillaria root rot. The general approach of avoidance seems to be the best approach to prevent Armillaria infections.

Physical removal of inoculum by removing diseased trees and uprooting even neighbouring uninfected stumps has been recommended. In France, trenches more that a metre wide were dug in vineyards and fruit orchards infected with Armillaria to control the spread of Armillaria root disease. This method was also adopted for cocoa and coffee plantations in Africa. Laying a plastic barrier in a trench and then filling it with the removed soil has been used in New Zealand. These physical control methods are, however, very labour intensive and impractical in established orchards (Shaw, 1978).

Soil fumigation can be used but is only effective where deep penetration can be achieved. Methyl bromide is currently the most extensively used fumigant that is applied between crops because of its non-specific action and good penetrability in the soil. Chloropicrin will destroy even the most resistant soil pathogen but penetration through the soil profile is much more difficult to achieve. Penetration is the most important aspect necessary because Armillaria has been found viable at a depth of up to almost 3 m in forest areas.

Biological control options were also investigated and it was found that mycorrhizal fungi do not protect the roots from Armillaria (Baumgartner, 2000). To control Armillaria, a wood-inhabiting organism might function by inhibiting or preventing rhizomorph and mycelial development by limiting the pathogen to the substrate already occupied by actively pre-empting the substrate, or by eliminating Armillaria from the substrate already occupied. Antagonistic organisms might not be able to prevent Armillaria from establishing in stumps, but they restrict further stump colonisation and thus limit the available food base. The most studied fungal antagonist of Armillaria is *Trichoderma* species, a common fungal hyperparasite in the majority of soils. Extensive studies around the world on their antagonistic activity against many plant pathogens has indicated that successful control of a number of diseases, both in glasshouse and field conditions, may be possible with several species and strains. *In vitro* interactions of Trichoderma and Armillaria suggest that Trichoderma must be considered a possible controlling factor in the spread of pathogenic fungi. Commercial

interest in the development of formulations based on Trichoderma has led to several products that are produced for sale, although there is currently none in use for controlling Armillaria. This might reflect more on business rather than biological constraints (Roland, 2003).

Armillotox, phenolic emulsion containing 48% cresylic acid as the active ingredient, is the only chemical product known that has been marketed for the control of Armillaria diseases. This formulation was developed following the successful suppression of Armillaria with creosote although the latter proved to be phytotoxic. Research to develop a new non–phytotoxic formulation has been studied *in vitro* and the most effective chemical against the Armillaria pathogen on 3% Malt Extract Agar was hexaconazole and flutriafol (both triazoles), fenpropidin (a piperidine), guazatine (a guanide), and phenyl phenol (a phenolic compound. The two triazoles and the piperidine are ergosterol biosynthesis inhibitors (EBI's). In wood blocks, the EBI fungicides were most effective protectant treatments, but the guazatine, phenyl phenol and cresylic acid were the most effective eradicant treatments. However, in a pear orchard no evidence could be found after application with any of the abovementioned chemicals products, applied as eradicant or protectant. Phytotoxicity was caused by cresylic acid and, to a lesser extent, by phenyl phenol. Additional experiments showed that the chemical failed to effectively eradicate rhizomorphs in soil even with higher concentrations. The reason for this reaction could be due to a thin layer of bark preventing the chemicals from eradicating the subcortical mycelium. Research done with fosetyl-Al (Aliette) indicated that this product was able to control Armillaria in strawberry plants (Turner, 1988).

The use of resistant plant material would assist producers to utilize indigenous or forest land infected with Armillaria, but it is known that all citrus cultivars are susceptible to this disease (Barkley, 2004). It was observed in citrus orchards in certain citrus producing regions in South Africa that The Swingle Citrange rootstock is severely affected and, in contrast, Rough Lemon rootstocks were less affected (Barkley, 2004).

Conclusion and recommendations

Armillaria root rot is commonly associated with drought-stressed trees and excess soil moisture as the latter may be as stressful as drought. Armillaria infects roots of healthy trees by rhyzomorph contact from diseased tissue, or by direct mycelial contact from diseased tissues. The pathogenic species have astonishingly wide host ranges that extend far beyond forest trees. Armillaria can cause serious damage to orchard crops whenever these are planted in sites where indigenous woodland or shrubland infected with Armillaria was cleared. The fungus will infect these crops from infected stumps or major root tissues that were left after clearing. All commonly grown citrus cultivars are susceptible to this disease. The fungus damages the root system, either affecting single trees or a group of trees. The disease will often move along the tree row, progressing from tree to tree as the infected roots of one tree come into contact with the next. A major impediment in the chemical and biological control approach for the control of Armillaria is the inability of the control agents to reach the site of inoculum inside the plant. Therefore an integrated management approach would be the most effective approach for the control of Armillaria root rot. The general approach of avoidance seems to be the best approach to prevent Armillaria infections.

<u>Recommendations for the control of Armillaria in citrus orchards</u>

- An integrated control management approach is recommended.
- When developing new planting sites for citrus on indigenous or forest areas possibly infected with Armillaria, soil preparation by means of deep ploughing is essential to remove all roots and old plant material to prevent recontamination of the new plantings with Armillaria.
- The newly identified area can be fumigated with methyl bromide if soil preparations have been done correctly.
- Newly developed land infected with Armillaria should be left fallow for at least one year in order to allow the soils to dry out.
- Although no resistant citrus cultivars are available, rough lemon rootstock should be considered, if possible, to replant Armillaria infested soils rather than with Swingle rootstocks.
- Limited chemical products are available but Armillotox, if available in South Africa, could be used. Registered Phosphonates could also be considered.
- By pruning low hanging branches and removing soil from around the trunk to expose structural roots to sunlight and air might reduce the presence of Armillaria in infected trees.

Future research

It will be essential to do an extensive survey in areas known to be infected with Armillaria to reconfirm the causal organism. This action should also include indigenous forest regions identified to be replanted with citrus to determine the host range of certain of these indigenous plants infected with Armillaria.

More effective integrated control measures in soils infected with Armillaria should therefore be investigated and this investigation should include existing orchards and new plantings.

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4.4.6 Evaluation of phosphonate–adjuvant mixtures to reduce the problem with possible phytotoxic damage to citrus fruit when applying a phosphonate for the control of *Phytophthora* brown rot on citrus

Experiment 861 by MC Pretorius (CRI)

Opsomming

Phytophthora nicotianae var. *parasitica* (Dastar) Waterhouse is ’n uiters aggressiewe grondgedraagde patogeen wat wortel-, kraag- en bruinvrot op sitruskultivars veroorsaak. Die fosfonate is tans die mees effektiewe produkte beskikbaar vir die beheer van *Phytophthora* spp. op sitrus en word tans met groot sukses in die bedryf gebruik. Die doel van hierdie proef was om fitotoksisiteit wat soms op vrugte voorkom moontlik te verminder deur fosfonate in tenkmengsels met sekere byprodukte toe te dien. Dit is bekend dat die fosfonate steeds die mees effektiewe bruinvrotbeheer-produkte is om die siekte te beheer. Die pH van die verskillende byvoegmiddels wat ge-evalueer is, het gewissel van 6.0 tot so laag as 4.7. In die potproef waar jong boompies met die mengsel gespuit is, is chemiese brand simptome op die blaarpunte waargeneem. Die resultate toon duidelik dat geen onnodige bymiddels by die fosfonate gevoeg behoort te word nie aangesien dit moontlike fitotoksiese reaksies kan bevorder. Hierdie resultate ondersteun verlede jaar se proefresultate waar ’n buffer by die fosfonaat oplossing gevoeg is en die pH van die mengsel dramaties verlaag het.

Introduction

Phytophthora nicotianae var. *parasitica* (Dastar) Waterhouse is an aggressive root rot and fruit rot pathogen in citrus. The pathogen has a wide range of host species and can be isolated from soil in most of the older citrus orchards in southern Africa as well as in young nursery trees. Most of the research on the chemical

control of fungal root pathogens of citrus includes *P. nicotianae* var. *parasitica* and *P. citrophthora* (Graham, 1998).

Potassium phosphonates are used worldwide for the control of Perenosporales such as *Phytophthora nicotianae*, the causal agent of citrus brown rot, collar rot and root rot. This product has proved itself to be the most effective control method not only in citrus but also on a wide variety of other crops including trees, shrubs (Azalea) and pastures (Clover). *Phytophthora* has decreased from being the most important citrus disease in SA in the 1970s to an almost forgotten problem in the year 2004. This can largely be attributed to the use of CIP certified trees in 1984, combined with phosphonate foliar applications and stem paints.The ED50 value for *in vitro* inhibition of *Phytophthora* root rot with H_3PO_3 is 30 µg/mℓ, and according to Afek & Sztejnberg (1989), H_3PO_3 is also 6-14 times more active than fosetyl-Al (Aliette) in inhibiting mycelial growth.

Phytophthora brown rot as a post harvest disease is a major problem in most of the citrus production areas of South Africa. Epidemic seasons are commonly associated with periods of prolonged wetness and with temperatures in the range of 20-32°C, coinciding with the maturation of early and mid-season citrus cultivars. The chances of developing new fungicides in the near future are slim. The current trend is to move towards products that induce the plant's own defence mechanisms, for instance the phosphonates to control *Phytophthora* on citrus (Schutte, 1990). Excellent results were obtained in controlling *Phytophthora* brown rot on citrus with the use of phosphonates. However, phytoxicity as a result of phosphonate sprays is the only aspect negatively influencing the use of these products on citrus trees. There is a need to evaluate different options in order to assist producers using the phosphonates with confidence without fear of phytotoxicity.

Materials and methods

A laboratory trial was laid out at CRI in Nelspruit, to determine the effect of different commonly used adjuvants that were mixed with two phosphonates, Fighter and Phytex. The two phosphonates were mixed with de-ionised water from the laboratory at the standard registered dosages. The phosphonates and water mixtures were poured into 600 mℓ glass beakers and 1 mℓ of the different adjuvants were applied to the phosphonate and water mixture. The pH of the mixtures was determined 1 hour after the products were mixed and again 24 hours later. The mixtures were left for 48 hours to determine if flocculation of the different mixtures is visible. Young citrus trees in pots outside the glasshouse at CRI were sprayed with these mixtures to establish if any of these products will have a phytotoxic reaction when sprayed to trees. A single application of these mixtures was applied and two trees per treatment were evaluated. The trees were evaluated 1, 2, 4, 6 and 8 days after application. The combination of the different phosphonates, Fighter and Phytex with the adjuvants and the mixtures rates are presented in Table 4.4.6.1.

Table 4.4.6.1. The mixture of Fighter and Phytex with different adjuvants and the rates of the mixtures evaluated at CRI's Diagnostic Centre at Nelspruit.

Treatments	Rates / 500 mℓ water
Fighter	3 mℓ
Phytex	5 mℓ
Aqua Right 3 + Fighter	1 mℓ + 3 mℓ
Aqua Right 3 + Phytex	1 mℓ + 5 mℓ
Aqua Right 5 + Fighter	1 mℓ + 3 mℓ
Aqua Right 5 + Phytex	1 mℓ + 5 mℓ
Aqua Right 7 + Fighter	1 mℓ + 3 mℓ
Aqua Right 7 + Phytex	1 mℓ + 5 mℓ
Aqua Wet + Fighter	1 mℓ + 3 mℓ
Aqua Wet + Phytex	1 mℓ + 5 mℓ
Tenderbuff + Fighter	1 mℓ + 3 mℓ
Tenderbuff + Phytex	1 mℓ + 5 mℓ
Wetcit + Fighter	1 mℓ + 3 mℓ
Wetcit + Phytex	1 mℓ + 5 mℓ

Results and discussion

None of the phosphonate–adjuvant mixtures had a flocculation effect 48 hours after the products were mixed together. The pH measured at 1 and 24 hours after the products were mixed are presented in Table 4.4.6.3.

Table 4.4.6.3. The effect of the different adjuvant mixtures on the ph of the phosphonate mixtures 1 and 24 hours after the adjuvants were mixed with the phosphonates.

Treatments	pH of the mixture	
	1 hour after mix	24 hours after mix
Fighter	6.9	6.9
Phytex	6.9	6.9
Aqua Right 3 + Fighter	5.4	5.4
Aqua Right 3 + Phytex	5.5	5.5
Aqua Right 5 + Fighter	4.7	4.7
Aqua Right 5 + Phytex	5.3	5.3
Aqua Right 7 + Fighter	5.8	5.8
Aqua Right 7 + Phytex	5.7	5.7
Aqua Wet + Fighter	6.0	6.0
Aqua Wet + Phytex	6.0	5.9
Tenderbuff + Fighter	5.8	5.8
Tenderbuff + Phytex	5.9	5.9
Wetcit + Fighter	6	6
Wetcit + Phytex	6	6

All the treatments, with the exception of the two standard phosphonates, reduced the pH of the mixture to a pH of 6 and less. Due to the low pH levels obtained in the laboratory trial, none of the adjuvants evaluated can be recommend to be added to a phosphonate mixture to reduce possible phytotoxic damage to citrus fruit when these products are applied for the control of *Phytophthora* brown rot.

The potted trees at CRI sprayed with the adjuvant and phosphonate mixture showed phytotoxic symptoms on the leaf edges 1 week after the trees were sprayed. Although the phytotoxic reaction on the non- bearing trees was not too serious it is not recommended to spray bearing trees with a mixture when the pH of the mixture is less that 6.

Conclusion

A field trial planned to determine if the use of adjuvants in combination with phosphonates will reduce the incidence of phytotoxicity to citrus fruit was not executed as a result of the laboratory and potted trees trial's results. The pH of 6 and less is not recommended to an already sensitive producing community regarding the use of phosphonates on bearing trees and possible phytotoxic damage to export citrus fruits.

These results supported last year's trial where a buffer added to phosphonates reduced the pH of the mixtures to unacceptable levels. It is clear from these results that no unnecessary products should be added to the phosphonates to either enhance the effectivity or to reduce the phytotoxic damage of these products when it is applied for the control of *Phytophthora* brown rot.

Future research

All possible means must be investigated to reduce problems that may occur when the phosphonates are used to control *Phytophthora* because it is still the most cost effective non-toxic fungicide available to citrus producers.

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4.4.7 **Screening of nursery isolates of *Phytophthora* for resistance to metalaxyl**

Experiment QMS 2006 / SBD 10 by J.J. Serfontein, S. Serfontein and S.H. Swart (QMS)

Opsomming

Phytophthora wortelvrot is 'n wesentlike probleem in sitruskwekerye. Metalaxyl-M is die primêre swamdoder wat vir kuratiewe beheer van die siekte in Suid-Afrikaanse sitruskwekerye gebruik word. Die status van metalaxyl-M sensitiwiteit van *Phytophthora* in sitruskwekerye in Suid-Afrika is onbekend. 'n Totaal van 40 isolate vanaf verskillende kwekerye en enkele isolate uit boorde is geselekteer vir die studie. Isolate is hoofsaaklik afkomstig vanaf twee kwekerye uit die Limpopo provinsie, terwyl isolate uit die Wes-Kaap en Oos-Kaap ook ingesluit is. Isolate se groei op aartappel dekstrose agar (ADA) en ADA bevattende 1 µg/ml, 10 µg/ml en 100 µg/ml metalaxyl-M onderskeidelik, is vergelyk. Uit die resultate is dit duidelik dat daar wel verskille in metalaxyl-M toleransie is met 'n enkele isolaat wat 'n hoë mate van weerstand toon, selfs by 100 µg/m. Hierdie bevinding plaas 'n beperking op die uitsluitlike gebruik van metalaxyl-M vir die beheer van *P. nicotianae* in sitruskwekerye en beklemtoon die noodsaaklikheid om ander sistemiese swamdoders vir die beheer van die siekte te evalueer en te registreer.

Introduction

Phytophthora root rot remains to be a constant problem in South African citrus nurseries, despite preventative measures taken. The use of metalaxyl/metalaxyl-M as a curative fungicide often fails to eliminate the pathogen from containerised trees grown in bark. In Florida, Timmer *et al.* (1998), did a survey in 8 field nurseries and found 39 of 42 isolates to be resistant to metalaxyl-M. Strains of *P. nicotianae* in South African tobacco production fields also varied in sensitivity to metalaxyl (Van Jaarsveld *et al.*, 2002). The sensitivity of citrus nursery strains is not known. In this study, *Phytophthora* isolates from citrus nurseries were evaluated for their sensitivity to metalaxyl-M.

Materials and methods

Phytophthora isolates used in this study were isolated from citrus nurseries as part of a routine nursery screening, supplied by Laura Huisman of the CRI and field isolates from citrus orchards. Isolates were obtained by leaf baiting from nursery growth media using Rough Lemon leaf discs and plating on selective PARPH medium. Isolates were purified by plating on PARPH medium once and thereafter on PDA (Biolab) until no bacterial growth was present. Cultures were stored at room temperature on agar discs in McCartney bottles containing sterile soil water.

Metalaxyl-M (Subdue Maxx, Syngenta) was incorporated in PDA medium to obtain concentrations of 0, 1.0, 10 and 100 µg/ml. The metalaxyl-M was added to molten (45^{o}C) PDA, mixed and poured into 65 mm diameter petri plates. Plates were inoculated by cutting discs, 5 mm diameter, from the margins of 5-day-old PDA grown cultures and placing onto the center of the media. There were two duplicates per isolate per concentration. Plates were incubated at approximately 28°C for 6 days. The colony diameters were measured and the colony area minus the diameter of the inoculum plug were calculated and expressed as percentage of the colony diameter of the no-fungicide control plates. The mean of the two replicates were used. A preliminary trial was done and 40 isolates selected to include in the final trial, eleven from Nursery A, Limpopo Province, 19 from nursery B, Limpopo Province, 5 from other nurseries (Western and Eastern Cape) and 5 from orchards. For the 10 µg/ml and 100 µg/ml concentrations, strains were divided into different categories based on their respective percentage colony sizes compared to the controls. The categories for the 10 µg/ml concentration were 1: 10%-15%, 2: 16%-20%, 3: 21%-25%, 4: 26%-30%; 5: 31%-35%, 6: 36%-40%, 7: >40%. The categories for the 100 µg/ml concentration were 1: ≤5%, 2: 6%-10%, 3: 11%-15%, 4: 16%-20%, 5: >21%.

Results

Only one isolate out of 40 tested grew as well or better on plates containing 100 µl/ml metalaxyl-M than on control plates indicating total resistance to the fungicide. The isolate originated in nursery B. The distribution of isolate sensitivity against the different concentrations is given in Fig. 4.4.7.1 for the 10 µg/ml concentration and Fig. 4.4.7.2 for the 100 µg/ml concentration. In nursery A, isolates were distributed between categories 1 (most sensitive) and 4 (a total 7 categories) in the 10 µg/ml metalaxyl-M scale and categories 1 and 4 (a total of 5 categories) in the 100 µg/ml metalaxyl-M scale, indicating a trend to increased insensitivity when compared to the other nurseries and field isolates. In the case of nursery B, isolates were distributed between categories 1 and 7, 10 µg/ml metalaxyl-M scale, and categories 1 and 5, 100 µg/ml metalaxyl-M scale. The highest percentage of the isolates in this nursery was present in the second category, indicating a shift towards higher insensitivity. Only one isolate of the field isolates fell in category 4 for the 10 µg/ml

metalaxyl-M scale and the rest were in categories 1 and 2. The highest insensitivity rating for isolates from the other nurseries was category 3 for the 10 µg/ml metalaxyl-M scale. For the 100 µg/ml metalaxyl-M scale, all the field and other nursery isolates fell into category 1.

Discussion

The purpose of this study was to determine the status of metalaxyl-M insensitivity of *Phytophthora* strains isolated from South African citrus nurseries. Only one of the 19 selected isolates from Nursery B was resistant to metalaxyl-M, even at the highest concentration tested. This is in contrast with the findings of Timmer *et al.* (1998) who found 39 of 42 isolates from a Florida nursery to be resistant to metalaxyl. They did, however, select isolates from the nursery by incorporating metalaxyl into the selective PARPH medium and trees and nursery trees were grown in the field, not in containers. Although the situation is not as desperate in South Africa, the presence of resistance was shown and variation in metalaxyl-M sensitivity does exist in the local citrus *Phytophthora* population. It is especially the distribution of nursery isolates in the higher insensitivity categories that is cause for concern. These findings emphasise the need to register alternative systemic chemicals for control of Phytophthora root rot in citrus nurseries rather than to depend on a single chemical. Strategies for controlling possible metalaxyl resistance in citrus nurseries should be in place. The testing of metalaxyl-M resistance by the inclusion of PARPH medium containing metalaxyl-M is recommended. This will hasten the detection of metalaxyl resistance in nurseries so that resistance control strategies can be implemented. No specific recommendations are available to control Phytophthora root rot in nurseries where trees are grown in bark in containers. The efficacy of generic metalaxyl products should be compared with metalaxyl-M. An integrated approach where systemic fungicides, preventative chemicals like captan and chemicals that induce resistance, like phosphonates should be implemented.

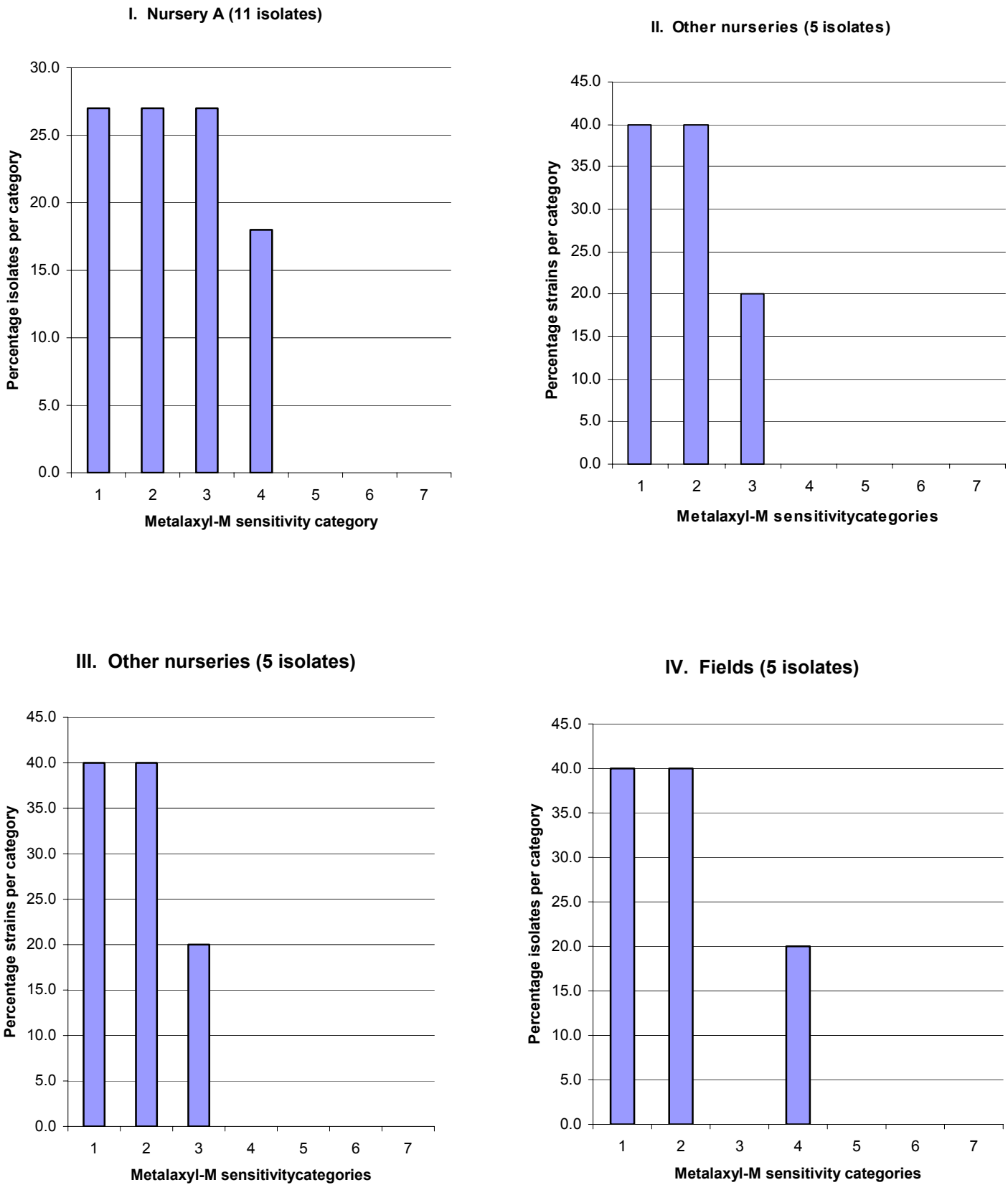
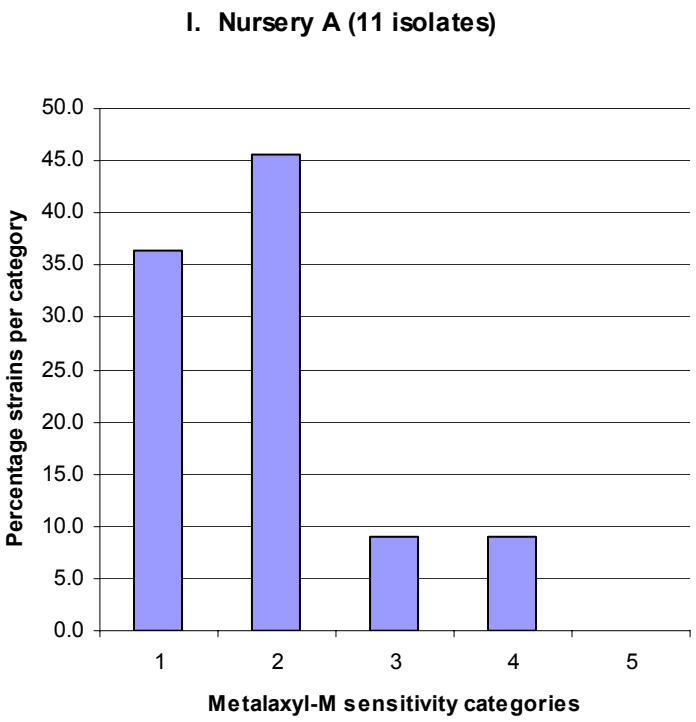
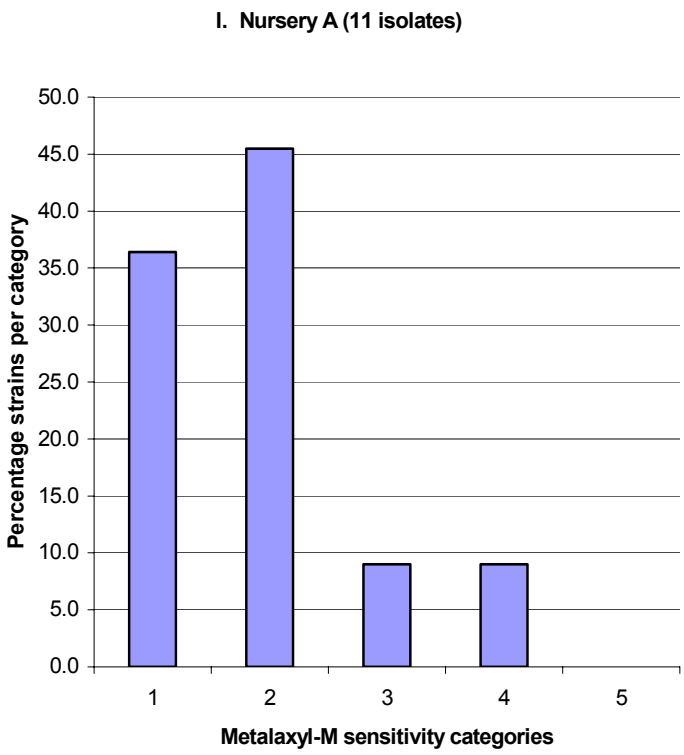
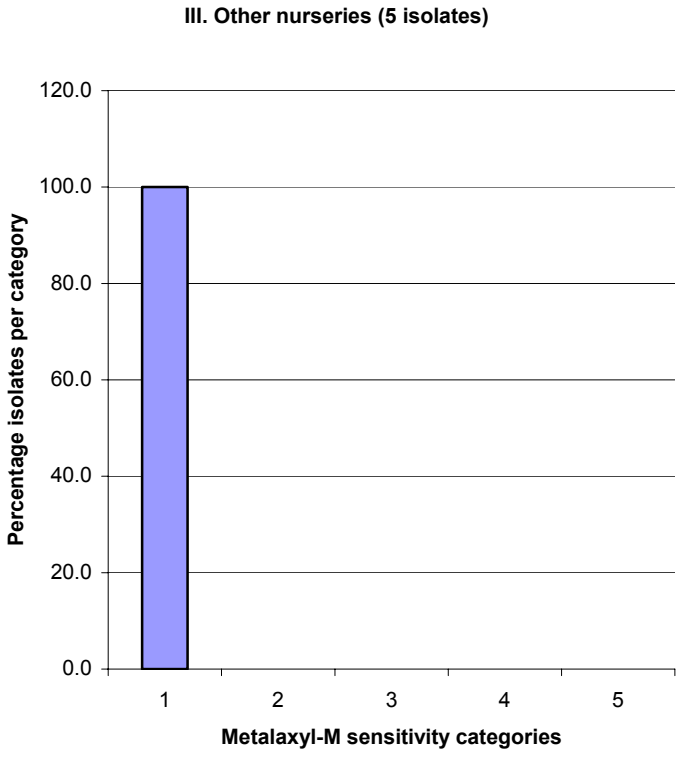


Fig. 4.4.7.1. The distribution of citrus *Phytophthora* isolates, obtained from different nurseries (I, II, III) and orchards (IV) in 7 categories according to the sensitivity to 10 µl/ml metalaxyl-M.







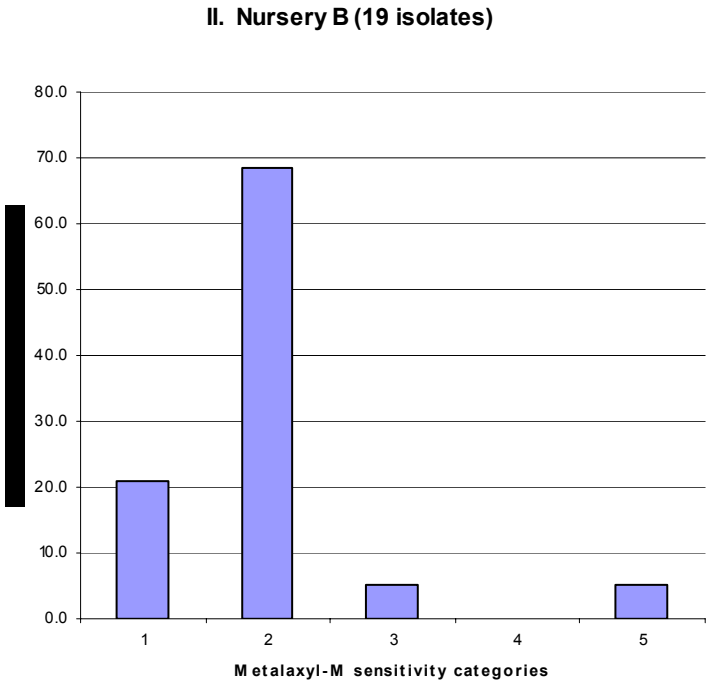


Fig. 4.4.7.2. The distribution of citrus *Phytophthora* isolates, obtained from different nurseries (I, II, III) and orchards (IV) in 5 categories according to the sensitivity to 100 µl/ml metalaxyl-M.

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4.4.8 Evaluate the efficacy of phosphonates applied through the irrigation system on citrus in the Letsitele area for control of *Phytophthora* root rot

Experiment QMS 2006/SBD11 by W. van de Pypekamp & S.H. Swart (QMS)

Opsomming

Phytophthora wortel- en kraagvrot is 'n belangrike siekte in die Suid-Afrikaanse sitrus industrie, en kan 'n nadelige effek op opbrengs en boomkondisie hê. Fosfonate word algemeen gebruik in die bedryf om weerstand teen die patogeen in die plant op te bou. Die produk is as blaarbespuiting en stamverf geregistreer. Die metodes is egter baie arbeidsintensief en kan in uitsonderlike omstandighede fitotoksies vir die plant wees. Deur die produk toe te dien deur die besproeiing, kan die koste van arbeid tot 'n groot mate uitgeskakel word, en kan dit ook minder fitotoksies vir die plant wees onder ekstreem toestande. Die doel van die projek was eerstens om te bepaal wat die optimale dosis is vir toediening deur die besproeiingstelsel, en die effek van verskillende besproeiingstelsels. Tweedens is die invloed van grondtekstuur en dosis op effektiwiteit bepaal, en of die produk onbeskikbaar raak in sekere grondtipes. Laastens is die kuratiewe effek van die verskillende toedieningsmetodes bepaal.

Introduction

The cost of labour and efficacy are two very important factors in the search for new and improved agrochemicals and/or application methods. Work done by Dr. S.H. Swart from QMS Agri Science showed much promise in applying potassium phosphonate through the irrigation system for the control of *Phytophthora* root and collar rot. By doing so the cost component of labour is greatly reduced and dosages are also lower compared to current registered application methods. The aims of this study were to determine the optimal dosage for application of phosphonates through irrigation systems, and the effect of soil texture and depth on efficacy.

Materials and methods

1. <u>The effect of tree age, dosages and type of irrigation system</u>

The trial was conducted in the Letsitele area, Limpopo Province, on drip and micro irrigation. Applications were done on trees of different tree age groups at three different phosphonate rates (Tables 4.4.8.1 and 4.4.8.2). Tree age groups included (if available) were 0-2 years, 3-6 years, 7-10 years and 15 plus years. Phosphonate was applied at rates of ½x, x and 2x and untreated control was also included. Specific rates (ℓ/ha) were used on different tree age groups (Tables 4.4.8.1 and 4.4.8.2). Only the x rates are shown in the tables.

Table 4.4.8.1. Potassium phosphonate dosages applied through micro irrigation for different tree ages.

Tree age	Root zone (m²)	Application rate (x) (ml/tree)	Application rate (x) (ml/m²)	Trees / ha	Application rate (ℓ/ha)	Additional application
0-2	1	21	21	476	10	
2-3	4	42	11	476	20	
4-6	4	42	11	476	20	
7-8	12	71	6	476	34	
9-15	12	71	6	476	34	
15-20	12	71	6	476	34	1
21-25	12	71	6	476	34	1
>25	12	71	6	476	34	2

Table 4.4.8.2: Potassium phosphonate dosages applied through drip irrigation for different tree ages.

Tree age	Root zone (m²)	Application rate (x) (ml/tree)	Application rate (x) (ml/m²)	Trees / ha	Application rate (ℓ/ha)	Additional application
0-2	2	21	5	476	10	
3-6	2	42	10	476	20	
7-15	2	42	10	476	20	1
16-25	2	42	10	476	20	2
>25	2	42	10	476	20	4

The first application was done in September/October 2005 (first season start), the second in December /January 2005/2006 and the third in March 2006.

2. The effect of soil texture (sand vs. clay) and dosage

In the second trial, the effect of clay and sandy soils on dosage and efficacy will be determined. The trial was done on two similar Valencia orchards (age, cultivar, tree spacing and proximity), one on clay and one on sandy soils in the Letsitele area. Dosages were ½x, x and 2x, with an untreated control. Treatments started in December 2005 (first season start) due to drought conditions. The second application was applied in February 2006 and the third and last application in the beginning of April 2006.

3. The effect of application methods on efficacy

The aim of the third trial was to determine the efficacy of the different application methods of potassium phosphonate on the control of *Phytophthora* in old Valencia orchard showing signs of decline. This trial started in October 2005 (first season start) and the second application was done in December 2005. The last application was applied in April 2006. Dosages applied are depicted in Table 4.4.8.3. The dosages for stem and foliar application are as per product registration label and dosages for irrigation application as per Table 4.4.8.1 and 4.4.8.2.

Table 4.4.8.3. Treatments applied in trial to determine the efficacy of application methods during the 2005/2006 growth season.

Treatment Number	Treatment Description	Application method	Dosage	Volume applied per tree
1	Phosguard 400 SL	Leaf application	500 ml/100l	3.8 ℓ
2	Phosguard 400 SL	Trunk paint application	1:1 diluted	Paint stem from ground to graft joint
3	Phosguard 400 SL	Irrigation application	350 ml/100l	10 ℓ
4	Untreated control	-	-	-

Preliminary results and discussion (First season)

Tree trunk diameters and vigour, for each of the three sub trials, was measured in November 2005 (first season) and September 2006, and will again be taken in April/May 2007 (second season) and September 2007 for all sub trials. Crop load and fruit size measurements were also measured in some orchards during the first season to study the preliminary effect of the different application methods. Fruit and soil samples were taken in June 2006 and sent to SGS for phosphorous acid analyses to determine the effect of soil types and different application methods on the uptake of phosphorous acid. Results obtained from SGS were contradictive to dosages applied, and numerous attempts were made to authenticate these readings with SGS, with no success. Phosphorous acid analyses for the second season will be done by SABS, utilizing new and more modern analyses equipment, to ensure accurate readings. Preliminary results obtained during the first season and comprehensive results from the second season will be compared and the effect of each sub trial on factors like fruit size and production, trunk diameter and tree vigour and phosphorous acid levels in the plant and fruit will be statistically determine.

Future research

The application of potassium phosphonate through the irrigation system for the control of *Phytophthora* root and collar rot is becoming more popular among producers. Positive results have been obtained commercially by continuous application over a number of years. Because of the diverse factors affecting the success of potassium phosphonate applications, the continuation of this trial for more than two seasons is necessary to ensure success on a commercial scale and being of value to the industry. Continuing with certain aspects based on future results is also an important part of this trial. The trial started in 2005 and is funded to 2007, second season applications started in September 2006.

4.5 PROJECT: POST-HARVEST PATHOLOGY

Project Co-ordinator: K.H. Lesar (CRI)

4.5.1 Project summary

In South African citrus packhouses, citrus fruits are treated with the post-harvest fungicides, imazalil, thiabendazole (TBZ) and guazatine (certain markets) to control infections caused by post-harvest pathogens. When using chemicals to control diseases, difficulties may be experienced when the pathogen becomes resistant to the pesticide or fungicide. The threat of the development of pathogen resistance to post-harvest fungicides has prompted the ongoing evaluation of new chemicals against the citrus pathogens in order to find new, safe compounds (GRAS compounds, biocontrol agents ec.), potential new chemistry fungicides and even formulated mixtures of two fungicides with different modes of action for more efficient waste control and also prevent the build up of resistance to one or more of the fungicides.

The new post-harvest fungicide Philabuster (from Janssen Pharmaceutica), a mixture of imazalil and pyrimethanil, demonstrated good control of infection by the post-harvest citrus pathogen *P. digitatum* (green mould), compared to the standard Fungazil sulphate 750 WSP (4.5.2).

Low levels of imazalil residues on citrus leads to unacceptable levels of post-harvest decay as well as a decrease in imazalil efficacy on sporulation of green and blue mould infections. Delta Valencia oranges were treated with a double application of imazalil, in a dip treatment at 500 ppm and in a wax application at 2000 ppm. Single applications of both of these treatments were also conducted. Residue levels of 3.2 and 3.4 ppm were recorded for the double application, 4.0 ppm for the single wax application and 0.7 and 0.9 for the single dip treatment. All the residue levels were below the required Minimum Residue Level of 5.0 ppm, but the residue levels in the single dip treatment were below 1.0 ppm. This double application of imazalil demonstrated that acceptable levels of imazalil on fruit will enable sporulation inhibition of green and blue mould and more effective waste control (4.5.3).

Variations in residue analysis results from different laboratories were observed. These variations in results lead to a trial being conducted to compare the results of the different “accredited” laboratories. Valencia oranges were treated with three different concentrations of imazalil and sent to the SABS. A homogenised sample of each treatment was forwarded to each of the “accredited” laboratories to determine any variation in these results (4.5.4).

Citrofresh is an organic sanitising agent. The product was evaluated in a simulated packhouse trial, but did not demonstrate the desired disinfection of a packhouse dump tank, nor the prevention of infection of injured fruit moving through the bath. The product did not demonstrate any fungicidal properties either (4.5.5).

The sanitising agent, Citrex 100 effectively sanitised a simulated packhouse dump tank at consentrations of 1mℓ/ℓ and 2 mℓ/ℓ, compared to the standard recommended quaternary ammonium compound Sporekill. Ozone was not able to be generated at the correct concentration to achieve the desired level of sanitation of the simulated dump tank (4.5.6).

Citrex and Croplife did not control *P. digitatum* infection at the recommended concentrations compared to the standard imazalil sulphate. This demonstrates that these products do not have fungicidal properties (4.5.7).

Wetcit did not demonstrate any activity against *P.digitatum* infection. Wetcit in combination with reduced concentrations of the standard imazalil did not contribute to increasing the efficacy of imazalil against *P. digitatum* infection (4.5.8).

The new fungicide Ortocil (ortho-phenylphenate) demonstrated good control of infection by *P. digitatum* and relatively good control of infection by *Geotrichum candidum* on Valencia oranges, compared to the standard

recommended imazalil sulphate and guazatine. These trials must be repeated and the efficacy of Ortocil must be screened on other citrus cultivars (4.5.9).

A yeast “antagonist”, isolated from the rind of oranges was screened agains the control of *P.digitatum* infections. The yeast demonstrated good preventative control of *P.digitatum* infection. No curative control was observed. Further trials will be conducted with the yeast (4.5.10).

The *in vitro* technique for resistance screening was optimised. Provisional screening of Penicillium spore samples have thusfar shown that not only have resistant spores against imazalil been detected, but also samples that are resistant to the other important post-harvest fungicide, guazatine. These screenings have however been conducted with potentially mixed cultures and the data is thus not conclusive. Single spore cultures will be made of all the Penicillium cultures that have been purified thusfar. They will then be screened against imazalil and guazatine. *In* vivo screenings will be conducted as soon as the resistance levels have been characterised to determine what resistance levels will result in practical resistance (i.e. loss of control) (4.5.11).

The plant growth regulators, Retain, Agromos, Bioboost and the new organic compound Croplife were screened on navel oranges and lemons in water dip treatments for calyx retention on citrus fruit after simulated shipping. Good calyx retention by Retain and Bioboost, compared to the standard recommended 2,4-D (Deccomone), was observed. A reasonable level of calyx retention by Croplife was also observed. Further trials will be conducted on other citrus cultivars (4.5.12).

Projekopsomming

Sitrusvrugte word behandel, in Suid Afrikaanse sitruspakhuise, met die na-oes swamdoders imazalil, thiabendazole (TBZ) en guazatine (sekere markte) om infeksies, veroorsaak deur na-oespatogene, te beheer. Tydens die gebruik van chemikalieë vir siektebeheer, probleme mag ondervind word sodra die patogeen bestand teen die plaagdoder of swamdoder word. Die dreiging van bestande Penicillium swamspoor populasies en moontlike algehele bestandheid teen die klein aantal na-oes swamdoders het die aangaande evaluering vir nuwe chemikalieë veroorsaak om nuwe, veiliger middels (GRAS chemikalieë, biologiesebeheermiddels ens.), nuwe chemie swamdoders en ook geformuleerde mengsels van twee swamdoders met verskillende werking, te ontdek, vir beter bederfbeheer en ook die vermeerdering van bestande spoor populasies te onderdruk.

Die nuwe na-oes swamdoder Philabuster (van Janssen Pharmaceutica), bestaande uit ’n mengsel van imazalil en pyrimethanil, het in vergelyking met die standaard Fungazil sulfaat 750 BP, goeie beheer teen infeksie deur die na-oes sitrus patogeen *P. digitatum* (groenskimmel) getoon. Philabuster kan vir registrasie aanbeveel word (4.5.2).

Lae residuele vlakke van imazalil op sitrusvrugte lei tot onaanvaarbare vlakke van na-oesverrotting, asook ’n verlies aan die onderdrukkende effek van imazalil op sporulering van groen- en blouskimmel infeksies. Delta Valencia vrugte is behandel met ’n dubbel aanwending van imazalil, eerstens in ’n doopbehandeling van 500 dpm en, tweedens, ’n waksaanwending van 2000 dpm, asook enkelaanwendings van hierdie behandelings. Residuvlakke van 3.2 en 3.4 dpm is getoon vir die dubbelbehandeling, 4.0 vir die enkel waksbehandeling, en 0.7 en 0.9 dpm vir die enkel doopbehandeling. Al die vlakke is heelwat onder die toegelate Minimum Residu Toleransie van 5.0 dpm, maar die enkel doopbehandeling het residu-vlakke van <1 dpm gehad. Hierdie dubbele aanwending van imazalil dui aan dat aanvaarbare residu vlakke van imazalil op vrugte sal sorg vir groen- en blouskimmel sporulering inhibisie en beter bederf beheer (4.5.3).

Variasie is opgemerk in residue-analise resultate vanaf verskillende laboratoria. Gevolglik is ’n proef gedoen om die resultate van verskillende ”geakrediteerde” laboratoria te vergelyk. Valencia lemoene is met drie verskillende konsentrasies van imazalil behandel en na SABS gestuur. ’n Gehomogeniseerde monster van elke behandeling is na elke “geakrediteerde” laboratorium gestuur om variasie in resultate te bepaal. Geen resulate is nog ontvang vanaf die laboratoria (4.5.4).

Citrofresh is ’n organiese saniteermiddel. Dit is in gesimuleerde pakproewe geevalueer, maar het nie die gewensde ontsmetting van ’n pakhuis dompelbad getoon nie, en ook nie die besmetting van beseerde vrugte deur die bad voorkom nie. Die produk het ook nie swamdodende eienskappe getoon nie (4.5.5).

Die saniteermiddel Citrex 100 het die gesimuleerde sitrus pakhuis dompelbad effektief teen konsentrasies van 1mℓ/ℓ and 2 mℓ/ℓ gesaniteer in vergelyking met die standaard aanbevole kwaternêre ammonium verbinding, Sporekill. Osoon is nie teen die regte konsentrasie gegenereer nie om die gewensde vlak van sanitasie te bereik nie (4.5.6).

Citrex en Croplife het nie infeksie deur *P. digitatum* teen die aanbevole konsentrasies in vergelyking met die standaard imazalil sulfaat inhibeer nie. Dit wys dan hierdie produkte nie swamdodende eienskappe toon nie (4.5.7).

Wetcit het geen effek op infeksie deur *P.digitatum* gehad nie. Wetcit in kombinasie met laer konsentrasies van die standaard imazalil het nie bygedra tot 'n verbetering in effektiwiteit van imazalil teen besmetting deur *P. digitatum* nie (4.5.8).

Die nuwe swamdoder Ortocil het goeie beheer van infeksie deur die sitrus patogeen *P. digitatum* en redelike goeie beheer van infeksie deur *Geotrichum candidum* op Valencia lemoene in vergelyking met die standard aanbevole imazalil sulfaat en guazatine getoon. Hierdie proewe moet herhaal word en die effektiwiteit van Ortocil op ander sitrus kultivars moet ondersoek word (4.5.9).

"n Gis "antagonis" geisoleer vanaf die skiloppervlak van lemoene is geevalueer teen die beheer van *P.digitatum* infeksies . Die gis "antagonis" het goeie beskermende beheer van *P. digitatum* infeksie getoon. Geen na-infeksie beheer is waargeneem nie. Verdere proewe met die gis sal uitgevoer word (4.5.10).

Die *in vitro* tegniek vir weerstandstoetsing is geoptimiseer. Voorlopige evaluasie van Penicillium spoormonsters tot dusvêr het gewys dat daar nie net bestande spore teen imazalil getoets is nie, maar wel ook monsters met weerstand teen die ander belangrike na-oes swamdoder, guazatine. Hierdie evaluasie is egter gedoen met potensiëel gemengde kulture, en die data is dus nie konklusief nie. Enkelspoorkulture sal van alle Penicillium kulture wat tot dusver gesuiwer is, gemaak word, waarna dit teen imazalil en guazatine getoets sal word. Sodra weerstandsvlakke gekarakteriseer is, sal *in vivo* toetse gedoen word om te bepaal watter weerstandsvlakke sal lei to praktyk-weerstand (i.e. verlies aan beheer) (4.5.11).

Die plantgroeireguleerders, Retain, Agromos, Bioboost en die nuwe organiese middel Croplife is op nawel lemoene en suurlemoene in water doopbehandelings aangewend vir blomkelk-behoud op sitrusvrugte na gesimuleerde verskeping. Goeie blomkelk behoud deur Retain en Bioboost in vergelyking met die standaard aanbevole 2,4-D (Deccomone) is waargeneem. 'n Redelike gehalte van blomkek behoud deur Croplife is ook waargeneem. Verdere proewe op ander sitruskultivars sal uitgevoer word (4.5.12).

4.5.2 **The evaluation of a new post-harvest fungicide Philabuster from Janssen Pharmaceutica against post-harvest disease for the purpose of registration**

Experiment 123 by K.H. Lesar (CRI)

Opsomming

Die nuwe na-oes swamdoder Philabuster (van Janssen Pharmaceutica), bestaande uit 'n mengsel van imazalil en pyrimethanil, het in vergelyking met die standaard Fungazil sulfaat 750 BP, goeie beheer teen infeksie deur die na-oes sitrus patogeen *P. digitatum* (groenskimmel) getoon. Philabuster kan vir registrasie aanbeveel word.

Introduction

In the South African citrus industry, the post-harvest fungicide imazalil is applied to export citrus for the control of *Penicillium digitatum* (green mould) and *Penicillium italicum* (blue mould). Guazatine, having a different mode of action than imazalil is also recommended for the control of green and blue mould. Both products are recommended together in a dip treatment for the purpose of controlling infections caused by these two pathogens, and also preventing the build-up of Penicillium resistant biotypes to these two fungicides.

Janssen Pharmaceutica (Belgium) has recently formulated two post-harvest fungicides, imazalil and pyrimethanil into a single mixture compound named Philabuster. Imazalil and pyrimethanil have different modes of action against *P. digitatum* and *P. italicum* and would also prevent the build-up of fungicide resistant biotypes. The added advantage of formulating the two fungicides into one compound allows for a more practical and efficient application of the two post-harvest fungicides. A sample of Philabuster was provided to Citrus Research International (CRI) by Janssen Pharmaceutica for evaluation of efficacy against post-harvest *Penicillium* infection of citrus.

Materials and methods

In vivo evaluation trials were conducted with the Janssen Pharmaceutica Philabuster 400 SC (200 g/ℓ imazalil and 200 g/ℓ pyrimethanil) on Navel and Valencia oranges that were inoculated with an **imazalil sensitive strain** of *P. digitatum*. Philabuster was compared with the standard Fungazil sulphate 750 WSP (Janssen Pharmaceutica) at the recommended commercial rate of 67 g/100 ℓ giving a treatment concentration of 500 ppm imazalil. Philabuster was evaluated at the rates of 0.125% (1/2x), 0.25% (1x) and 0.5% (2x), giving treatment concentrations of 250, 500 and 1000 ppm, respectively.

A spore suspension of *P. digitatum* was made up by suspending spores in sterile deionised water containing the surfactant, Tween 20. The spore suspension was spectrophotometrically adjusted to a concentration of $1x10^6$ spores/mℓ.

Untreated navel and Valencia oranges (Crocodile Valley Estate) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected. The fruit was washed in clean water and surface sterilised by dipping in a 1% NaOCl solution for 2 minutes. .The fruit was then allowed to dry prior to inoculation.

Inoculation

The inoculation of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides, giving a total of 60 inoculation sites per treatment. Each fruit was then infected with the pathogen by applying 35 µℓ of spore suspension to each injury site using a micropipette. The inoculated fruit was incubated for 12 hours at ±23°C (to simulate a 12-hour delay after harvest before packhouse treatments) before treatment with the chemical compound being evaluated.

Treatments

Inoculated fruit was divided into 3 replicates of 10 fruit per treatment. All the treatments involved a 3-min dip at ambient temperature in the following treatments:

1. Untreated control (*P. digitatum*) - water dip
2. Standard treated control – Fungazil WSP 67g /100 ℓ (500 ppm imazalil)
3. Philabuster – 0.125% (250 ppm imazalil and 250 ppm pyrimethanil)
4. Philabuster – 0.25% (500 ppm imazalil and 500 ppm pyrimathanil)
5. Philabuster – 0.5% (1000 ppm imazalil and 1000 ppm pyrimethanil)

After treatment, the fruit was incubated in paper packets at 20°C for 7 days or until such time as the untreated controls exhibited sufficient green mould. The treatments were then evaluated by counting the number of infected wounds per treatment and the results were recorded as percentage decay inhibition.

The trial was conducted twice on navel oranges and once on Valencia oranges.

Results

The new fungicide Philabuster 400 SC demonstrated good control of the citrus pathogen *P. digitatum* infection on navel and Valencia oranges, compared to the standard recommended Fungazil sulphate 750 WSP (Table 1). In al three trials, Philabuster and Fungazil effected 100% inhibition of green mould, except for the 0.125% Philabuster treatment, which showed between 80% and 90% inhibition. No phytotoxicity was evident on the fruit treated at the highest concentration (0.5%) of Philabuster.

Table 4.5.2.1. Percentage inhibition of green mould on Navel and Valencia oranges that were wounded and inoculated with *Penicillium digitatum* 12 hours before dip-treatment in water with 0.125%, 0.25% and 0.5% Philabuster and 67 g/100ℓ Fungazil.

Treatments	% Inhibition [a]		
	Navels (Trial 1)	Navels (Trial 2)	Valencia
1. Untreated control	0.0 b	0.0 b	0.0 b
2. Treated control: Fungazil (67 g/100 ℓ)	100.0 a	100.0 a	100.0 a
3. Philabuster (0.125%)	90.0 a	90.0 a	90.0 a
4. Philabuster (0.25%)	100.0 a	100.0 a	100.0 a
5. Philabuster (0.5%)	100.0 a	100.0 a	100.0 a

[a] Values represent the means of 3 replicates of 10 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; P≤ 0.05).

Conclusion

The Philabuster 400 SC by Janssen Pharmaceutica demonstrated good control of the citrus pathogen *P. digitatum* compared to the standard, recommended Fungazil sulphate 750 WSP. Treated Valencia orange fruit samples were submitted to SABS for residue analyses. At the recommended treatment concentration of 0.25% (1x), the residue levels recorded were 2.8 and 2.3 mg/kg for pyrimethanil and imazalil, respectively. At 0.5% (2x), residue levels were 3.6 and 4.7 mg/kg, respectively. The current MRL for imazalil is 5.0 mg/kg and the expected MRL for pyrimethanil would also be set at 5.0 mg/kg, as reported by Janssen Pharmaceutica. Based on these results the product can therefore be recommended by CRI for registration as a dip or drench application at a concentration of 500 ppm (a.i.).

4.5.3 **Residue analyses on fruit samples treated with imazalil sulphate 750 WSP and Fungazil 500 EC for sporulation inhibition**
Experiment 123 by K.H. Lesar (CRI)

Opsomming

Lae residuele vlakke van imazalil op sitrusvrugte lei tot onaanvaarbare vlakke van na-oesverrotting, asook 'n verlies aan die onderdrukkende effek van imazalil op sporulering van groen- en blouskimmel infeksies. Delta Valencia vrugte is behandel met 'n dubbel aanwending van imazalil, eerstens in 'n doopbehandeling van 500 dpm en, tweedens, 'n waksaanwending van 2000 dpm, asook enkelaanwendings van hierdie behandelings. Residuvlakke van 3.2 en 3.4 dpm is getoon vir die dubbelbehandeling, 4.0 vir die enkel waksbehandeling, en 0.7 en 0.9 dpm vir die enkel doopbehandeling. Al die vlakke is heelwat onder die toegelate Minimum Residu Toleransie van 5.0 dpm, maar die enkel doopbehandeling het residu-vlakke van <1 dpm gehad. Hierdie dubbele aanwending van imazalil dui aan dat aanvaarbare residu vlakke van imazalil op vrugte sal sorg vir groen- en blouskimmel sporulering inhibisie en beter bederf beheer.

Introduction

The citrus industry has been experiencing higher than normal levels of waste on export citrus for the past 2-3 seasons. An important contributing factor to the overall problem of the higher incidence of waste (specifically the Penicillium wound pathogens) on export citrus over the last two seasons was the overall low residue level of imazalil (< 1.0 mg/kg) retained on export citrus after treatment in the packhouses, as observed in the majority of residue analyses recorded by the accredited test laboratories. This resulted in Penicillium sporulation on infected fruit and excessive spread of infections in export cartons with high waste and also an increased risk of selection for resistant spores.

In order to investigate means of increasing residue levels to levels that would inhibit sporulation (1.0–2.0 mg/kg) without exceeding MRL levels (5.0 mg/kg), a trial was conducted with a double application of imazalil *viz.* a water dip treatment of imazalil sulphate 750 WSP (standard rate of 500 mg/kg), and a dip application of Fungazil 500 EC (2000 mg/kg) in citrus wax.

Materials and methods

Good sound, untreated Delta Valencia oranges (Crocodile Valley Estate) were obtained in bulk. The fruit was washed in clean water and surface sterilised by dipping in a 1% NaOCl solution for 2 minutes minutes. The fruit was then allowed to dry prior to treatment. Treatments involved the following:

1. Untreated control (Water dip)
2. 500 mg/kg imazalil sulphate (Fungazil 750 WSP) in dip treatment
3. 2000 mg/kg imazalil EC (Fungazil 500 EC) in wax application
4. 500 mg/kg Fungazil 750 WSP (dip) and 2000 mg/kg Fungazil 500 EC (wax)

The treatments, with imazalil sulphate 750 WSP were done in a water dip at ambient temperature. After treatment the fruit was left to dry in order to retain a residue of the imazalil sulphate on the fruit prior to the second application of imazalil incorporated in the wax. All the treatments in the water and wax dips were immersed for 3 minutes. After treatment with the second imazalil application in the wax dip the fruit was allowed to dry overnight. Treated samples were placed in paper bags, labelled and stored for 2-3 days in the deep freeze until frozen and then couriered to the SABS for residue analyses.

Results

Residue levels of all the imazalil treatments were below the MRL of 5.0 mg/kg for imazalil in all markets (Table 4.5.3.1). Dip treatment with imazalil (Fungazil 750 WSP) resulted in residue levels below 1 mg/kg.

Table 4.5.3.1. The recorded results of duplicate imazalil residue analyses conducted by SABS.

	Treatments	Imazalil residue level (mg/kg)
1	Water dip	Nil
2	500 mg/kg imazalil 750 WSP dip	0.7 ; 0.9
3	2000 mg/kg imazalil 500 EC in wax	4.0 ; 4.0
4	500 mg/kg imazalil 750 WSP + 2000 mg/kg imazalil 500 EC in wax	3.2 ; 3.4

Conclusion

The recorded imazalil residue results above indicate that the double application of imazalil in a citrus packhouse can be safely recommended without exceeding the MRL of 5.0 mg/kg for imazalil in all markets.

The imazalil residue levels attained after double application in the packhouse will also achieve the desired inhibition of Penicillium sporulation and reduce the selection for resistant spores, should there be Penicillium infections in export cartons of citrus (Figure 4.5.3.1).

A single imazalil application by means of 2000 mg/kg imazalil 500 EC in wax also resulted in effective residue levels without exceeding MRL levels. A single dip-application of imazalil did, however, not attain residue levels of higher than the desired 1 mg/kg.

Figure 4.5.3.1. Green mould decay on imazalil-treated Valencia oranges with residue levels below (left) and above (right) 1 mg/kg. Note the absence of sporulation in the latter instance.

4.5.4 **Imazalil residue "ring test" to determine if the test procedure is standardised at all accredited test laboratories**
Experiment 123 by K.H.Lesar (CRI)

Opsomming

Variasie is opgemerk in residue-analise resultate vanaf verskillende laboratoria. Gevolglik is 'n proef gedoen om die resultate van verskillende "geakrediteerde" laboratoria te vergelyk. Valencia lemoene is met drie verskillende konsentrasies van imazalil behandel en na SABS gestuur. 'n Gehomogeniseerde monster van elke behandeling is na elke "geakrediteerde" laboratorium gestuur om variasie in resultate te bepaal. Geen resulate is nog ontvang vanaf die laboratoria.

Introduction

Over the last 3-4 seasons, imazalil residue results, as determined by the two NDA laboratories on fruit samples routinely drawn by PPECB from consignments of export cartons in packhouses, have indicated inconsistencies between the two laboratories. Results from fruit samples submitted to the other "accredited" laboratories also revealed discrepancies. This has resulted in the recording of high residue levels and the holding back of export consignments of citrus. In many cases, this delay was unnecessary because of questionably "high" residue levels, and caused consternation and frustration among producers. Moreover, current recommended concentrations and applications of imazalil in packhouses does not warrant many of the high (>5 up to as high as 30.0 mg / kg) residue levels recorded.

This experiment was conducted to determine the variability in residue results conducted by the different test laboratories. A “ring test” was conducted where oranges were treated with three different concentrations of imazalil. A homogenised sample of each treatment was sent to each “accredited” test laboratory to determine if the testing procedure in all the labs is a standardised procedure

Materials and methods

Good sound, untreated Valencia oranges (Crocodile Valley Estate) were obtained in bulk. The fruit was washed in clean water and surface sterilised by dipping in a 1% NaOCl solution for 2 minutes minutes. The fruit was then allowed to dry prior to treatment.

Three treatments, with imazalil sulphate 750 WSP were done in a water dip at ambient temperature. All the treatments were dipped for 3 minutes. Treatments were an untreated control (water dip), 250 mg/kg, 500 mg/kg and 1000 mg/kg imazalil sulphate. Each treatment consisted of ten fruit. After treatment the fruit was left to dry in order to retain a residue of the imazalil sulphate on the fruit. After drying, all the treated samples were placed in paper bags, labelled and stored for 2-3 days in the deep freeze until frozen and then couriered to the SABS for preparation of the homogeneous samples. Sub-samples were subsequently distributed to the “accredited” laboratories for imazalil residue analyses.

Results

These results are still pending.

4.5.5 The evaluation of Citrofresh in a citrus packhouse dumptank washing system as a sanitising agent against post-harvest disease

Experiment 123 by K.H. Lesar (CRI)

Opsomming

Citrofresh is ’n organiese saniteermiddel. Dit is in gesimuleerde pakproewe geevalueer, maar het nie die gewensde ontsmetting van ’n pakhuis dompelbad getoon nie, en ook nie die besmetting van beseerde vrugte deur die bad voorkom nie. Die produk het ook nie swamdodende eienskappe getoon nie.

Introduction

The sanitising agent Citrofresh 14P (an organic sanitiser/biocide) was evaluated in a simulated dump tank washing system for efficacy of sanitising the system and preventing possible infection of injured fruit moving through the system. The product was compared with the standard recommended Sporekill (a quaternary ammonium compound). The simulated dump tank was seeded with a suspension of the post-harvest pathogen *Penicillium digitatum* (green mould) in this evaluation. In terms of fungicidal properties against *P. digitatum*, Citrofresh was also *in vivo* evaluated and compared with the standard recommended imazalil sulphate 750 WSP.

Materials and methods

Evaluation as sanitising agent. A spore suspension of this pathogen was made up by suspending spores in sterile deionised water containing the surfactant, Tween 20. The spore suspension was spectrophotometrically adjusted to a concentration of 1 x 10^6 spores/mℓ. Good, sound, untreated Valencia oranges from Crocodile Valley Citrus Co. were obtained in bulk. The fruit was divided into lots of 10 fruit per treatment and all the fruit was washed in clean water and surface sterilised by dipping in a 1% NaOCl solution for 2 minutes minutes. The fruit was allowed to dry prior to the treatments being conducted.

A clean water dump tank washing system in a packhouse was simulated. The dump tank water was maintained at ambient temperature for this trial. The clean, surface sterilised Valencia oranges were injured by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured three times, twice equatorially on opposite sides and once at the stylar end of the fruit, giving a total of 30 injury sites per treatment.

The 10 fruit of the untreated control treatment were dipped in water in the clean dump tank for 3 minutes. The system was then seeded with the 1x10^6 spores/ml concentration of *P. digitatum*. Untreated, injured fruit dipped in the contaminated water for 3 minutes (Untreated, inoculated control). The contaminated dump tank was then sanitised with concentrations of 5, 7 or 10% solutions of Citrofresh for 10 minutes. Injured fruit was dipped in each of the ‘sanitised’ systems for 3 minutes. In a similar manner, injured fruit was also

exposed to the standard recommended quaternary ammonium compound Sporekill at concentrations of 1 ℓ and 2 ℓ/1000 ℓ for 3 minutes.

To summarise, treatments involved the following:
1. Untreated control - injured fruit dipped in clean water.
2. Untreated, inoculated control – injured fruit dipped in water + $1x10^6$ *P. digitatum* spores / mℓ.
3. Injured fruit dipped in water + $1x10^6$ *P. digitatum* spores / mℓ + 5% Citrofresh.
4. Injured fruit dipped in water + $1x10^6$ *P. digitatum* spores / mℓ + 7% Citrofresh.
5. Injured fruit dipped in water + $1x10^6$ *P. digitatum* spores / mℓ + 10% Citrofresh.
6. Untreated Control as in 1.
7. Untreated, inoculated control as in 2.
8. Injured fruit dipped in water + $1x10^6$ *P. digitatum* spores / mℓ + 1 ℓ/1000 ℓ Sporekill.
9. Injured fruit dipped in water + $1x10^6$ *P. digitatum* spores / mℓ + 2 ℓ/1000 ℓ Sporekill.

All the treated fruit were placed in paper packets and incubated for 7-10 days at 23°C. After incubation the fruit was evaluated for decay and the results were recorded as percentage decay (number of infected wounds).

In vivo evaluation for fungicidal properties. For this aspect of the experiment, Navel oranges (obtained and pre-treated as above) were wounded with a rubber bung with two protruding nails (2mm long). Each fruit was prick-injured twice equatorially on opposite sides giving 20 infection sites per treatment. Each fruit was then infected with the pathogen by applying 35 µℓ of a $1x10^6$ *P. digitatum* spore suspension to each injury site using a micropipette.

After 4 hours incubation at ±23°C, the following treatments were conducted:
1. Untreated control (clean water).
2. Treated control 500 ppm imazalil sulphate WSP.
3. 5% Citrofresh.
4. 7% Citrofresh.
5. 10% Citrofresh.

Each treatment involved an immersion in a water bath at ambient temperature for 3 minutes. After treatment, the fruit was incubated in paper packets at 20°C for 7-10 days or until such time as the controls had sufficiently rotted. The treatments were then evaluated and the results recorded as percentage decay (number of infected wounds).

Results

Evaluation as sanitising agent. All the fruit in the Citrofresh-treated dump tank were infected with green mould, indicating that Citrofresh did not effectively sanitise a citrus packhouse simulated dump tank washing system at concentrations of 5, 7, and 10% (Table 4.5.5.1). The standard recommended quaternary ammonium compound Sporekill at 1 ℓ and 2 ℓ/1000 ℓ reduced green mould decay incidence to 6.7% and 0%, respectively.

Table 4.5.5.1. The percentage decay incidence recorded on Valencia fruit that were injured and dip-treated in a simulated dump tank system with clean water, or water with $1x10^6$ *P. digitatum* spores that had been treated with different concentrations of Citrofresh or Sporekill.

Treatments	% Decay
1. Untreated Control	Nil
2. Treated Control	96.6
3. 5% Citrofresh	100
4. 7% Citrofresh	100
5.10% Citrofresh	100
6. Untreated control	Nil
7. Treated Control	93.3
8. Sporekill 1.0 ℓ / 1000 ℓ	6.7
9. Sporekill 2.0 ℓ / 1000 ℓ	Nil

In vivo evaluation for fungicidal properties. The results (Table 4.5.5.2) indicate that Citrofresh did not inhibit infections by the pathogen *P. digitatum* at the concentrations of 5, 7, and 10% compared to the standard

recommended imazalil sulphate WSP. This demonstrates that Citrofresh does not exhibit any fungicidal properties.

Table 4.5.5.2. The percentage decay incidence recorded on Navel fruit that were injured and inoculated with 35 µℓ of a 1x10^6 *P. digitatum* spore suspension to each injury site and subsequently treated with clean water, 500 ppm imazalil sulphate WSP or Citrofresh (5%, 7% and 10%).

Treatments	% Decay
1. Untreated Control (clean water)	100
2. Treated control 500 ppm imazalil sulphate WSP	Nil
3. 5% Citrofresh	100
4. 7% Citrofresh	100
5.10% Citrofresh	100

Conclusion

The organic sanitiser/biocide Citrofresh 14P did not effectively sanitise a simulated citrus packhouse dump tank washing system at concentrations of 5, 7, and 10%, compared to the standard recommended quaternary ammonium compound Sporekill. Moreover, Citrofresh did not inhibit infection by the post-harvest citrus pathogen *P. digitatum* thereby not demonstrating any fungicidal properties. No further work is planned with this product.

4.5.6 **The evaluation of Citrex and Ozone in a citrus packhouse dumptank washing system as sanitising agents**
Experiment 123 by K.H. Lesar (CRI)

Opsomming

Citrex 100 het die gesimuleerde sitrus pakhuis dompelbad effektief teen konsentrasies van 1mℓ/ℓ and 2 mℓ/ℓ gesaniteer in vergelyking met die standaard aanbevole kwaternêre ammonium verbinding, Sporekill. Osoon is nie teen die regte konsentrasie gegenereer nie om die gewensde vlak van sanitasie te bereik nie.

Introduction

The two sanitising agents Citrex 100 (an organic "fungicide") and Ozone (generated on site by means of a mini generator), were evaluated in a simulated dump tank washing system for efficacy of sanitising the system and preventing possible infection of injured fruit moving through the system. The two products were compared with the standard recommended Sporekill (a quaternary ammonium formulation). The simulated dump tank was seeded with a suspension of the post-harvest pathogen *Penicillium digitatum* (green mould) in this evaluation.

Materials and methods

A spore suspension of this pathogen was made up by suspending spores in sterile deionised water containing the surfactant, Tween 20. The spore suspension was spectrophotometrically adjusted to a concentration of 1 x 10^6 spores/mℓ. Untreated Valencia oranges from Crocodile Valley Citrus Co. were obtained in bulk. Blemish free, sound fruit was selected for experimentation. The fruit was divided into lots of 10 fruit per treatment, washed in clean water and surface sterilised by dipping in a 1% NaOCl solution for 2 minutes. The fruit was allowed to dry prior to further treatment.

A clean water dump tank washing system in a packhouse was simulated. The dump tank water was maintained at ambient temperature for this trial. The clean, surface sterilised Valencia oranges were injured by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured three times, twice equatorially on opposite sides and once at the stylar end of the fruit, giving a total of 30 injury sites per treatment of 10 fruit. Each treatment was left in the dump tank for 3 minutes. The first lot of 10 injured fruit in the clean water bath served as the untreated control. The system was then seeded with the 1x10^6 spores/ml concentration of *P. digitatum.*

For the Citrex treatments, the seeded dump tank was treated with recommended concentrations of 0.5, 1.0 and 2.0 mℓ/ℓ of Citrex 100. For the ozone treatment, 5 ℓ of clean water was ozonated by continuous bubbling of ozone from the generator into the water for 45 minutes. A maximum Oxidation Reduction Potential (ORP) reading of 450 Mv was recorded and after a further 15 minutes ozonation, the desired reading of 600-700 Mv

could not be attained. Injured fruit was then dipped in the ozonated water for 3 minutes (untreated control), which reduced the ORP reading to 400 Mv. Thereafter the system was seeded with the *P. digitatum* spores as above. Injured fruit was dipped in the contaminated water for 3 minutes and removed. The contaminated system was then again treated with ozone. The starting ORP reading was recorded at 300 Mv. After 1hr 15min ozonation, the maximum ORP reading that could be recorded was 450 Mv. Injured fruit were again allowed to stand in the 'sanitised' system for 3 minutes. The standard quaternary ammonium compound Sporekill was used as control at concentrations of 1 L and 2 L/1000 L for 3 minutes exposure as well.

Treatments

1. Untreated control – injured fruit dipped in clean water.
2. Treated control – injured fruit dipped in water + 1 x 10^6 spores / mℓ.
3. Injured fruit dipped in water + spores + 2.5 mℓ / 5 ℓ Citrex 100.
4. Injured fruit dipped in water + spores + 5.0 mℓ / 5 ℓ Citrex 100.
5. Injured fruit dipped in water + spores + 10.0 mℓ / 5 ℓ Citrex 100.
6. Untreated Control as in 1.
7. Treated control as in 2.
8. Injured fruit dipped in water + spores + 1ℓ / 1000 ℓ Sporekill.
9. Injured fruit dipped in water + spores + 2ℓ / 1000 ℓ Sporekill.
10. Untreated control - injured fruit dipped in ozonated water.
11. Treated control - injured fruit dipped in water + 1 x 10^6 spores / mℓ.
12. Injured fruit dipped in "contaminated" water after ozonation.

All the treated fruit were placed in paper packets and incubated for 7-10 days at 23°C. After incubation the fruit was evaluated for decay and the results were recorded as percentage decay (number of infected wounds)

Results and discussion

The organic antimicrobial compound (organic "fungicide") Citrex 100 at concentrations of 1mℓ / ℓ and 2 mℓ / ℓ demonstrated an effective level of disinfection of a packhouse dump tank washing system, and the product was also able to achieve a high level of preventing the infection of injured fruit moving through the system, compared with the standard recommended Sporekill (Table 4.5.6.1). Ozone, however, did not attain the same level of disinfection of the dump tank nor did it prevent infection of injured fruit moving through the system.

Table 4.5.6.1. The mean percentage decay incidence recorded on Valencia oranges that were injured and dip-treated in a simulated dump tank system containing clean water, a *P. digitatum* spore suspension, and/or different concentrations of Citrex 100 or Sporekill, ozonated clean water, or ozonated contaminated water.

Treatments	% Decay
1. Untreated Control	Nil
2. Treated Control	100
3. 2.5 ml / 5L Citrex 100	20
4. 5.0 ml / 5L Citrex 100	6.7
5. 10.0 ml / 5L Citrex 100	6.7
6. Untreated control	3.3
7. Treated Control	100
8. Sporekill 1.0L / 1000L	6.7
9. Sporekill 2.0L / 1000L	Nil
10. Untreated Control	6.7
11. Treated Control	100
12. Injured fruit in ozonated water	100

One of the most important requirements for the use of such a sanitising agent in the washing systems of a citrus packhouse is a suitable test kit. The movement of large quantities of citrus fruit through a washing system leads to the rapid depletion of the sanitising agent and the soiling of the system. Therefore, the concentration of the sanitising agent needs to be monitored regularly and the system needs to be topped up with the correct concentration of the sanitiser to maintain the desired and effective level of disinfection at all times during production.

Although promising results were obtained with Citrex, a suitable titration-type concentration test kit (**no dip sticks are recommended)** will need to be designed before further trials, including packhouse trials, could be

proposed. It would also be appropriate to evaluate the product at higher concentrations than those used in this evaluation.

Ozone was not effective in this experiment as effective concentrations could not be attained in the simulated dump tank system. Moreover, research in the Florida (USA) citrus industry has shown that the higher the build-up of organic matter in a water-washing system, the more flighty the ozone becomes and more difficult it is to maintain an effective residual concentration in solution. This could also explain why it was not possible to achieve the correct ORP reading in this trial, and thus not an efficient disinfection system. With regards to the use of ozone in postharvest systems, the following was stated in literature: "Effective but safe concentrations of ozone are difficult to maintain in typical post-harvest applications because automated detection systems have not been highly reliable in complex (dissolved or suspended inorganic and organic constituents) process water. Newer electrode probes that measure oxidation-reduction potential (ORP) of the water are being used to monitor ozone concentrations more accurately, **but problems in practical application still exists** (University of Florida "Packinghouse Newsletter" August 2003)

4.5.7 **The evaluation of Citrex and Croplife *in vivo* against post-harvest citrus disease**
Experiment 123 by K.H. Lesar (CRI)

Opsomming

Citrex en Croplife het nie infeksie deur *P. digitatum* teen die aanbevole konsentrasies in vergelyking met die standaard imazalil sulfaat inhibeer nie. Dit wys dan hierdie produkte nie swamdodende eienskappe toon nie.

Introduction

The organic compounds Citrex 100 (organic "fungicide") and Croplife (nutrient synergist) were evaluated *in vivo* against *P. digitatum* to determine if the products had any fungicidal properties. The products were compared with the standard recommended imazalil sulphate 750 WSP.

Materials and methods

A spore suspension of this pathogen was made up by suspending spores in sterile deionised water containing the surfactant, Tween 20.The spore suspension was then spectrophotometrically adjusted to a concentration of 1 x 10^6 spores / mℓ. Good, sound, untreated navel oranges from Crocodile Valley Citrus Co. were obtained in bulk. The fruit was divided up into lots of 10 fruit per treatment and all the fruit was washed in clean water and surface sterilised by dipping in a 1% NaOCl solution for 2 minutes minutes. The fruit was allowed to dry prior to treatment.

The inoculation of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides, giving 20 infection sites per treatment. Each fruit was infected with the pathogen by applying 35 µℓ of spore suspension to each injury site using a micropipette.

Four hours after inoculation, fruit was treated with the chemical compounds. Treatment involved immersion in the treatment suspensions at ambient temperature for 3 minutes. The following treatments were conducted:

1. Untreated Control (clean water).
2. Treated Control (500 ppm Imazalil Sulphate WSP).
3. 0.5 mℓ/ℓ Citrex.
4. 1.0 mℓ/ℓ Citrex.
5. 2.0 mℓ/ℓ Citrex.
6. 30 mℓ/100 ℓ Croplife.
7. 70 mℓ/100 ℓ Croplife.
8. 140 mℓ/100 ℓ Croplife.

After treatment, the fruit was incubated in paper packets at 20°C for 7-10 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay (number of infected wounds).

Results

Neither Citrex nor Croplife inhibited infections by the pathogen *P. digitatum* at the recommended concentrations compared with the standard recommended imazalil sulphate WSP (Table 4.5.7.1). This demonstrates that neither of these compounds have fungicidal properties.

Table 4.5.7.1. Mean percentage decay on navel oranges that were wounded, inoculated with a *P. digitatum* spore suspension and subsequently treated with clean water, imazalil sulphate and various concentrations of Citrex and Croplife.

Treatments	% Decay
1. Untreated Control	100
2. Treated control 500 ppm Imazalil Sulphate WSP	Nil
3. 0.5 mℓ/ℓ Citrex	100
4. 1.0 mℓ/ℓ Citrex	100
5. 2.0 mℓ/ℓ Citrex	100
6. 30 mℓ/100 ℓ Croplife	100
7. 70 mℓ/100 ℓ Croplife	100
8. 140 mℓ/100 ℓ Croplife	100

Conclusion

Neither of the organic compounds, Citrex nor Croplife, inhibited infections by the post-harvest citrus pathogen *P. digitatum*, thereby not demonstrating any fungicidal properties. Citrex 100 will be further evaluated in a simulated dumptank washing system and Croplife as a nutrient synergist.

4.5.8 **The evaluation of Wetcit against the control of post-harvest disease**
Experiment 123 by K.H. Lesar (CRI)

Opsomming

Wetcit het geen effek op infeksie deur *P.digitatum* gehad nie. Wetcit in kombinasie met laer konsentrasies van die standaard imazalil het nie bygedra tot 'n verbetering in effektiwiteit van imazalil teen besmetting deur *P. digitatum* nie.

Introduction

The surfactant Wetcit, which has excellent wetting properties, is particularly suitable for application of pesticides/fungicides at reduced concentrations for more effective utilisation of a fungicide in controlling plant diseases. Wetcit also contains the inorganic compound Borax that has been reported to demonstrate some fungicidal properties against plant pathogens.

Wetcit was evaluated alone and in combination with reduced concentrations of the fungicide imazalil for efficacy against infections caused by the post-harvest pathogen *P. digitatum.*

Materials and methods

A spore suspension of this pathogen was made up by suspending spores in sterile deionised water containing the surfactant, Tween 20. The spore suspension was spectrophotometrically adjusted to a concentration of 1 x 10^6 spores / mℓ. Good, sound, untreated Valencia oranges from Crocodile Valley Citrus Co. were obtained in bulk. The fruit was divided up into 3 replicates of 10 fruit per treatment and all the fruit was washed in clean water and surface sterilised by dipping in a 1% NaOCl solution for 2 minutes. The fruit was then allowed to dry prior to the treatments being conducted.

Each fruit was wounded by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides giving 60 infection sites per treatment. Each fruit was then infected with the pathogen by applying 35 µℓ of spore suspension to each injury site using a micropipette.

The inoculated fruit was treated 4 hours after inoculation with the chemical compounds by immersion in a water bath containing each treatment suspension at ambient temperature for 3 minutes. The following treatments were conducted:

1. Untreated control (clean water).
2. Treated control (500 ppm imazalil sulphate 750 WSP).
3. Wetcit (50 mℓ/100 ℓ).
4. Wetcit (100 mℓ/100 ℓ).
5. Imazalil sulphate (25 ppm).
6. Imazalil sulphate (50 ppm).
7. Imazalil sulphate (100 ppm).
8. 100 mℓ/100ℓ Wetcit + 25 ppm imazalil sulphate.
9. 100 mℓ/100ℓ Wetcit + 50 ppm imazalil sulphate.
10. 100 mℓ/100ℓ Wetcit + 100 ppm imazalil sulphate.

After treatment, the fruit was incubated in paper packets at 20°C for 7-10 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay (number of infected wounds).

Results

Wetcit on its own had no effect in controlling infections by this pathogen (Table 4.5.8.1). Wetcit in combination with lower dosages than the standard dosage of imazalil, did not aid in any increased efficacy of imazalil in controlling *P. digitatum* infection.

Table 4.5.8.1. Mean percentage decay on Valencia oranges that were wounded, inoculated with a *P. digitatum* spore suspension and subsequently dip-treated in clean water, Wetcit (50 and 100 mℓ/100ℓ) and various concentrations of imazalil sulphate with or without Wetcit at 100 mℓ/100 ℓ.

Treatments	% Inhibition[a]
1. Untreated control (clean water)	0.0 e
2. Treated control (500 ppm imazalil sulphate 750 WSP)	100.0 a
3. Wetcit (50 mℓ/100ℓ)	0.0 e
4. Wetcit (100 mℓ/100ℓ)	0.0 e
5. Imazalil sulphate (25 ppm)	20.0 d
6. Imazalil sulphate (50 ppm)	53.3 c
7. Imazalil sulphate (100 ppm)	80.0 b
8.100 mℓ/100ℓ Wetcit (borax) + 25 ppm imazalil sulphate	15.0 d
9.100 mℓ/100ℓ Wetcit (borax) + 50 ppm imazalil sulphate	48.3 c
10. 100 mℓ/100ℓ Wetcit (borax) + 100 ppm imazalil sulphate	80.0 b

[a]Values represent the means of 3 replicates of 10 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

Conclusion

Wetcit had no effect in controlling infections by the post-harvest pathogen *P. digitatum*. Wetcit, in combination with reduced dosages of imazalil did not aid in any increased efficacy of imazalil in controlling *P. digitatum* infection. No further work is planned with this product.

4.5.9 **The evaluation of the post-harvest fungicide Ortocil (ortho-phenylphenate) for the control of post-harvest diseases**
Experiment 123 by K.H. Lesar (CRI)

Opsomming

Die nuwe swamdoder Ortocil het goeie beheer van infeksie deur die sitrus patogeen *P. digitatum* en redelike goeie beheer van infeksie deur *Geotrichum candidum* op Valencia lemoene in vergelyking met die standard aanbevole imazalil sulfaat en guazatine getoon. Hierdie proewe moet herhaal word en die effektiwiteit van Ortocil op ander sitrus kultivars moet ondersoek word.

Introduction

The water-soluble post-harvest fungicide SOPP (Sodium ortho-phenylphenate) provides fairly good protection against *P. digitatum* (green mould) and *P. italicum* (blue mould), and demonstrates limited activity against Diplodia stem-end rot, Alternaria core rot and G. *candidum* (sour rot). SOPP is not compatible with TBZ and imazalil sulphate in water and incorrect usage of SOPP may lead to serious phytotoxicity (burn) on

citrus rinds. SOPP concentration, pH of the mixture, temperature of the mixture and time of exposure of the fruit to the mixture must be controlled accurately. Any deviations within the recommended parameters of these variables could also lead to serious "burn" on fruit.

The standard pre-degreening drench mixture includes the use of guazatine for the protection of the fruit against green and blue mould and sour rot. Unfortunately, guazatine has to be withdrawn from the drench mixture where fruit is being treated and exported to countries without permitted MRL's, such as USA, Canada, Korea and Japan. Without guazatine in the pregreening drench, fruit will be exposed to infections by these three pathogens and full development of these infections will be compounded during degreening.

Therefore, it has become important to find a compound, with a different mode of action to guazatine, that can be alternated with guazatine in the drench for protection against the Penicillium organisms and sour rot and also play a role in reducing the risk of the build up of resistant green and blue mould and sour rot fungal spores to both compounds and minimise the risk of the build up of imazalil resitant Penicillium spores.

A Spanish formulation of ortho-phenylphenate (Ortocil) was especially formulated for use in a mixture, specifically a pre-degreening drench mixture. The product was evaluated for efficacy against infections caused by imazalil sensitive and resistant *P. digitatum* and *G. candidum*.

Materials and methods

Spore suspensions of *P. digitatum* (imazalil sensitive and resistant spores) and *G. candidum* were prepared by suspending spores in sterile deionised water containing the surfactant, Tween 20. The spore suspensions were spectrophotometrically adjusted to concentrations of $1x10^6$ spores/mℓ. Good sound, untreated Valencia oranges (Crocodile Valley Estate) were obtained in bulk. The fruit was divided into 3 replicates of 10 fruit per treatment and all the fruit was washed in clean water and surface sterilised by dipping in a 1% NaOCl solution for 2 minutes. The fruit was allowed to dry prior to inoculation.

Each fruit was wounded by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides giving 60 infection sites per treatment. Each fruit was then infected with the pathogen by applying 35 µℓ of spore suspension to each injury site using a micropipette.

The inoculated fruit was treated 4 hours after inoculation with the chemical compounds by immersion in a water bath containing each treatment suspension at ambient temperature for 3 minutes. The following treatments were conducted:

<u>Imazalil sensitive *P.digitatum* spores</u>
1. Untreated control (*P. digitatum*)
2. Treated control – 500 ppm imazalil sulphate
3. 500 ppm Ortocil
4. 1000 ppm Ortocil
5. 2000 ppm Ortocil
6. 3000 ppm Ortocil

<u>Imazalil resistant *P. digitatum* spores</u>
7. Untreated control (*P. digitatum*)
8. Treated control – 500 ppm imazalil sulphate
9. 500 ppm Ortocil
10. 1000 ppm Ortocil
11. 2000 ppm Ortocil
12. 3000 ppm Ortocil

<u>*G. candidum*</u>
13. Untreated control (*G. candidum*)
14. Treated control – 1000 ppm guazatine
15. 500 ppm Ortocil
16. 1000 ppm Ortocil
17. 2000 ppm Ortocil
18. 3000 ppm Ortocil

After treatment, the fruit was incubated in paper packets and plastic bags (sour rot) at 20°C for 7-10 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay inhibition (number of infected wounds).

Results and discussion

Ortocil (ortho phenylphenate) demonstrated good control of infection caused the imazalil sensitive spores of the pathogen *P. digitatum* compared with the standard imazalil sulphate (Table 4.5.9.1). The product also inhibited infections caused by imazalil resistant *P. digitatum* spores effectively, and demonstrated fairly good activity against sour rot infections caused by the pathogen *G. candidum*, compared with the standard guazatine. These trials need to be repeated and the efficacy of Ortocil will be evaluated on other citrus cultivars.

Table 4.5.9.1. Mean percentage inhibition of infection of Valencia oranges following wounding, inoculation with imazalil-sensitive and -resistant *P. digitatum* and *G. candidum* and subsequent dip-treatment with various concentrations of Ortocil (ortho phenyphenate) and the standard fungicides imazalil sulphate and guazatine.

Treatments	% inhibition[a]
1. Untreated control (*P. digitatum* **sensitive spores**)	3.3 c
2. Treated control – 500 ppm imazalil sulphate	100.0 a
3. 500 ppm Ortocil	13.3 c
4. 1000 ppm Ortocil	76.7 b
5. 2000 ppm Ortocil	90.0 a
6. 3000 ppm Ortocil	90.0 a
7. Untreated control (*P. digitatum* **resistant spores**)	0.0 d
8. Treated control – 500 ppm imazalil sulphate	26.7 c
9. 500 ppm Ortocil	6.7 d
10. 1000 ppm Ortocil	46.7 b
11. 2000 ppm Ortocil	86.7 a
12. 3000 ppm Ortocil	100.0 a
13. Untreated control (*G. candidum*)	10.0 d
14. Treated control – 1000 ppm guazatine	100.0 a
15. 500 ppm Ortocil	40.0 c
16. 1000 ppm Ortocil	40.0 c
17. 2000 ppm Ortocil	70.0 b
18. 3000 ppm Ortocil	96.7 a

[a]Values represent the means of 3 replicates of 10 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

4.5.10 **The evaluation of a yeast antagonist against the possible control of post-harvest diseases**
Experiment 123 by K.H. Lesar (CRI)

Opsomming

Die gis "antagonis" het goeie beskermende beheer van *P. digitatum* infeksie getoon. Geen na-infeksie beheer is waargeneem nie. Verdere proewe met die gis sal uitgevoer word.

Introduction

Consumers are concerned over chemical products that are applied to perishable commodities during post-harvest stages. Eventually, this might result in discontinuation of many chemical treatments for the control of post-harvest diseases. Without the use of these treatments, producers and suppliers of many commodities could face serious threats of increased spoilage and economic losses. Moreover, with the increasing build-up of pathogen resistance to post-harvest fungicides, citrus packhouses are yearning for alternatives to break the cycle of standard chemical controls, while still getting adequate control of diseases.

Biological control through competition or antagonism by another micro-organism has been extensively studied. Many systems work well in the laboratory but few have been recommended as commercial treatments. In many instances, environmental conditions in the field do not suit the biocontrol agent. However, when fruit is harvested and placed into storage, the environment can be stabilised. Under these controlled storage conditions, the activity of biocontrol agents can be optimised.

A yeast “antagonist”, isolated from the rind surface of oranges was evaluated for protective and post-infection control of *P. digitatum* infections. In the first of two trials a suspension of the yeast was first added to injury sites on the rind of oranges, and then a spore suspension of the pathogen was added to the same injury sites 2, 4 and 16 hours after the yeast. In the second trial the fruit was first infected with the pathogen and the yeast was then added 2, 4 and 16 hours after infection.

Materials and methods

Spore suspensions of *P. digitatum* (imazalil sensitive spores) and the yeast “antagonist” were made up by suspending spores, from pure cultures on Potato Dextrose agar (PDA) in sterile deionised water containing the surfactant, Tween 20. The spore suspensions were spectrophotometrically adjusted to concentrations of $1x10^6$ spores/mℓ. Good sound, untreated Valencia oranges (Crocodile Valley Estate) were obtained in bulk. The fruit was divided into 4 replicates of 10 fruit per treatment and all the fruit was washed in clean water and surface sterilised by dipping in a 1% NaOCl solution for 2 minutes. The fruit was allowed to dry prior to inoculation.

Each fruit was wounded by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides giving 80 infection sites per treatment. Each fruit was then infected with the pathogen and treated with the yeast by applying 35 µℓ of spore suspension to each injury site using a micropipette, in the sequence described below.

Protective control
1. Untreated control (*P. digitatum*).
2. Treated control (500 ppm imazalil sulphate).
3. *P. digitatum* spores added to injuries 2 hours after the yeast “antagonist”.
4. *P. digitatum* spores added to injuries 4 hours after the yeast “antagonist”.
5. *P. digitatum* spores added to injuries 16 hours after the yeast “antagonist”.

Post-infection control
1. Untreated control (*P. digitatum*).
2. Treated control (500 ppm imazalil sulphate).
3. Yeast “antagonist” added to injuries 2 hours after the pathogen spores.
4. Yeast “antagonist” added to injuries 4 hours after the pathogen spores.
5. Yeast “antagonist” added to injuries 16 hours after the pathogen spores.

After treatment, the fruit was incubated in paper packets at 20°C for 7-10 days or until such time as the controls had grown. The treatments were evaluated and the results recorded as percentage decay inhibition (number of infected wounds).

Results and discussion

Good activity in controlling the infection was observed where the pathogen was added to the injury site 16 hours after the yeast (Table 4.5.10.1). No control of the infection was achieved when the yeast was added to infected fruit.

This evaluation was a pilot trial to determine if there was any activity by the yeast antagonist against one of the major citrus pathogens, *P. digitatum.* Much work has already been done by other researchers worldwide on biocontrol agents and the indications are that the antagonist yeast cells must be present in sufficient numbers in an injury prior to pathogen infection. The yeast colonises the wound and prevent the pathogen from infecting through competition for space and nutrients or otherwise neutralising the pathogen by directly acting against pathogen.

The results obtained indicate that the longer the yeast is in the wound before the pathogen, the more effective the protection against subsequent infection by the pathogen will be. The trials on this yeast isolate are ongoing to determine the actual potential of the organism in an integrated postharvest disease control program.

Table 4.5.10.1. Mean percentage inhibition of infection of Valencia oranges following wounding, and inoculation with the yeast "antagonist" followed by inoculation with *P. digitatum* (protective control), or inoculation with the pathogen followed by the yeast (post-infection control).

Treatments		% inhibiton[a]
Protective control		
1.	Untreated control (*P. digitatum*)	0.0 e
2.	Treated control (500 ppm imazalil sulphate)	100.0 a
3.	*P. digitatum* spores added to injuries 2 hours after the yeast	27.5 d
4.	*P. digitatum* spores added to injuries 4 hours after the yeast	45.0 c
5.	*P. digitatum* spores added to injuries 16 hours after the yeast	87.5 b
Post-infection control		
1.	Untreated control (*P. digitatum*)	0.0 b
2.	Treated control (500 ppm imazalil sulphate)	100.0 a
3.	Yeast added to injuries 2 hours after the pathogen spores	0.0 b
4.	Yeast added to injuries 4 hours after the pathogen spores	0.0 b
5.	Yeast added to injuries 16 hours after the pathogen spores	0.0 b

[a]Values represent the means of 4 replicates of 10 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

4.5.11 The screening of South African isolates of Penicillium fungal spores from all citrus production areas for resistance to the post-harvest fungicides imazalil and guazatine

Experiment 850 by K.H. Lesar (CRI), J. Mildenhall and L. Trollope (Katco)

Opsomming

Die *in vitro* tegniek vir weerstandstoetsing is geoptimiseer. Voorlopige evaluasie van Penicillium spoormonsters tot dusvêr het gewys dat daar nie net bestande spore teen imazalil getoets is nie, maar wel ook monsters met weerstand teen die ander belangrike na-oes swamdoder, guazatine. Hierdie evaluasie is egter gedoen met potensiëel gemengde kulture, en die data is dus nie konklusief nie. Enkelspoorkulture sal van alle Penicillium kulture wat tot dusver gesuiwer is, gemaak word, waarna dit teen imazalil en guazatine getoets sal word. Sodra weerstandsvlakke gekarakteriseer is, sal *in vivo* toetse gedoen word om te bepaal watter weerstandsvlakke sal lei to praktyk-weerstand (i.e. verlies aan beheer).

Introduction

Blue and green mould of citrus fruit, caused by *Penicillium italicum* and *Penicillium digitatum* respectively, are the major contributors to post harvest decay in South African export citrus fruit. There are currently only two fungicides registered for the control of these pathogens on export fruit, namely, imazalil and guazatine. Certain countries, however, do not permit the use of guazatine on fresh fruit, leaving imazalil as the sole fungicide available for the control of post harvest Penicillium infections. Development of resistance to imazalil in populations of *P. italicum* and *P. digitatum* poses a serious threat to the export of citrus fruit and resistance has already been found in the Citrusdal area. In order to determine the extent of resistance development and to implement anti-resistance strategies, it is necessary to survey the incidence of imazalil resistance in orchards and packhouses in the major citrus producing areas of southern Africa.

In 2000, Kat River Citrus Co-operative (Katco) began monitoring fungal levels in the tip baths and fungicide baths by conducting in-house tests in a small microbiological laboratory. The Research Department of Outspan, then Capespan and thereafter CRI, randomly *in vivo* screened Penicillium spores samples for resistance development from the early 1990's onwards. The first imazalil resistant spores were detected in the Western Cape in 1999. Since then there has been a steady increase in the detection of imazalil resistant Penicillium biotypes in the citrus industry. This increase in resistant spores prompted the initiation of a joint strategy between CRI and Katco to assess the incidence of imazalil and guazatine resistance by *in vitro* screening of *Penicillium* spore samples collected throughout the citrus producing areas of Southern Africa. The results obtained from screening over 100 isolates for their sensitivity to imazalil and guazatine are presented in this report.

Materials and methods

Development of a suitable *in vitro* screening method

Gradient Plate Method for testing fungicide resistance. The conventional method for evaluating resistance of fungi to fungicides involves the preparation of agar plates amended with different concentrations of fungicide. Plates are inoculated by placing an agar plug of mycelium from the parent culture in the centre of fungicide-amended plates (Holmes & Eckert 1999). After a suitable incubation period the diameter of the colonies are measured. The disadvantage of this method is that large numbers of plates and large quantities of media have to be prepared. The objective of investigating the use of gradient plates was to develop a rapid screening process to determine whether the culture was sensitive, moderately resistant or totally resistant to the fungicide (Gerhardt *et al.*, 1994). A single Petri dish would suffice for each culture.

Petri dishes were placed on the bench and a 7 mm dowel rod was inserted under one side in order to angle the dishes. Ten millilitres of the basal Potato Dextrose Agar (PDA) medium was pipetted into each plate and allowed to set, forming a wedge-shaped layer across the plate. Thereafter plates were placed flat on the bench and 10 ml of the fungicide-amended medium was poured over the basal layer. Plates were incubated for 24 hours in order to allow the gradient to develop.

Two methods of inoculating the plates were investigated. In the first method, gradient plates were inoculated by cutting a 5 mm wide strip of culture from the parent plate, and transferring the strip on to the gradient plate aligning it across the gradient i.e. from the region of high concentration to the low concentration. In the second method, gradient plates were inoculated by pipetting 0.2 ml spore suspension in 0.01% Agral 90 (v/v) on to the centre of a gradient plate and spreading the suspension over the surface of the plate using a sterile bent glass rod.

Plates were incubated for 7 days and inspected visually for differences in growth of the colony over the surface of the plate (Fig. 4.5.11.1). Although some plates (Fig. 4.5.11.1) indicated sensitivity to the fungicide, the results were too erratic to determine whether the cultures were sensitive, moderately resistant or totally resistant (Fig 4.5.11.2). Because of the long incubation period (7 days) necessary to allow sufficient growth, the fungicide diffused throughout the plate thereby eliminating the gradient. This method was therefore discarded.

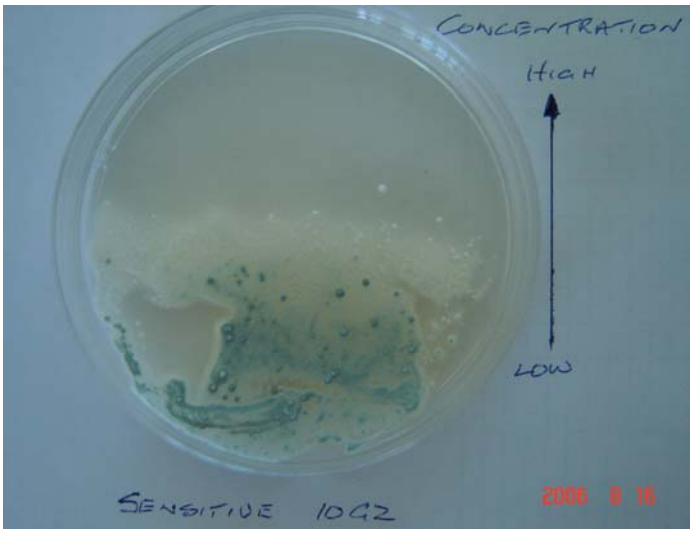

Figure 4.5.11.1. Growth of Penicillium italicum on a gradient plate after 7 days incubation at 24^{o} C indicating sensitivity to 10 mg/l guazatine.

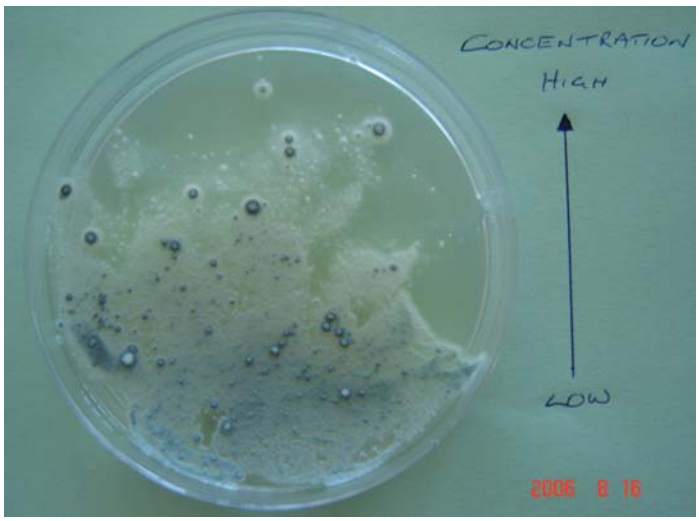

Figure 4.5.11.2. Growth of Penicillium italicum on a gradient plate indicating elimination of the gradient and the erratic growth after 7 days incubation.

In vitro *growth study with single spore cultures on a range of fungicide amended PDA plates.* Fungicide resistance is determined genetically (Schmidt *et al.*, 2006). Mass transfer cultures are genetically heterogeneous. Assessment of fungicide resistance/sensitivity using such cultures may yield conflicting results because of the genetic differences within the culture itself. Single spore cultures are genetically uniform and the expression of resistance/sensitivity to fungicides should be uniform for such cultures. Recent studies (Schmidt *et al.*, 2006) have used single spore cultures to investigate thiabendazole resistance in *Penicillium digitatum*. In order to continue with the current project on resistance in *Penicillium italicum* and *P. digitatum* to imazalil and guazatine, we considered it essential to single spore the stock cultures before undertaking further evaluation for fungicide resistance.

Test tubes containing sterile 0.9 ml Agral 90 (0.01% v/v aqueous) were prepared. A platinum wire loop was heated and cooled by insertion into the agar of a Penicillium culture. The agar-coated loop was drawn over the surface of the culture and the spores and mycelial fragments adhering to the loop were transferred to a test tube by rotation of the loop. This suspension was designated 10° dilution. Further dilutions were made by serial transfer of 0.1 ml to each of four tubes, giving dilutions of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} respectively. From the 10^{-2} and 10^{-3} dilutions, 0.1 ml was transferred to PDA plates, carefully placing the suspension to one side of the side of the agar (Marasas, W.F.O., personal communication). The plates were placed vertically (on edge) and the suspension was allowed to spread downwards over the agar surface. A sterile platinum loop was used to gently streak across the direction of flow. Plates were incubated in this vertical position for 24 hours at ambient temperature. Thereafter the plates were inspected for germinating spores under a light microscope using the 10x objective (i.e. 100x magnification). When single germinated spores were observed, the Petri dish base under the spore was marked by racking down the condenser of the microscope and circling the bottom of the plate with a fine tipped marker pen. The agar over this area was cut out using a sterile scalpel and the agar block was transferred to a new PDA plate. After 3 days incubation at 24°C a single spore culture was obtained.

Single spore cultures will be centre-inoculated on PDA plates amended with a range of imazalil (0.2, 1.0 and 10.0 ppm) and guazatine (0.2, 1.0 and 10.0 ppm) concentrations. Following an incubation period of 18 hours, colony diameters will be measured twice perpendicularly. Data will be collected and percentage inhibition for each concentration relative to the unamended control will be determined.

Isolation of new Penicillium cultures from the 2006 season

Packhouses in South Africa were requested by CRI to submit spore samples from fruit infected with *Penicillium italicum* and *P. digitatum* to Katco for testing for resistance to imazalil and guazatine. Cotton buds containing spores collected by the various packhouses were sent to Katco during 2006. Spores were plated onto the semi-selective sodium polypectate citrus rind medium (NaPP agar) developed at KATCO (NaPP agar: 20 g orange or lemon rind homogenate, 50 g homogenized in 100 ml distilled water. Filter through cheesecloth. Add 50 ml homogenate per litre of medium. Agar 12g/l; water 1 litre). and the cultures were subsequently transferred to PDA containing chloramphenicol (500 mg/l) to eliminate bacterial contaminants. Because of heavy contamination from *Rhizopus* all cultures were transferred onto PDA amended with 0.01% (m/v) pentachloronitrobenzine.

Results

A probable further 100 cultures have been isolated and are currently being purified from the spore samples collected during 2006. Penicillium cultures were not recovered from all the samples submitted. Single spore cultures are in the process of being obtained and will provide genetically uniform cultures for subsequent resistance testing.

Future research

Single spore cultures will be identified to species level and *in vitro* tested for sensitivity against imazalil and guazatine. The results obtained from the single spore cultures will be compared to the parent cultures, some of which have already been evaluated. This screening of spore samples is ongoing. Subsequent to the characterisation of imazalil and guazatine sensitivity, representative isolates from the characterised sensitivity classes will be evaluated in *in vivo* packhouse trials to ascertain which resistance levels constitute practical resistance (i.e. control failure).

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4.5.12 **The evaluation of plant growth regulators (PGRs), applied post-harvest, as possible alternatives to 2,4-D sodium salt (Deccomone) for calyx retention on citrus fruit**
Experiment 754 by K.H.Lesar (CRI)

Opsomming

Die plantgroeireguleerders, Retain, Agromos, Bioboost en die nuwe organiese middel Croplife is op nawel lemoene en suurlemoene in water doopbehandelings aangewend vir blomkelk-behoud op vrugte na gesimuleerde verskeping. Goeie blomkelk-behoud deur Retain en Bioboost in vergelyking met die standaard aanbevole 2,4-D (Deccomone) is waargeneem. 'n Redelike gehalte van blomkek behoud deur Croplife is ook waargeneem. Verdere proewe op ander sitruskultivars sal uitgevoer word.

Introduction

In the eventuality of 2,4-D being discontinued as a post-harvest treatment in the not too distant future, there is currently no alternative product registered as a post-harvest treatment on citrus for the preservation of fruit buttons (calyx). Therefore it is imperative to evaluate new safe products that could prevent calyx abscission. Button abscission on citrus fruit could possibly expose the fruit to infection by one or more of the latent citrus pathogens, *viz.* Anthracnose, *Diplodia* and *Alternaria*, as was evident in the 2003 production season. Button abscission also detracts from the eye-appeal of the fruit on the market. In this simulated export trial, the following PGRs were evaluated: Retain (aminoethoxyvinylglycine), Agromos (phytoalexin enhancer), Bioboost (phytoalexin enhancer), Croplife (a nutrient synergist).

Materials and methods

Good, sound, untreated navel oranges (Crocodile Valley Citrus Co.) and lemons (Larten Estate) were obtained in bulk. The fruit was washed and surface sterilised on the packline at CRI in a high-pressure spray using a suitable sanitising agent (Prasin, a quaternary ammonium compound). The fruit was then dried in the drying tunnel on the packline. All the fruit for this trial were selected with firm green buttons (calyxes).

Navel oranges were used in the first trial. All the treatments were immersed in water dip solutions for 3 minutes. Each treatment consisted of 3 replicates of 15 fruit each. The following treatments were conducted:

1. Untreated control – water dip only.

2. Treated control (250 ppm 2,4-D (Deccomone).
3. Treated control (500 ppm 2,4-D (Deccomone).
4. Retain (250 ppm).
5. Retain (500 ppm).
6. Agromos (2.4 ℓ/100 ℓ).
7. Bioboost (1.2 ℓ/100 ℓ).
8. Agromos (4.8 ℓ/100 ℓ).
9. Bioboost (2.4 ℓ/100 ℓ).
10. Croplife (30 ml/100 ℓ).
11. Croplife (70 ml/100 ℓ).
12. Croplife (140 ml/100 ℓ).
13. Croplife (270 ml/100 ℓ).

After dipping, the treated fruit was allowed to dry overnight and then all the treatments were placed into paper packets and stored under simulated shipping conditions, i.e. 1 week at 20°C, 6 weeks at 8°C and 1 week at 20°C. After the simulated shipping period, the treatments were evaluated and the results recorded as percentage button retention. The fruit was also evaluated for any stem-end infections caused by any of the latent pathogens. The trial was repeated with lemon and Valencia fruit. In the latter trial, the concentrations of certain products were adjusted (see Table 4.5.12.2).

Results and discussion

On Navel (Table 4.5.12.1) and Valencia (Table 4.5.12.2) fruit, good calyx retention by Retain and Bioboost at the higher concentrations compared with the standard recommended 2,4-D (Deccomone) was observed. In the Valencia-trial, the higher concentrations of Croplife did not differ from the lower concentrations in the Navel-trial. No stem-end infections by any of the latent pathogens were observed on the fruit evaluated. No results were available from the lemon-trial as the incidence of secondary green mould infection was high, thus preventing the recording of any accurate results. This trial will be repeated on lemons and grapefruit.

Table 4.5.12.1. Mean percentage button retention of Navel oranges following dip-treatment with various concentrations of Deccomone, Retain, Agromos, Bioboost and Croplife and 8-week storage at simulated shipping conditions.

Treatments		Mean Button Retention (%) [a]
1.	Untreated Control	13.4 c
2.	Treated Control - 250 ppm 2,4-D (Deccomone)	100.0 a
3.	Treated Control - 500 ppm 2,4-D (Deccomone)	100.0 a
4.	Retain - 250 ppm	73.3 ab
5.	Retain - 500 ppm	80.0 ab
6.	Agromos - 2.4 ℓ/100 ℓ	60.0 b
7.	Bioboost - 1.2 ℓ/100 ℓ	60.0 b
8.	Agromos - 4.8 ℓ/100 ℓ	73.3 ab
9.	Bioboost - 2.4 ℓ/100 ℓ	80.0 ab
10.	Croplife - 30 mℓ/100 ℓ	60.0 b
11.	Croplife - 70 mℓ/100 ℓ	66.7 b
12.	Croplife - 140 mℓ/100 ℓ	66.7 b
13.	Croplife - 270 mℓ/100 ℓ	73.3 ab

[a] Means followed by the same letter are not significantly different (Fisher's Unprotected LSD; P≥ 0.05)

Table 4.5.12.2. Mean percentage button retention of Valencia oranges following dip-treatment with various concentrations of Deccomone, Retain, Agromos, Bioboost and Croplife and 8-week storage at simulated shipping conditions.

Treatments		Mean Calyx Retention (%) [a]
1.	Untreated Control	35.0 e
2.	Treated Control - 250 ppm 2,4-D (Deccomone)	85.0 ab
3	Treated Control - 500 ppm 2,4-D (Deccomone)	90.0 a
4.	Retain - 250 ppm	75.0 abcd
5.	Retain - 500 ppm	85.0 ab
6.	Agromos - 2.4 ℓ/100 ℓ	75.0 abcd
7.	Bioboost - 1.2 ℓ/100 ℓ	80.0 abc
8.	Agromos - 4.8 ℓ/100 ℓ	65.0 abcde

9.	Bioboost - 2.4 ℓ/100 ℓ	75.0 abcd
10.	Croplife - 140 mℓ/100 ℓ	50.0 cde
11.	Croplife - 270 mℓ/100 ℓ	45.0 de
12.	Croplife - 350 mℓ/100 ℓ	55.0 bcde
13.	Croplife - 500 mℓ/100 ℓ	70.0 abcd

[a] Means followed by the same letter are not significantly different (Fisher's Unprotected LSD; P≥ 0.05)

4.6 PROJECT: FRUIT AND FOLIAR DISEASES

Project Co-ordinator: G.C. Schutte (CRI)

4.6.1 Project summary

New spray programmes consisting of new fungicides were evaluated for Alternaria brown spot control on 'nova' mandarins in the winter rainfall region of South Africa. Copper oxychloride (Fynox) spray programmes sprayed at monthly intervals, performed well at the registered rate of 200 g/hl water against Alternaria brown spot. When the copper fungicide rate was lowered from 200 g/hl water to 100 g/hl water and combined in a tank mix with Sporekill (100 ml/hl water) then sprayed at monthly intervals (7 applications in total), equivalent results were obtained. Both the copper oxychloride alone and copper oxychloride with Sporekill, resulted in 95-98% clean exportable fruit. No stippling due to copper was observed in the latter treatment with Sporekill but it did occur when copper oxychloride was sprayed at a rate of 200 g/hl. The lower rate of copper with Sporekill has a very favourable MRL and pre-harvest interval (4.6.2).

New spray programmes consisting of new fungicides were also evaluated for Alternaria brown spot control on 'nova' mandarins in the summer rainfall region of South Africa. Three applications consisting of strobilurins and mancozeb with either mineral spray oil or Sporekill gave good control of Alternaria brown spot versus eight applications of the other spray programmes that consisted mainly of contact fungicides. Sporekill can therefore be recommended to replace mineral spray oil in tank mixtures with strobilurins. Copper oxychloride (but not mancozeb) sprayed at monthly intervals, performed well at registered rates of 200 g/hℓ water against Alternaria brown spot. Where either the copper oxychloride or mancozeb rates were lowered from 200 g/hℓ water to 100 g/hℓ water but in a tank mix with Sporekill (100 mℓ/hℓ water) and sprayed at monthly intervals (8 applications in total from September to April), the results were just as good as the standard registered rate of copper oxychloride. No stippling due to copper was observed in a commercial application consisting of Sporekill + mancozeb (MZ) (a) and Sporekill + copper oxychloride (Cu) (b) in the adjacent orchard where the trials were conducted, showing that this type of alternation (a:b:a:b:a:b:a:b). The 3-way tank mixture of copper oxychloride (50 g) plus mancozeb (50 g) plus Sporekill (100 mℓ) in 100ℓ water did not perform that well for Alternaria control and should be re-evaluated. Another DDAC formulation did not perform as well as Sporekill did against Alternaria brown spot (4.6.3).

The new trunk disease on Clementines in the winter rainfall regions of South Africa was investigated in different facets. To determine their susceptibility, different Clementine cutivars such as 'Marisol', 'Clemlate', 'Oroval' and 'Oroblanco' were all successfully inoculated with *Phytophthora citrophthora* showing that they are susceptible to *P. citrophthora*. The latter was also isolated from commercial orchards consisting of 'Orovals' en 'Clemenpons' (4.6.4).

To control the disease, a different experiment was also conducted in the Western Cape. Isolations from the experimental orchard in Swellendam tested all positive for *Phytophthora citrophthora*. A spray programme consisting of a soil treatment in spring with Ridomil Gold, a foliar spray also in spring with Fighter with two monthly follow-on treatments also with Fighter as well as a trunk application with a tank mixture consisting of Captan and Sporekill starting in the winter (with the onset of the first winter rain), gave good control of Phytophthora trunk rot. Trees with a thin canopy (>6/10) and with a heavily infected trunk (>60%) did not react to the treatments and should rather be removed. Micro-sprayers should be installed in such a manner that the trunks are not wetted during irrigation. Snails should also be controlled as they may contribute to the spread of the disease. An alternative method for control is the painting of the trunks with 2.5 kg copper oxyhloride plus 5 liter PVA in 5 liter water (4.6.5).

Projekopsomming

Koperoksichloried wat met maandelikse intervalle gespuit is vir die beheer van Alternaria bruinvlek, het goed teen die geregistreerde dosis van 200 g/hℓ water gevaar. Waar die dosis verlaag is van 200 g/hℓ water na 100 g/hℓ water in kombinasie met Sporekill teen 'n dosis van 100 mℓ /hℓ water en ook met maandelikse intervalle toegedien is (7 bespuitings in total), het dit ook uitstekende beheer tot gevolg gehad. Geen stippelvorming is met hierdie mensel waargeneem nie, terwyl die gewone geregistreerde dosis van koperoksichloried, uitermatige stippelvorming tot gevolg gehad het (4.6.2).

Drie behandelings bestaande uit strobilurine en mancozeb in 'n tank mengsel met óf minerale spuitolie óf Sporekill, het goeie beheer van Alternaria bruinvlek tot gevolg gehad teenoor die ander spuitprogramme wat uit agt bespuitings met kontak swamdoders bestaan het. Sporekill kan daarom aanbeveel word as plaasvervanger vir minerale spuitolie. Koperoksichloreid wat met maandelikse intervalle gespuit is (agt bespuitings in total) teen 'n dosis van 200 g/hℓ water, het goeie beheer van Alternaria bruinvlek tot gevolg gehad. Waar die koperoksichloried of mancozeb dosisse verlaag is van 200 g/hℓ water na 100 g/hℓ water, maar in 'n tankmengsel met Sporekill (100 mℓ/hℓ water) en maandeliks toegedien is (8 toedienings in totaal van September tot April), was die resultate net so goed soos die standaard registreerde dosis van koperoksichloried. Geen stippelvorming as gevolg van koperoksichloried was waargeneem in 'n kommersiële toediening bestaande uit Sporekill + mancozeb (MZ) (a) en Sporekill + koperoksichloried (Cu) (b) in die aangrensende boord waar die proewe uitgevoer is nie waar die produkte afgewissel is nie (a:b:a:b:a:b:a:b). Die tankmengsel van koperoksichloried (50 g) plus mancozeb (50 g) plus Sporekill (100 mℓ) in 100ℓ water het nie aan die verwagtinge voldoen nie en moet her-evalueer word. 'n Ander DDAC formulasie het gladnie so goed presteer soos Sporekill nie teen Alternaria bruinvlek nie (4.6.3).

Clementine kutivars soos 'Marisol', 'Clemlate', 'Oroval' en 'Oroblanco' kon almal kunsmatig met *Phytophthora citrophthora* geïnokuleer word en sodoende bewys dat hulle vatbaar is vir *P. citrophthora*. In kommersiële boorde is 'Orovals' en 'Clemenpons' ook positief met die siekte geïdentifiseer (4.6.4).

Isolasies van die eksperimentele boord in Swellendam het almal positief getoets vir *Phytophthora citrophthora*. 'n Spuitprogram bestaande uit 'n grondbehandeling in die lente met Ridomil Gold, 'n blaarbespuiting beginnende in die lente (September) en opgevolg met twee maandelikse bespuitings met Fighter asook 'n stambespuiting met 'n mengsel van Captan en Sporekill beginnende in die winter (aanvang van die eerste reën), het uitstekende beheer van Phytophthora stamvrot gegee. Bome wat 'n yl blaredak het (>6/10) en waarvan die stam ook hewig geïnfekteer was (>60%) het geensins gereageer op die spuitprogram nie en moet liefs verwyder word. Mikrospuite moet ook so geplaas word dat die bome se stamme nie benat word nie en slakke moet ook beheer word omdat hulle moontlik kan bydra tot die verspreiding van die siekte tot op die stamme. 'n Alternatiewe metode wat ook kan help met die voorkoming van die siekte is die stamverf met 2.5 kg koperoksichloried plus 5 liter PVA in 5 liter water (4.6.5).

4.6.2 **Evaluation of new spray programmes for the control of *Alternaria* brown spot in the winter rainfall region of South Africa**

Experiment 749 by G.C. Schutte (CRI)

Opsomming

Koperoksichloried wat met maandelikse intervalle gespuit is vir die beheer van Alternaria bruinvlek, het goed teen die geregistreerde dosis van 200 g/hℓ water gevaar. Waar die dosis verlaag is van 200 g/hℓ water na 100 g/hℓ water in kombinasie met Sporekill teen 'n dosis van 100 mℓ /hℓ water en ook met maandelikse intervalle toegedien is (7 bespuitings in total), het dit ook uitstekende beheer tot gevolg gehad. Geen stippelvorming is met hierdie mensel waargeneem nie, terwyl die gewone geregistreerde dosis van koperoksichloried, uitermatige stippelvorming tot gevolg gehad het.

Introduction

Brown spot disease of citrus caused by *Alternaria alternata* is one of the most prevalent fungal diseases in all production areas in South Africa. Minneola tangelos, Novas, mandarins and their hybrids, tangors and grapefruit are the most susceptible cultivars. The disease can affect both fruit and foliage and is most prevalent under wet, humid conditions. The fruit lesions are very unsightly and readily reduce crop value. Toxin formation in foliar and twig infection also causes defoliation.

South Africa has both winter and summer rainfall areas. In both areas, wet, humid periods can occur which favour brown spot disease. This applies particularly to the autumn in southern areas with a Mediterranean climate. Heavy dew can also create suitable conditions for disease development (Timmer *et al*., 2000) and again the southern areas are more susceptible to this climatic factor. Nevertheless, due to the unpredictability of seasonal climatic trends, it is necessary to annually protect the most susceptible cultivars against the disease in all areas. This typically requires a multiple spray programme to cover the possible infection periods from spring to autumn. In this regard, the strategy employed by South African growers is to use the more expensive systemic fungicides during the wet, high-disease-pressure periods (Schutte *et al*., 1994) and the less expensive contact fungicides during dry, low-disease-pressure periods. However, not all fungicides have acceptable MRLs in the European Union.

Our aim was to evaluate coppers in a modified brown spot spray programme to assist growers in the winter rainfall region of the Western Cape with more effective spray programmes that will result in more acceptable residues during the wet, high disease periods in September/ October and April/May. Sporekill (a patented didecyl dimethylammonium chloride compound; 12%), a disinfectant or plant sanitiser, showed promise against citrus black spot in tank mixtures with copper fungicides and mancozeb and was therefore also evaluated for control of Alternaria brown spot.

Materials and methods

A trial site was selected on the farm, Sovereign Estates, near Swellendam on 'novas' with a high incidence of brown spot. Different cost effective spray programmes (as specified below) were selected comprising copper oxychloride (Fynox) and Sporekill. A randomised row design with 25 trees per row was used per treatment and sprayed with an automatic spray machine. Buffer rows were allowed between each of the treatments. Trees were thoroughly sprayed to the point of run-off. A total of seven applications were made at monthly intervals between 20 September 2005 and 7 March 2006. All sprays were applied during good weather conditions without wind or rain.

The evaluation of brown spot (200 fruit per replicate) on the fruit rind was conducted just prior to harvesting, during mid-June. Criteria used for rating the fruit were: 0 = fruit with no brown spot lesions, 1 = fruit with one to five brown spot lesions, 2 = fruit with six or more brown spot lesions. The results were expressed as percentages and the means compared using Fisher's LSD test for significance

Results and discussion

Results from the field trial conducted at Sovereign Estates at Buffeljachts (Table 4.6.2.1) show that there were no significant differences ($P > 0.01$) between the standard registered copper oxychloride and the tank mixtures of reduced copper oxychloride with Sporekill at the rates tested. These treatments yielded 98% and 95% clean exportable fruit, respectively. All the other criteria (fruit with 1-5 brown spot lesions per fruit and fruit with 6 or more brown spot lesions per fruit) were also not significantly different ($P > 0.01$) from each other but all the treatments were significantly different from the control. More spray programmes consisting of different mixtures with Sporekill will be evaluated in the new season. Stippling was observed in the registered copper oxychloride programme (Fig. 4.6.2.1) but not in the copper oxychloride plus Sporekill treatment.

Conclusion

Copper oxychloride (Fynox) spray programmes sprayed at monthly intervals, performed well at the registered rate of 200 g/hℓ water against Alternaria brown spot. A combination of Sporekill (100 mℓ/hℓ water) and copper oxychloride (100 g/hℓ water) was as effective as the registered programme but did not cause stippling. Copper residues on fruit were also reduced.

Future research

More spray programmes consisting of different mixtures with Sporekill will be evaluated in the new season.

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Fig. 4.6.2.1. Stippling due to frequent copper oxychloride spray application at a rate of 200 g/hℓ water.

Table 4.6.2.1. Application of copper oxychloride (Fynox) alone or in a tank mixture with Sporekill for *Alternaria* brown spot control on 'novas' at Buffeljachtsrivier, near Swellendam S.A. during 2005 and 2006.

Treatments							**Percentage of fruit in each class** [z]		
20 September 2005	**18 October 2005**	**15 November 2005**	**13 December 2005**	**10 January 2006**	**7 February 2006**	**7 March 2006**	**Lesions/fruit**		
							0	**1-5**	**≥6**
Fynox 200 g	Fynox 200 g	Fynox 200 g	Fynox 200 g	Fynox 200 g	Fynox 200 g	Fynox 200 g	98.00a	1.00a	1.00a
Fynox + Sporekill 100 g + 100 mℓ	Fynox + Sporekill 100 g +100 mℓ	Fynox + Sporekill 100 g + 100 mℓ	Fynox + Sporekill 100 g + 100 mℓ	Fynox + Sporekill 100 g +100 mℓ	Fynox + Sporekill 100 g + 100 mℓ	Fynox + Sporekill 100 g + 100 mℓ	95.33a	2.00a	2.67a
Not sprayed	Not sprayed	Not sprayed	Not sprayed	Not sprayed	Not sprayed	Not sprayed	48.33b	19.00b	32.67b

[z]Means in a column, based on 200 fruit per replicate, followed by the same letter are not significantly different (P > 0.01) according to Fisher's least significant difference test.

4.6.3 **Positioning and evaluation of new spray programmes consisting of strobilurins for the control of *Alternaria* brown spot in the summer rainfall regions of South Africa**
Experiment 750 by G.C. Schutte (CRI)

Opsomming

Drie behandelings bestaande uit strobilurine en mancozeb in 'n tank mengsel met óf minerale spuitolie óf Sporekill ['n gepatenteerde didesiel dimetielammonium chloried (DDAC) produk], het goeie beheer van Alternaria bruinvlek gegee, vergelykend met die ander spuitprogramme wat uit 8 bespuitings van kontak swamdoders bestaan het. Vir hierdie toepassing, kan Sporekill dus as plaasvervanger vir minerale spuitolie aanbeveel word. Koperoksichloried, wat met maandelikse intervalle teen 'n dosis van 200 g/hℓ water gespuit is (8 bespuitings in totaal), het goeie beheer van Alternaria bruinvlek gegee. Waar die koperoksichloried of mancozeb dosisse verlaag is van 200 g/hℓ water na 100 g/hℓ water, maar in 'n tenkmengsel met Sporekill (100 mℓ/hℓ water) toegedien is (8 toedienings in totaal), was die resultate net so goed soos die standaard registreerde dosis van koperoksichloried. Laer koperoksichloried of mancozeb dosisse (50 g/hℓ) in kombinasie met Sporekill (100 mℓ/hℓ) het nie aan die verwagtinge voldoen nie en moet her-evalueer word. Sporekill + mancozeb (a) en Sporekill + koperoksichloried (b) is afwissellend gespuit (a:b:a:b:a:b:a:b) in die aangrensende boord en geen stippelvorming as gevolg van koperoksichloried is waargeneem nie. 'n Ander DDAC formulasie het gladnie so goed soos Sporekill teen Alternaria bruinvlek presteer nie.

Introduction

Alternaria brown spot is a serious disease of tangerines (*Citrus reticulata*) and their hybrids in all South African regions. The causal agent was originally described as *Alternaria citri* Ellis & N. Pierce, and later renamed *A. alternata* pv. *citri*. Simmons (1999) described 10 new species from a worldwide collection of *Alternaria* isolates from citrus. According to his study, isolates from Turkey, Israel, Australia and South Africa were the same and were renamed *A. turkisafria*. Isolates from the USA and Colombia were morphologically different and were named *A. tangelonis* and *A. colombiana*, respectively. Peever *et al*. (2002) confirmed the research of Simmons by determining the worldwide phytogeography of the brown spot pathogen using molecular markers and sequence data, as well as determining the quantitative differences in virulence among isolates from different citrus growing areas of the world. Recently, Vincent *et al*. (2000) reported that *Alternaria* brown spot (sp. unknown) was detected in Spain.

Alternaria brown spot attacks young leaves, twigs and fruit, causing small black necrotic spots after a 24 to 36 h incubation period. The pathogen produces a host-specific toxin that causes lesions to expand, often resulting in leaf and fruit drop and twig dieback. On more mature fruit, lesions may vary from small necrotic spots to large, sunken pockmarks. Thus, this disease may affect tree growth, cause considerable crop loss, and produce blemishes on fruit that are unacceptable to the consumer. Leaves are susceptible to infection from the time of formation until they are fully expanded and hardened, while fruit are susceptible from petal fall until harvest. In the USA, however, fruit are only susceptible from petal fall until they reach about 5 cm in diameter.

Cultural measures, such as wider tree spacing to allow air movement and dry-off of trees, the elimination of overhead irrigation and avoidance of excess nitrogen fertilizer, can assist in reducing disease severity in some orchards. However, fungicide applications are essential for disease control and production of blemish-free fruit. In South Africa, it is important to protect fruit and flushes of cultivars such as tangerines and their hybrids with fungicides from September to April/May, often requiring 8+ spray applications. The cost of sprays and products are not economically sustainable. Moreover, the large number of applications might result in unacceptable residues on fruit. It is therefore a research priority to identify more effective fungicides with potentially longer lasting residues for more effective control of *Alternaria* brown spot.

Our aim was to evaluate the use of strobilurin fungicides (with EU MRLs) in a 2- or 3-spray programme during the high disease pressure period from October to January. Sporekill, a patented didecyl dimethylammonium chloride compound (DDAC), showed promise against citrus black spot in tank mixtures with copper fungicides and mancozeb and was therefore included in these trials.

Materials and methods

Ten single-tree plots per treatment were selected randomly from a 'nova' orchard at Belmont, 40 km west of Nelspruit. The trees were 13 years old and planted in 2x5 m tree spacing in rows that ran from north to south.

Trees were selected for uniformity in canopy density and tree size. Single untreated guard trees were located between plots within rows. Several fungicide programmes, as specified below, were evaluated. Fungicides were applied with a trailer-mounted, high-volume, high-pressure (2500-3000 kPa) sprayer with two hand-held spray guns. The weather was fine and dry on all spray occasions with minimal wind. Spray volumes varied according to the size and canopy density of the tree but all trees were sprayed to the point of run-off. At fruit maturity in June, Alternaria brown spot severity was rated on 100 fruits per tree according to a 3-point index: 0 = clean fruit with no brown spot lesions; 1 = one to five brown spot lesions per fruit; and 2 = six and more brown spot lesions per fruit. Data was analysed by ANOVA, using Fisher's LSD test (P = 0.05).

Results and discussion

Sporekill in tank mixtures with mancozeb and copper oxychloride

Results from the field trial conducted at Belmont showed that there were no significant differences between the copper oxychloride and mancozeb when their rates were lowered from 200 g/hℓ water to 100 g/hℓ water when applied in a tank mix with Sporekill (100 mℓ /hℓ water) and sprayed at monthly intervals (8 applications in total) from September to March. Copper oxychloride and mancozeb in tank mixtures with Sporekill resulted in 96% and 93.5% clean exportable fruit respectively. This shows that the 2x rates do not contribute to more disease control and that the 100 mℓ/hℓ water rate. None of the Sporekill applications either alone or in combination with other products resulted in any phytotoxicity on fruit of foliage. An interesting observation was that the copper oxychloride tank mixtures with Sporekill at rates of 100 mℓ/hℓ water resulted in less stippling due the low rates of copper used and the lower amount of free copper-ions, while copper oxychloride resulted in severe stippling at registered rates (Fig. 4.6.3.1.). Even the 3-way mixture of copper oxychloride (50 g) plus mancozeb (50 g) plus Sporekill (100 mℓ) in 100 ℓ water had no stippling. On the other hand, a commercial application of Sporekill + mancozeb (MZ) (a) and Sporekill + copper oxychloride (Cu) (b) in the adjacent orchard where the trials were conducted, showed that this type of alternation (a:b:a:b:a:b:a:b) will not cause copper stippling (Fig. 4.6.3.2).

There was a 12% difference between the standard copper oxychloride and mancozeb resulting in 98.3% and 86.2% clean exportable fruit respectively. The 3-way tank mixture of copper oxychloride (50 g) plus mancozeb (50 g) plus Sporekill (100 mℓ) in 100ℓ water did not perform that well and only achieved 84.5% clean exportable fruit. Another DDAC in a tank mixture with mancozeb also not performed that well and in this regard only had 81.1% clean exportable fruit. All the treatments were significantly different from the control with regards to all criteria used for evaluation (Table 4.6.3.1.)

Strobilurin and mancozeb tank mixtures with Sporekill and mineral spray oil

Results from Table 4.6.3.2 showed that there were no significant differences between any of the spray programmes evaluated. There was however is significant difference between the two standard treatments, copper oxychloride and mancozeb, both sprayed at registered rates of 200 g/hℓ water. Azoxystrobin sprayed at registered rates as recommended for CBS in tank mixtures with mancozeb with either mineral spray oil or Sporekill, performed exceptional well as three applications and this in a year with high rainfall. This new approach is exciting as azoxystrobin was sprayed with 60 day intervals with three applications only, thereby saving the growers 5 spray rounds. The reason for this phenomenon is because strobilurins do have a systemic or local systemic mode of action and their long lasting residual action plays an important role in the good fungicidal action against Alternaria brown spot (Häuser-Hahn, Pontzen & Baur, 2003). Furthermore, the strobilurin, Flint, has a mesostemic mode of action whereby it has a high affinity for the plant's waxy layer and is thus stored there very effectively. This results in a fungicide reservoir from which the active ingredient penetrates continuously into the deep-lying tissue of the plant. Due to this reservoir, a continuous protective effect is exerted against fungal-spore attack (Krieg, Weile & Göhlich, 2003) explaining why they are so effective in Alternaria brown spot control. Sporekill successfully replaced mineral spray oil in the tank mixture with azoxystrobin and mancozeb and can therefore be recommended to limit the problems associated with colouring of early maturing cultivars such as 'novas'. All the spray programmes were significantly different from the untreated control with regards to all criteria used for evaluation.

There were also no significant differences between the spray programmes with regards to the criteria 1 to 5 Alternaria brown spot lesions per fruit and 6 and more Alternaria brown spot lesions per fruit. However, all the treatments were significantly different from the control (Table 4.6.3.2).

Conclusion

Sporekill in tank mixtures with mancozeb and copper oxychloride

Sporekill (100 mℓ and 200 mℓ) was successfully mixed with reduced rates (100 g) of copper oxychloride and mancozeb for the control of Alternaria brown spot. Sporekill at 2x rates did not contribute to more disease control. Less stippling was noticed on the fruit. Commercial applications of Sporekill + mancozeb (a) and Sporekill + copper oxychloride (b) in an adjacent orchard showed that alternation of these applications (a:b:a:b:a:b:a:b) will not cause copper stippling.

Strobilurin and mancozeb tank mixtures with Sporekill and mineral spray oil

Three strobilurin + mancozeb applications with either mineral spray oil or Sporekill, gave good control of Alternaria brown spot compared with 8 applications of the other spray programmes that consisted mainly of contact fungicides. This type of spray programme will save the growers five spray rounds if compared with the contact/preventative type of spray programme that one has to spray with monthly intervals. However, growers should be warned that the frequent spraying of strobilurins can lead to resistance and that they should alternate it with other fungicides with different modes of action.

Future research

More spray programmes consisting of different DDAC's will be evaluated in the new season as well as three applications of all the registered strobilurins. The 3-way tank mixture of copper oxychloride (50 g) plus mancozeb (50 g) plus Sporekill (100 mℓ) in 100ℓ water did not perform that well and should be re-evaluated. New fungicides with different modes of action should also be investigated.

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Table 4.6.3.1. Evaluation of Sporekill (1x and 2x rates) and DDAC X (didecyldimethylammonium chloride) in tank mixtures with copper oxychloride (Fynox) and/or mancozeb applied from September to April during 2005 and 2006 for the control of Alternaria brown spot on 'nova' mandarins at Belmont, Schagen, South Africa.

Treatment	Rate /hℓ water	Percentage of fruit in each class [x]		
		Lesions/fruit		
		0	1-5	≥6
Copper oxychloride	200 g	98.3 a	0.6 a	1.1 a
Copper oxychloride + Sporekill	100 g + 200 mℓ	98.1 a	1.5 a	0.4 a
Mancozeb+ Sporekill	100 g + 200 mℓ	96.0 ab	2.0 a	2.0 a
Copper oxychloride + Sporekill	100 g + 100 mℓ	96.0 ab	2.0 a	2.0 a
Mancozeb+ Sporekill	100 g + 100 mℓ	93.5 abc	4.3 ab	2.2 a
Mancozeb	200 g	86.2 abc	6.6 abc	7.2 a
Copper oxychloride + mancozeb + Sporekill	50 g + 50 g + 100 mℓ	84.5 bc	11.3 c	4.2 a
DDAC X + mancozeb	100 mℓ + 100 g	81.1 c	9.5 bc	9.4 a
Control		29.6 d	22.3 d	48.1 b

[x] Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.
[y] 8 applications: spray dates were 19 September 2005, 19 October 2005, 14 November 2005, 12 December 2005, 17 January 2006, 15 February 2006, 15 March 2006 and 12 April 2006

Table 4.6.3.2. Evaluation of Sporekill (didecyldimethylammonium chloride) in tank mixtures with copper oxychloride (Fynox), mancozeb and azoxystrobin applied from September to April during 2005 and 2006 for the control of Alternaria brown spot on 'nova' mandarins at Belmont, Schagen, South Africa.

Treatment	Rate /hℓ water	Percentage of fruit in each class [x]		
		Lesions/fruit		
		0	1-5	≥6
Copper oxychloride [z]	200 g	98.3 a	0.6 a	1.1 a
Copper oxychloride + Sporekill [z]	100 g + 100 mℓ	96.0 ab	2.0 ab	2.0 a
Mancozeb+ Sporekill	100 g + 100 mℓ	93.5 ab	1.8 ab	4.7 a
Azoxystrobin + mancozeb + oil [y]	20 mℓ + 150 g + 250 mℓ	93.0 ab	4.8 ab	2.2 a
Azoxystrobin + mancozeb + Sporekill [y]	20 mℓ + 150 g + 100 mℓ	91.6 ab	5.0 ab	3.4 a
Azoxystrobin + Controller + oil [y]	20 mℓ + 100 g + 250 mℓ	89.6 ab	5.3 ab	5.1 a
Mancozeb [z]	200 g	86.2 b	6.6 b	7.2 a
Control		29.6 c	22.3 c	48.1 b

[x] Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.
[y] 3 applications: spray dates were 19 October 2005, 12 December 2005 and 15 February 2006
[z] 8 applications: spray dates were 19 September 2005, 19 October 2005, 14 November 2005, 12 December 2005, 17 January 2006, 15 February 2006, 15 March 2006 and 12 April 2006

Fig. 4.6.3.1. Severe stippling of 'nova' mandarins due to 8 applications of copper oxychloride at the registered rate of 200 g/hℓ water (left), mild stippling also due to 8 applications of copper oxychloride at a reduced rate of 100 g/hℓ water in a tank mixture with Sporekill (middle) and no stippling due to monthly alternation of mancozeb + Sporekill with copper oxychloride + Sporekill (right).

Fig. 4.6.3.2. Stippling-free fruit from trees that were subjected to 8 alternating applications of Sporekill + mancozeb (MZ) (a) and Sporekill + copper oxychloride (Cu) (b) as a demonstration that this programme (a:b:a:b:a:b:a:b) will most likely not cause copper stippling.

4.6.4 **Evaluation of spray programmes for the control of *Phytophthora citrophthora* on Clementines in the Western Cape**

Experiment 836 by G.C. Schutte (CRI)

Opsomming

Diagnose van die terugsterwingsituasie is bevestig deurdat isolasies uit die eksperimentele boord in Swellendam almal positief getoets vir *Phytophthora citrophthora* het. Verskeie spuitprogramme is getoets en 'n program bestaande uit 'n grondbehandeling in die lente met Ridomil Gold, 'n blaarbespuiting in die lente (September), opgevolg met twee maandelikse bespuitings met Fighter, asook 'n stambespuiting met 'n mengsel van Captan en Sporekill in die winter (aanvang van die eerste reën) het uitstekende beheer van Phytophthora stamvrot gegee. Bome wat 'n yl blaredak het (>6/10) en waarvan die stam ook hewig geïnfekteer was (>60%) het geensins gereageer op die spuitprogram nie en moet liefs verwyder word. Mikrospuite moet ook so geplaas word dat die bome se stamme nie benat word nie. Slakke moet ook beheer word omdat hulle moontlik kan bydra tot die

verspreiding van die patogeen tot op die bostamme. 'n Alternatiewe metode wat ook kan help met die voorkoming van die siekte is die verf van stamme met 2.5 kg koperoksichloried plus 5 liter PVA in 5 liter water.

Introduction

Tree die-back was observed in the Knysna region of the Eastern Cape on the Nules cultivar. According to new surveys, the problem seems to occur in other regions such as Patensie, Swellendam, Stellenbosch, and Citrusdal with devastating effects. The die-back starts right on the scion (30 cm above ground level) as gumming and is accompanied by anthracnose and *Diplodia* fungal growth (secondary wound pathogens). Die-back was diagnosed as caused by *Phytophthora citrophthora*.

Although 10 *Phytophthora* species have been reported from diseased trees around the world, three species cause the most serious disease, stem gummosis, as well as root and fruit rot: *P. citrophthora (*R.E. Sun & E.H. Sun) (Leonian 1906), *P. nicotianae* (syn *P. parasitica*) and *P. palmivora* (Erwin & Ribeiro, 1996; Graham & Menge, 2000). They have distinct temporal and climatic requirements, so that their relative distribution and influence vary in the different production areas (Matheron *et al.*, 1997). *P. citrophthora* is extremely sensitive to high temperatures of above 33°C and this explains why *P. citrophthora* is so active in the mediterranean type of climate experienced along the Eastern and Western Cape coastline.

Rootstocks like the sour orange (*Citrus aurantium*) which appeared to be resistant to *Phytophthora* following the mid-1800s gummosis epidemics in the Mediterranean area (Laviola *et al.*, 1990) was later shown to be highly susceptible to other pathogens such as the citrus tristeza virus (CTV) (Bar-Jospeh *et al.*, 1981), nematodes and 'mal secco' (Laviola et al., 1990). Replacing the sour orange rootstock with resistant rootstocks such as Troyer citrange, Cleopatra mandarin and Carrizo citrange helped to curb the disease in countries such as Corsica. However, there has been a resurgence of Phytophthora in Corsican groves, probably due to the change in soil and climatic conditions or changing cultural practices or the adaptation of *Phytophthora* to the new rootstocks (Cohen *et al.*, 2003).

Fungicides such as metalaxyl or fosetyl-Al control *P. citrophthora* (Davis, 1982) but require several applications and must be timed correctly (Davino *et al.*, 1990). Other management practices include irrigation management, foliar and trunk application of fungicides and fumigation. Registered fungicides (Fighter and Ridomil Gold) that are effective against *Phytophthora* have been selected for the field trial using them at their registered rates and times of application. Captan, registered in Argentina for use against gummosis at a rate of 200 g/hℓ water (Ref?), was also included in this trial. It is reported from Argentina that this fungicide is not that effective against the disease and it was therefore decided to boost it with Sporekill to be used as a trunk application during the winter period.

The aims of this study were to reconfirm the diagnosis of tree dieback in the Western Cape, and to determine the efficacy of soil, trunk and/or foliar applications of these fungicides against *P. citrophthora* in citrus growing areas with optimal conditions for the pathogen.

Materials and methods

Isolations

During all the visits to Swellendam, soil samples were taken close to the trunks and placed into plastic bags and maintained at 20 – 25°C during transport to the laboratory and storage prior to processing. After 5 days, ±6-7 g of soil was placed into small containers (3x5 cm), diluted with ±5 ml water and floating citrus leaf discs were used as bait. After 3 days, these leaf discs were placed onto PARP selective media for *Phytophthora* spp. and incubated at 24°C. Of the *Phytophthora* isolates growing from the leaf discs, one sub-culture of each isolate was send to the Plant Protection Research Institute in Pretoria for identification.

Fungicidal treatments

Five rows of orchard "Vlei" G2 (planted in 1990) at Sovereign Estates east of Swellendam were selected and marked. The following treatments were applied at the following rates:

a) Ridomil Gold (2 mℓ/m²) – soil application; applied as registered
b) Fighter (570 mℓ/hℓ water) – foliar application; applied as registered

c) Captan + Sporekill (200 g + 100 mℓ/hℓ water) – trunk spray application; applied as a new experimental treatment

The exact timing of each treatment is presented in Fig. 4.6.4.1.

General tree and trunk health of the first five affected trees with visible infections was assessed in the 'nules' orchard ("Vlei" G2) in 2005 (before the application of the spring fungicides) and in 2006 (after harvest). Tree health was assessed by rating the tree canopy to the following scale: 0 = healthy tree and 10 = dead tree. Trunk health was assessed by rating the region from the scion upwards to just below the first branches (± 50 cm in length) to the following scale: 10% to 90% at 10% increments = where 10% to 90% of total outside circumference of the trunk showed infection. Trees that showed 100% were already dead at that stage.

Results and discussion

Isolates

All the *Phytophthora* isolates obtained from the soil samples were identified as *Phytophthora citrophthora*.

Fungicidal treatments

The remaining trees in the 'nules' orchard ("Vlei" G2) all recovered from the trunk infection. Although not tested individually, the combined effect of the soil, foliar and trunk treatments were effective in controlling the disease. Interesting to note is that all the tree that had a canopy rating of >6/10 died, most likely because the ambimobile phoshonate could not be adequately absorbed into the foliage. Where the trunks had an infective rating of 60%, the trunk applications could not protect the tree from further infection. Therefore the recommendation to the growers would be that trees with a canopy rating of >6/10 and a trunk infection ration rating of 60% should eliminated as no treatment will be effective in the recovery of the tree (Fig. 4.6.4.2, Fig. 4.6.4.3 and Fig. 4.6.4.4).

Conclusion

Diagnosis of tree dieback in the Western Cape was confirmed by this study as all *Phytophthora* isolates obtained were identified as *Phytophthora citrophthora*. *P. citrophthora* seems to be limited to the cool coastal areas of South Africa that are subjected to the winter rainfall. The temperature growth requirements for this pathogen are: minimum: <5°C, optimum: 24-28°C, maximum: 32-33°C. This explains why the disease was not prevalent on young trees with small canopy diameters: light penetration onto the soil will effect warmer soils surrounding the trunk and therewith conditions not suitable for the pathogen. However, when the trees are about 15 years old and the canopies closes between the trees, little light penetration occurs onto the soil and the prevailing microclimate is conducive for the disease. The placement and type of micro irrigation is also important. Growers should prevent splash-on and wetting of the trunk from micro irrigation as irrigation water can be the source of inoculum. Although not proven, but the huge amount of snails that occur in the orchards can serve as a vector for the disease onto the trunks.

Future research

Reports were also received of similar problem in the Gamtoos Valley, as well as certain Clementine groves in the Western Cape. These farms should be inspected to determine the extent of the problem on Clementines. Control programmes consisting of different fungicides with different modes of action should be investigated and the possibility that snails can severe as a vector, should also be investigated.

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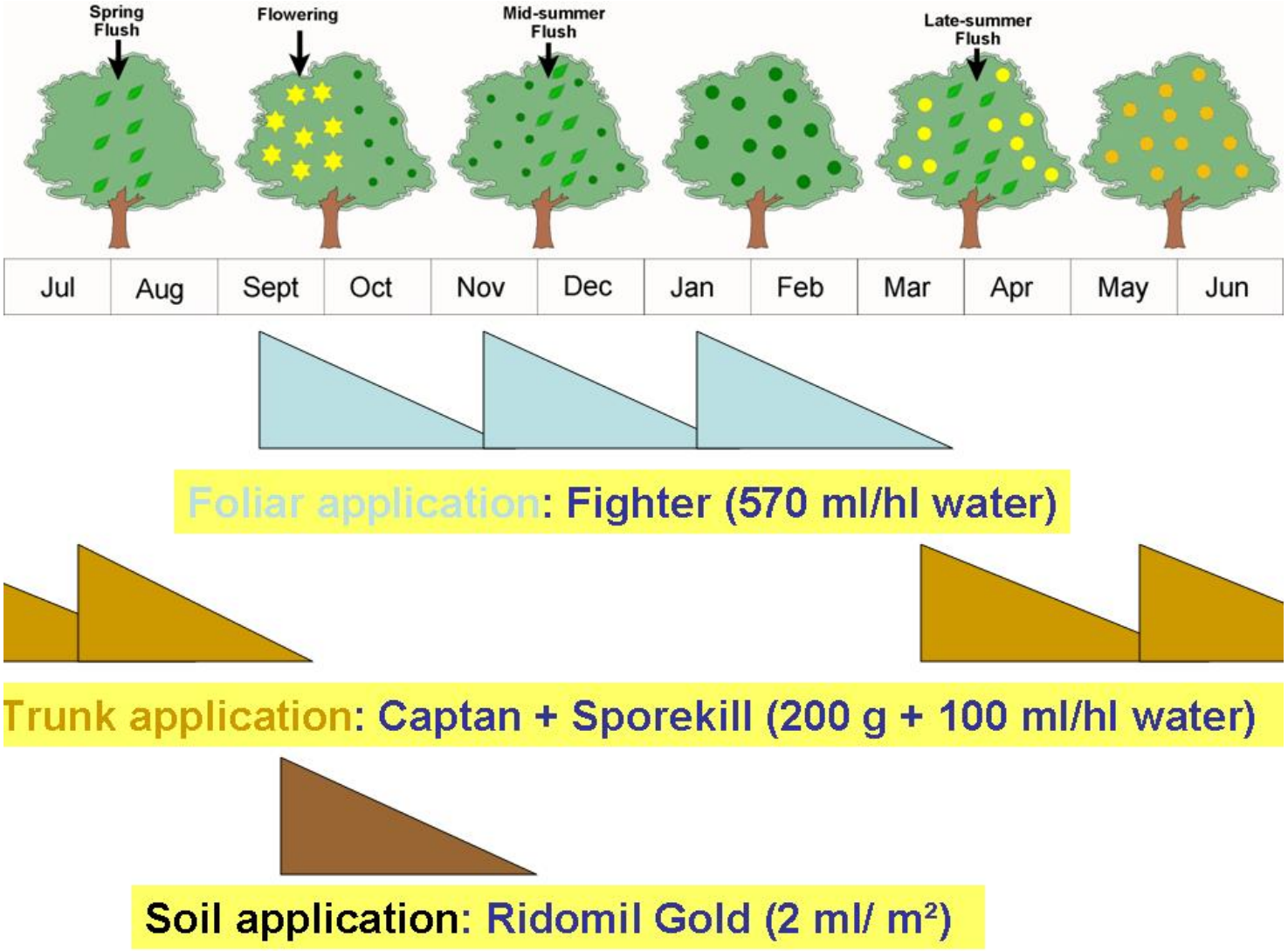

Fig. 4.6.4.1. Control programme used on Nules Clementines for the control of *Phytophthora citrophthora* during 2005 -2006.

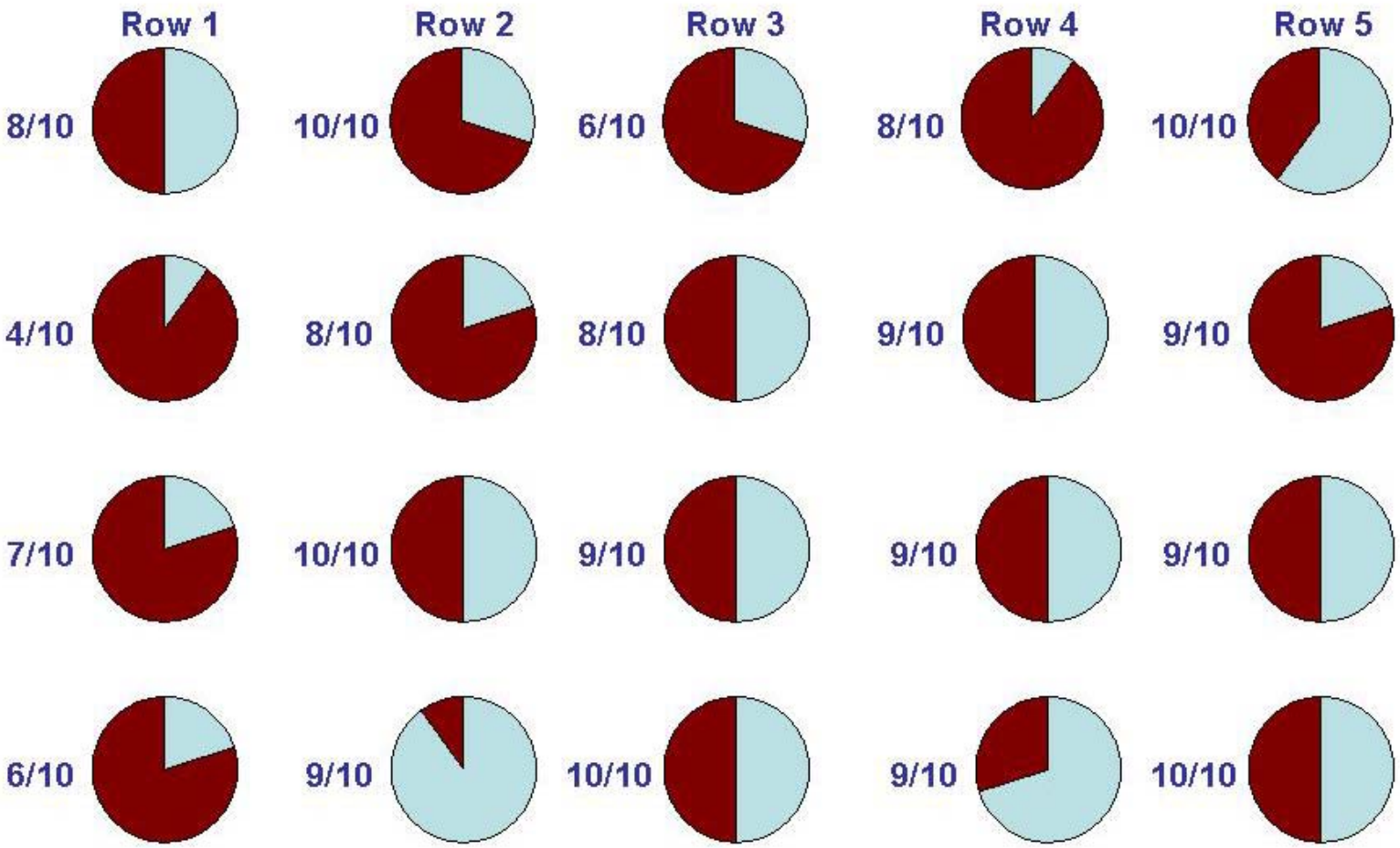

Fig. 4.6.4.2. Nules Clementine canopies affected by *Phytophtora citrophthora* was rated on a scale from 0 – 10 (where 0 = healthy and 10 = dead). If the tree trunk was girdled by the disease, a % scale (where 0% = healthy and 100% = totally infected) was used (illustrated in the circles). Evaluation date: 28 September 2005.

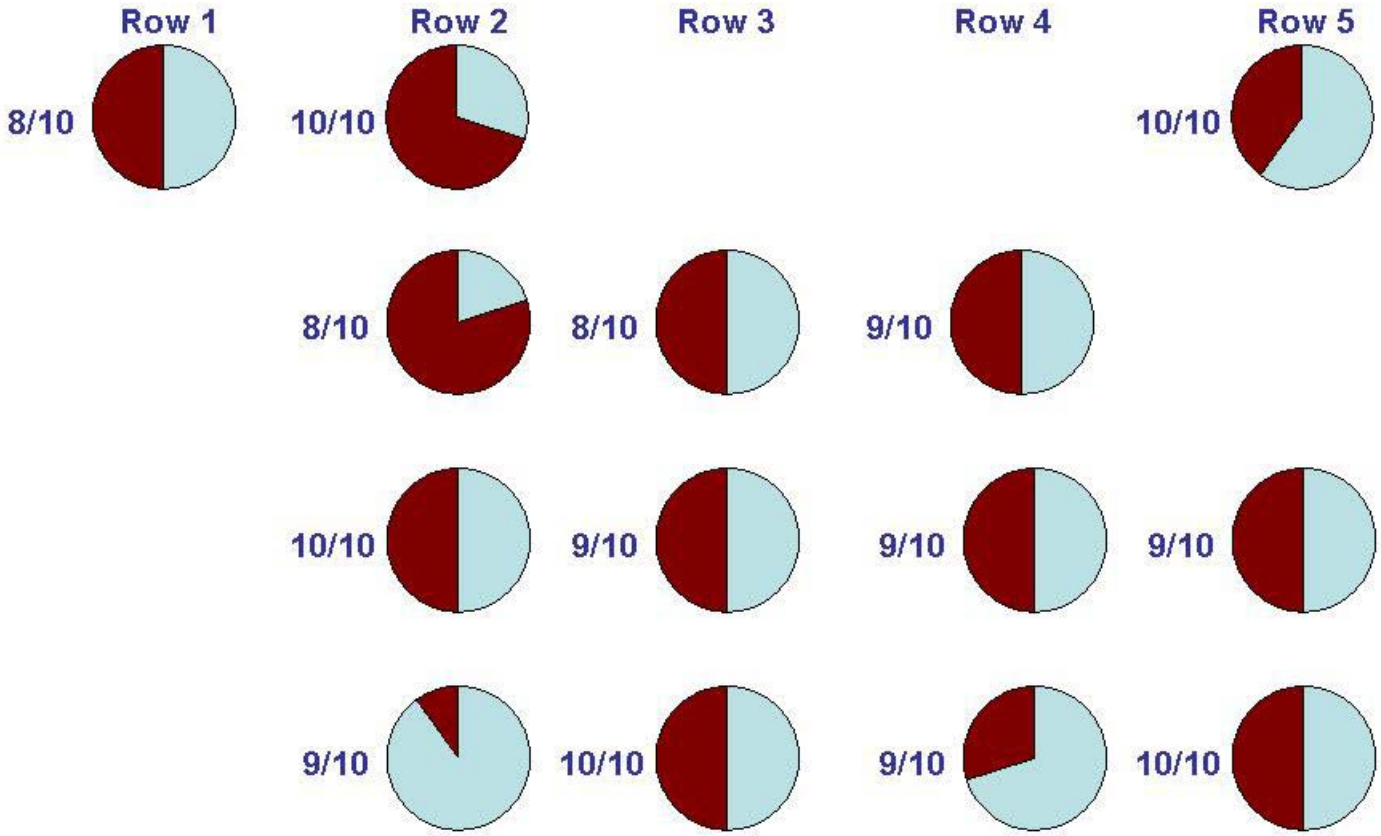

Fig. 4.6.4.3. Nules Clementine canopies affected by *Phytophtora citrophthora* was rated on a scale from 0 – 10 (where 0 = healthy and 10 = dead). If the tree trunk was ringulated with the disease, a % scale (where 0% = healthy and 100% = totally infected) was used (illustrated in the circles). Trees missing from this diagram, died and were removed from the orchard. Evaluation date: 6 July 2006.

Fig. 4.6.4.4. Callus formation on a Nules Clementine branch (left) and trunk (right) after a control programme was followed during the 2005-2006 season. Date of evaluation: 6 July 2006.

Fig. 4.6.4.5. Recovery of old Nules Clementine trees of a commercial block (not part of experiment) after trunk paint with 2.5 ℓ copper oxychloride plus 5 ℓ PVA in 5 ℓ water for the control of *Phytophthora citrophthora.*

4.6.5 **Susceptibility of different Clementine cultivars to *Phytophthora citrophthora***
Experiment 855 by G.C. Schutte (CRI)

Opsomming

Die vrees dat ander Clementine kultivars ook deur *Phytophthora citrophthora* geïnfekteer kan word, moes ondersoek word. Clementine kutivars soos 'Marisol', 'Clemlate', 'Oroval' en 'Oroblanco' kon almal kunsmatig met *Phytophthora citrophthora* geïnokuleer word en sodoende bewys dat hulle vatbaar is vir *P. citrophthora*. In kommersiële boorde is 'Orovals' en 'Clemenpons' ook positief met die siekte geïdentifiseer.

Introduction

Tree die-back was observed in the Knysna region of the Eastern Cape on the Nules cultivar. According to growers, the problem seems to occur in other regions such as Patensie, Swellendam, Stellenbosch and Citrusdal as well and is spreading each year to adjacent trees resulting in die-back of the trees within one month. Recently, die-back of 'Orovals' was also recorded in the Knysna region, but a cultivar such as 'Satsumas' appears not be affected. All these cultivars were budded onto 'Troyer' rootstocks and the rootstock is not affected. The die-back starts on the scion (30 cm above ground level). The aim of this study was to determine whether trunk inoculation can be used to determine the relative susceptibility of different Clementine cultivars.

Materials and methods

Isolations

During a visit to the farm "Buffeljachts" near Swellendam, soil and bark samples were taken from infected trees and taken to CRI in Nelspruit. Soil samples were taken close to the trunks and placed into plastic bags and maintained at 20 – 25°C during transport to the laboratory and storage prior to processing. After 5 days, ±6-7 g of soil was placed into small containers (3x5 cm), diluted with ±5 ml water and floating citrus leaf discs were used as bait. After 3 days, these leaf discs were placed onto PARP selective media for *Phytophthora* spp. and incubated at 24°C. Of the *Phytophthora* isolates growing from the leaf discs, one sub-culture of each isolate. One of the sub-cultures of each isolate was send to the Plant Protection Research Institute in Pretoria for identification. The other sub-samples were kept for later use to fulfil Koch's postulates.

Koch's postulates

Five nursery trees consisting of Nules Clementine (indicator tree), 'Clemlate', 'Marisol', 'Oroblanco' and 'Oroval' (all on Troyer rootstocks were obtained from the OFB in Uitenhage and taken to CRI in Nelspruit where they were kept in a glasshouse. On the stems of these trees (above the scion), a 3x3 cm² area was surface sterilized with ethanol using *P. citrophthora*. Directly afterwards, a 1x1cm² section was wounded by means of a sterile scalpel where only the bark was slightly scraped to allow a site for entry into the stem. A 1x1 cm² mycelial plug consisting of *P. citrophthora* was placed on the bark and wrapped with parafilm. Trees were placed in the laboratory where a constant temperature of 24-25°C was maintained and the trees were monitored for dieback on a daily basis.

Isolations from a commercial 'Clemenpons' orchard

During a visit to the farm 'Mandaryn' west of Riviersonderend, a new cultivar, *viz*. 'Clemenpons' showed similar die-back as the 'nules' from Swellendam. Soil and bark samples were taken for identification purposes.

Results and discussion

Isolations

All the isolates were identified as *P. citrophthora* by Dr. W.J. Botha (ARC-PPRI, Pretoria).

Koch's postulates

All the trees showed typical secretion of gum and died within two weeks (Fig. 4.6.5.1.) *P. citrophthora* was also re-isolated from the bark from all these trees.

Isolations from a commercial Clemenpons orchard

The soil and bark samples tested positive for *P. citrophthora* (Fig. 4.6.5.2.).

Conclusion

Under optimal conditions for *P. citrophthora* infection (24-28°C), all Clementine cultivars that were screened for susceptibility, were affected by *P. citrophthora*. These findings are confirmed by a similar study conducted in Valencia, Spain, showing that 'nules', 'Marisol' and 'Clemenpons' were susceptible to *P. citrophthora* (Alvarez *et al*., 2006). They also showed that other cultivars such as the 'Fortune' (mandarin), 'navelina' and 'Hernandina' are also susceptible. Furthermore, all the rootstocks on which these cultivars were grafted, viz. Çarrizo citrange', 'Volkameriana', and 'Ámargo', were resistant to *P. citrophthora*. In a separate investigation, isolations made from 'Oroval' trees in an orchard at Sovereign Estates near Swellendam, also tested positive for the disease (Fig. 4.6.5.3).

Future research

All citrus orchards will be monitored to determine the spread of the disease and all references regarding susceptible cultivars (Table 4.6.5.1.) will accumulated and list will be updated annually.

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Table 4.6.5.1. World wide distribution of citrus hosts susceptible to *Phytophthora citrophthora* (gummosis).

Host	Cultivar	Country	Reference
Citrus limon	Lemon	USA	Fawcett, 1936
			Matheron & Matjeka, 1988
		New Zealand	Cockayne & Cunningham, 1921
		Argentina	Fawcett, 1915
		Australia	Darnell-Smith, 1918
		Italy	Traverso, 1921
		France	Cohen, 2003
		Corsica	Cohen, 2003
Citrus paradise	Grapefruit	South Africa	Wager,1931
		USA	Fawcett, 1915
		Congo	Staner, 1929
		Puerto Rico	Guiscafre-Arrillaga, 1931
		India	Sharangapani, 1930
		Australia	Fraser, 1942
		New Zealand	Brien, 1946
		Israel	Schiffman-Nadel & Cohen, 1968
		Corsica	Cohen, 2003
Citrus reticulate	Clementine:		
	Nules	South Africa	Schutte, 2007
	Oroval	Spain	Alvarez, 2006
	Clemenpons	Corsica	Cohen, 2003
	Clemlate		
	Oroblanco		
Citrus reticulata hybrids	Mandarins:		
	Nova	Spain	Alvarez, 2006
	Fortune		
	Orlando tangelo	USA	Matheron & Matjeka, 1988
Citrus sinensis	Sweet oranges:		
	Salustiana	Spain	Alvarez, 2006
		USA	Fawcett, 1915
		Taiwan	Sawada, 1919
		South Africa	Doidge, 1925
		Congo	Staner, 1929
		Philippines	Anon. 1938
		Australia	Fraser, 1942
		Argentina	Frezzi, 1950
		New Zealand	Fletcher, 1957
		Morocco	Cohen, 2003
Citrus spp.	Citrus	Brazil	Averna-Sacca, 1912
		Sardinia	Petri, 1925

Host	Cultivar	Country	Reference
		Spain	Fawcett, 1930
		Sicily	Fawcett, 1930
		Egypt	Fawcett, 1930
		Puerto Rico	Guiscfre, 1932
		Tunisia	Chabrolin, 1932
		Mozambique	Cardoso, 1934
		Australia	McLennan, 1936
		Argentina	Frezzi, 1940
		South Africa	Loest, 1950
		Mauritius	Orian, 1951

Fig. 4.6.5.1. A young 'Marisol' nursery tree inoculated with *Phytophthora citrophthora,* showing typical gum secretion after one week and death within 2 weeks.

Fig. 4.6.5.2. Sampling bark from a 'Clemenpons' with an increment borer for the isolation of *Phytophthora citrophthora*. Soil samples were also taken from the same trees.

Fig. 4.6 5.3. The trunk of an ‘Oroval’ showing typical symptoms of *Phytophthora citrophthora* in Swellendam.

4.7 **CRI Diagnostic Centre**

By Laura Huisman and Timothy Zulu (CRI)

Analysis		Citrus nurseries	Commercial samples	Other crops	Research samples
Nematodes:	Roots		326	26	378
	Soil	73	39	242	338
Sugar flotation					40
Phytophthora:	Soil	1501	309	198	106
	Nursery water	82			1
Black spot			13		
Red scale			2		
Citrus greening (UP)			2		9
Internal fruit quality			10		

Citrus Accredited Nurseries

One thousand five hundred samples were received by the DC for *Phytophthora* analyses. Only 3.4% tested positive. No citrus nematodes were extracted from any CIP nursery samples.

Commercial samples

Samples were received from the following citrus areas: Western Cape 50.6%, Mpumalanga 18.3%, Limpopo 10.3%, Eastern Cape 8.7%, North-West 8.1% and Natal 4%. Most of the samples received from citrus growers were analysed for *Phytophthora nicotianae* and citrus nematodes. *Phytophthora citrophthora* was isolated in some samples received from coastal areas. Fifty-six percent of samples had citrus nematode counts above the threshold value of 1000 females per 10 grams of roots, and nematicide treatments were recommended. Forty-four percent of the samples tested positive for *Phytophthora*.

Other crops

Nematode counts were done on soil or root samples of grapes, leather leaf ferns, bananas, litchis and vegetables. *Phytophthora* and *Pythium* analyses were done on palms and macadamia samples. The macadamia industry is planning a nursery improvement plan similar to the Citrus Nursery Improvement Plan. The DC will analyse some of the nursery samples for *Phytophthora cinnamomi.*

Avocado nursery samples

A total of 90 avocado nursery samples were analysed for *Phytophthora cinnamomi.*

Research samples

Laboratory trials were conducted to test different treatments and combinations of treatments to stimulate citrus nematode egg hatching. During August/September, black spot fruit from Crocodile Valley Citrus Co. was tested to determine the percentage resistance to benzimidazole and the percentage infected with *Guignardia citricarpa*.

5 PROGRAMME: CROP LOAD AND FRUIT QUALITY MANAGEMENT

5.1 PROGRAMME SUMMARY

By Tim G Grout (Manager: Research & Technical)

During 2006, horticultural research was conducted in the three projects rind condition, fruit quality enhancement, and crop load management. Most resources were spent on the rind condition project due to the extreme losses that can be experienced from creasing, rind breakdown, chilling injury and Peteca spot. One of the reasons why the causes of these conditions have not yet been identified is due to their sporadic intensity and 2006 emphasised this fact. The levels of creasing experienced were high and perhaps masked remedial effects of weak treatments, while the levels of chilling injury and peteca spot were low to non-existent. Much of this research therefore requires further attention in 2007. However, valuable advances were made in the understanding of rind breakdown and the importance of carbohydrate levels. Pruning may be important in reducing both rind breakdown and creasing by allowing more light into the tree. Different approaches to improving fruit colour at harvest were also investigated. Preharvest treatments of Prohexadione-calcium and postharvest cold and heat-shock treatments showed promise, although the response to the latter treatments was cultivar dependent. Further research on an alternative to calcium arsenate for lowering fruit acidity again showed that 1 or 2% MAP reduces acid levels significantly, although not to the same extent as calcium arsenate. Various approaches to improving crop load and fruit size had some measure of success but were not always economically beneficial. Late in 2006 a decision was taken to establish a research project focusing on cold chain management and packaging. Future research in this project should dove-tail with current advances in rind condition and fruit quality research.

PROGRAMOPSOMMING

Deur Tim G Grout (Bestuurder: Navorsing en Tegnies)

Die drie projekte waarop navorsing in hierdie program gedurende 2006 gefokus het, was skilkondisie, verbetering van vrugkwaliteit en die bestuur van oeslading. Die meeste fondse is aan die projek op skilkondisie gespandeer omdat geweldige verliese gelei kan word as gevolg van kraakskil, skilafbraak, koue skade en Peteka vlek. Een van die redes waarom die oorsake van hierdie toestande nog nie bepaal kon word nie, is die sporadiese voorkoms en intensiteit daarvan wat weereens in 2006 bevestig is. Die vlakke van voorkoms van kraakskil was hoog en het moontlik die beskermende effekte van swak behandelings verbloem. Die vlakke van koue skade en Peteka vlek was laag tot feitlik afwesig. Baie van hierdie navorsing benodig dus verdere aandag in 2007. Waardevolle vordering is egter gemaak in die verstaan van skilafbraak en die belang van koolhidraat vlakke. Snoei mag belangrik wees in die vermindering van beide skilafbraak en kraakskil deurdat daar meer lig in die boom ingelaat word. Verskillende benaderings om vrugkleur tydens oes te verbeter is ook ondersoek. Voor-oes behandelings met Proheksadioon-kalsium en na-oes koue en hitte-skok behandelings het potensiaal getoon, alhoewel die reaksie op laasgenoemde behandelings kultivar afhanklik was. Verdere navorsing op ’n alternatief vir kalsium arsenaat vir die verlaging van vrugsure het weereens getoon dat 1 of 2% MAP die suurvlakke betekenisvol kan verminder, alhoewel nie tot dieselfde mate as kalsium arsenaat nie. Verskeie benaderings om die oeslading en vruggrootte te verbeter het ’n mate van sukses opgelewer maar was nie altyd ekonomies voordelig nie. Daar is aan die einde van 2006 besluit op ’n navorsingsprojek wat sal fokus op die bestuur van die koue ketting en verpakking. Verdere navorsing in hierdie projek sal moet inskakel by die vordering wat reeds gemaak is met navorsing op skilkondisie en vrugkwaliteit.

5.2 PROJECT: RIND CONDITION

Project Co-ordinator: J P Bower (UKZN)

5.2.1 Project summary

The project has concentrated on four areas of research, those being creasing, rind breakdown including puffiness, chilling injury and Peteca spot in lemons. Three trials relating to creasing were conducted. In the first (5.2.2), the products AVG (Retain®), Decrease and Goëmar were tested. None were commercially useful, and future work will concentrate on other products. In experiment 863 (5.2.3) creasing incidence was identified as greatest in inside fruit, with Ca and K being correlated, with Ca negatively and K positively (opposite to leaf analysis results). It is suggested that light and temperature play a role, and that pruning is a useful management option. Future work should further study albedo nutrients. Work was started on the role and manipulation of carbohydrate relations (5.2.4), but no results are as yet available. The effect of ethylene and CO_2 on Clementine puffiness development (5.2.5) was tested, but without clear results due to low puffiness development. In 2007 work will be repeated with ethylene and CO_2 combinations. A number of experiments were conducted to decrease chilling injury in various cultivars. In section 5.2.6, results of

various chemical applications to lemons, Clementines and Valencias are outlined. Hot water treatments were also included. The best result was a 50°C water bath treatment, with 40% less chilling injury. Work will be repeated in 2007. A study of carbohydrate levels in Clementines (5.2.7) showed lower concentrations in the flavedo of inside fruit, which is considered to be more susceptible to rind breakdown, supporting the theory that lower carbohydrate levels enhance the chance of the postharvest disorder. A simulated cold sterilization trial of Oroblanco (5.2.8) resulted in high levels of Diplodia and an oleo-like symptom rather than chilling injury. The pathogen incidence is of concern, but the trial will be repeated in 2007. The use of hot water treatment on lemons (5.2.9) for chilling injury prevention was investigated. Unfortunately, after treatment at 53°C, fruit wilted in storage, and the trial was terminated. A repeat will be conducted in 2007. Five experiments were conducted on Peteca spot. The effect of storage temperature and duration were studied (5.2.10). Low levels of Peteca spot occurred, and no clear trends could be established, suggesting temperature is not a primary cause. The role of relative humidity and rind water status (5.2.11) was studied, but it could not be demonstrated that a 24 h delay in packing with low relative humidity induced the disorder. However, in the late harvested fruit wilted for more than 24 h before degreening (5.2.12) Peteca spot did increase. The effect of preharvest chemicals and wax (5.2.13) did not result in any Peteca spot development. When various types of wax applications were applied to lemon fruit, plus hot water treatments and rough handling added, no symptoms occurred (5.2.14). Thus, no conclusions could be made.

Projekopsomming

Die projek het op vier velde van navorsing gekonsentreer, naamlik kraakskil, skil afbraak insluitende pofferigheid, die efek van koue, veral op suurlemoene en Peteca vlek op suurlemoene. Drie proewe in verband met kraakskil is gedoen. In die eerste (5.2.2) is die produkte AVG (Retain®), Decrease en Goëmar getoets. Geen van hulle was kommersieel aanvaarbaar nie, en toekomstige werk sal op ander produkte toegespits word. In eksperiment 863 (5.2.3), was die voorkoms van kraakskil die grootste by binne vrugte, met kalsium en kalium daarmee gekorreleer, kalsium negatief en kalium positief, die laasgenoemde anders as by blare. Dit is voorgestel dat lig en temperatuur 'n rol speel, en dat snoei 'n waardevolle rol kan speel. Toekomstige werk sal op albedo elemente konsentreer. Werk is op die rol van koolhidrate en die manipulering daarvan (5.2.4) begin, maar resultate is nog nie beskikbaar nie. Die efek van etileen en CO_2 op pofferigheid van Clementines (5.2.5) is getoets, maar geen duidelikke resultate as gevolg van die min ontwikkeling van pofferigheid, is gekry. Gedurende 2007 sal die werk met die gebruik vam kombinasies van CO_2 en etileen gedoen word. Verskeie experimente vir die voorkoming van koueskade by 'n verskeidenheid kultivars is gedoen. In afdeling 5.2.6 word die resultate van die aanwending van verskeie gemikalië by suurlemoene, Clementines en Valencias aangetoon. Warm water behandeling is ook gebruik. Die beste resultaat was 'n warm water behandeling van 50°C wat tot 'n 40% verlaging van koueskade gelei het. Die werk sal in 2007 herhaal word. 'n Studie van die vlakke van koolhidrate by die skille van Clementines (5.2.7) het gewys dat die konsentrasies in die flavedo van binne vrugte, wat ook bekend is vir meer ontwikkeling van skilafbraak, laer is. Dit ondersteun die teorie dat laer konsentrasies van koolhidrate in die skil van vrugte verhoog die kans van skil afbraak. 'n gesimuleerde proef van koue sterilisasie van Oroblanco (5.2.8) het hoë vlakke van Diplodia gehad, sowel as 'n oleo-tiepe simptoom, maar minder koueskade. Die voorkoms van die patogeen is van kommer, maar die proef sal in 2007 herhaal word. Die gebruik van 'n warm water behandeling by suurlemoene (5.2.9) teen die ontwikkeling van koueskade was verondersoek. Ongelukkig, na 'n behandeling van 53°C en opberging, het die vrugte tot so 'n mate verwelk, dat die proef gestaak was. Dit sal in 2007 herhaal word. Vyf proewe is op Peteca vlek gedoen. Die efek van opbergings temperature en tydsduur is ondersoek (5.2.10). Lae vlakke van Peteca vlek het voorgekom, en geen tendense kon gevind word. Tempweratuur is dalk nie 'n primêre oorsaak van die problem nie. Die rol van relatiewe vogtigheid en skil water verhoudings (5.2.11) is ondersoek, maar dit kon nie bewys word dat 24 h se vertraging in die pak van vrugte wat by 'n lae vogtigheid gestaan het, die afwyking laat voorkom het. Nietemin, vrugte wat gepluk en vir meer as 24 h voor ontgroening laat staan het (5.2.12) het meer Pateca gewys. Die efek van 'n aantal chemikalië en wakse wat aangewent was, het geen ontwikkeling van peteca vlek veroorsaak nie. Toe 'n verskeidenheid wakse saam met rowwe hantering van vrugte en warm water behandeling toegepas was (5.2.14), het geen simtome voorgekom nie, en geen gevolgtrekkings kon gemaak word.

5.2.2 **Evaluation of alternative means of controlling creasing (albedo breakdown)**

Experiment 849 by Stephan Verreynne (CRI at SU)

Opsomming

Kraakskil is 'n fisiologiese abnormaliteit wat krake in die albedo van vrugte veroorsaak en lei tot kanale op die oppervlak van die vrug. Gibberelliensuur, wat skilveroudering vertraag, is al vir jare geregistreer and verminder kraakskil as oestyd nie vertraag word nie, maar kan skilkleurontwikkeling vertraag. Die doel van die studie was om alternatiewe beheermaatreëls teen kraakskil te evalueer. AVG (Retain®), De-Crease en Goëmar is toegedien op verskillende proefpersele, teen verskillende konsentrasies en op verskillende tye op

Navel bome in Citrusdal, Addo en Marble Hall en op Washington Navel bome in Citrusdal. Geen behandeling het 'n betekenisvolle verlaging in kraakskil veroorsaak nie, maar Retain® het 'n verhoogte set met kleiner vrugte tot gevolg gehad. Dus, geen behandeling is 'n kommersiële oplossing vir kraaksil nie en toekomstige navorsing sal fokus op ander ongetoetsde produkte, vroeër as normale toediening van GA_3, en alternatiewe Ca bronne en ander kombinasies van produkte.

Introduction

Creasing or albedo breakdown is a physiological disorder resulting in cracks in the albedo and showing channels on the surface of the fruit (Treeby et al., 1995). It starts when cells in the albedo separate at the cell wall junctions (Storey and Treeby, 1994). Pectin that makes up the "cement" (middle lamella) between individual cells in the albedo is broken down as fruit matures (Monselise et al., 1976). Many minerals, namely Mo, Zn, Ca, S, B and Mg, are known to form complexes with the pectin, increasing its strength to hold individual albedo cells together. Creasing incidence is normally correlated with low pectin levels and low complex formation with minerals in cell walls as well as an increase in water soluble pectins (Monselise et al., 1976). The condition was already recorded in 1938 in South Africa, and although the contributing factors have been studied extensively and are known, the exact cause of creasing is still unresolved (Le Roux and Crous, 1938). Creasing yearly causes tremendous losses in both Navels and Valencias. Gibberellic Acid (GA_3) which delays rind senescence has been registered for a number of years and reduces creasing if harvest is not delayed, but it sometimes results in delayed colour development (Embleton et al., 1973; Gilfillan et al., 1980; Gilfillan and Cutting, 1992). AVG (Retain®) is an ethylene inhibitor and preliminary work in California resulted in a decrease in creasing (Gonzalez and Lovatt, 2004). The objective of this study was to evaluate alternative measures to reduce creasing. AVG (sold as Retain®) (an ethylene inhibitor), De-Crease (a formulation of Mg, Mo and Zn), and Goëmar (a formulation containing Mo, B, Mg, S, auxins and cytokinins) were tested at different sites, different concentrations and at different application times (petal fall, 2 weeks after petal fall and after physiological fruit drop).

Materials and methods

Plant material. Palmer Navel trees in Citrusdal, Addo and Marble Hall as well as Washington Navel trees in Citrusdal were used for this study.

AVG (Retain®) is an ethylene inhibitor and was applied at 125 ppm. De-Crease is a formulation containing Mg (30 g/ℓ), Mo (45 g/ℓ) and Zn (20g/ℓ) and is applied at 50 ml/100 ℓ. Goëmar BM 86 E is a GA14 seaweed cream containing Mo (0.02%), B (2.07%), Mg (4.8%), S (3.8%), auxins (18.5 mg/ℓ) and cytokinins (0.052 mg/ℓ).

Treatments. De-Crease was applied as foliar sprays at different concentrations and at different times on Palmer Navels in Citrusdal (Table 5.2.2.1) and Addo (Table 5.2.2.2). Treatments consisted of the following: At petal fall (14 Oct 2005 for Citrusdal and 26 Oct 2005 for Addo), De-Crease at 50 ml/100 ℓ water or at 100 ml/100 ℓ water, Goëmar to which Zn was added, or De-Crease in combination with Goëmar was applied. Two weeks after petal fall (WAPF) (4 Nov 2005 for Citrusdal and 8 Nov 2005 for Addo), De-Crease at 50 ml/100 ℓ water was applied. After physiological fruit drop (5 Dec 2005 for Citrusdal and 9 Dec 2005 for Addo), De-Crease at 50 ml/100 ℓ water was applied. Stopit ($CaCl_2$) was added to some of the treatments (Tables 5.2.2.1 and 2).

In Marble Hall the following treatments were applied as foliar sprays at petal fall (7 Oct 2005): AVG (Retain®) at 125 ppm, De-Crease at 50 ml/100 ℓ water, or Goëmar at 200 ml/100 ℓ water, to which Zn was added (Table 5.2.2.3).

In Citrusdal the following treatments were applied as foliar sprays: At petal fall (5 Oct 2005) AVG (Retain®) at 125 ppm, De-Crease at 50 ml/100 ℓ water, or Goëmar at 200 ml/100 ℓ water, to which Zn was added, with a second Goëmar and Zn application on 4 Nov 2005 (Table 5.2.2.4). After physiological fruit drop (5 Dec 2005), AVG (Retain®) at 125 ppm was applied.

All treatments were applied on 10 single-tree replicates per treatment in a randomized complete block design.

Measurements. Shoots of Palmer Navel trees in Citrusdal were tagged at petal fall to determine percentage fruit set. At harvest, yield (kg/tree), fruit size and fruit number per tree were determined at specific sites using a sample grader. Creasing incidence (percentage fruit per tree with creasing) and severity (score 0-4

per fruit; 0 being no creasing and 4 being 100% creasing) were determined in the field using 50 arbitrary fruit per tree around the tree.

Results and discussion

None of the treatments had a significant effect on fruit set percentage or percentage creasing incidence and none of the treatments significantly reduced creasing severity of Palmer Navel fruit in Citrusdal (Table 5.2.2.1).

Table 5.2.2.1. Effect of De-Crease applied at different times and different concentrations on creasing incidence of Palmer Navel fruit in Citrusdal

Treatments	Concentration	Set	Creasing Incidence	Creasing Severity
	--ml/100 l--	*--%--*	*---%---*	*--(0-4)--*
Control		16.8 ab[z]	37.8	0.8 c
Petal fall (14 Oct 2005)				
De-Crease	50	13.9 b	46.0	1.0 a
De-Crease	100	13.9 b	39.3	0.9 abc
Goëmar, Zinc Max, Stopit	200, 150, 200	23.8 a	39.0	0.9 abc
De-Crease, Goëmar, Zinc Max	50, 200, 150	13.6 b	40.0	0.9 abc
De-Crease, Stopit	50, 200	11.7 b	45.5	1.0 ab
2 WAPF (4 Nov 2005)				
De-Crease	50	--	45.0	1.0 a
After fruit drop (5 Dec 2005)				
De-Crease	50	--	37.5	0.8 bc
P-value		0.0454	0.4448	0.0166

[z]Means in the same column followed by different letters are significantly different at the 5% level

None of the treatments had a significant effect on total yield (kg/tree), number of fruit per tree or average fruit weight per tree (g/fruit) of Palmer Navels in Addo (Table 5.2.2.2). Also, none of the treatments significantly reduced the percentage creasing incidence or the creasing severity of Palmer Navel fruit in Addo (Table 5.2.2.2).

Table 5.2.2.2. Effect of De-Crease applied at different times and different concentrations on creasing incidence of Palmer Navel fruit in Addo

Treatments	Concentration	Yield	Fruit	Fruit weight	Creasing Incidence	Creasing Severity
	--ml/100 l--	*Kg/tree*	*-no.-*	*--g/fruit--*	*---%---*	*--(0-4)--*
Control		110	554	198	53.8 abc[z]	1.3 bc
Petal Fall (26 Oct 2005)						
De-Crease	50	101	622	169	47.6 c	1.2 c
De-Crease	100	117	614	192	53.5 abc	1.3 bc
Goëmar, Zinc Max, Stopit	200, 150, 200	86	472	182	51.5 bc	1.2 c
De-Crease, Goëmar, ZincMax	50, 200, 150	116	636	182	58.7 ab	1.4 ab

De-Crease, Stopit	50, 200	98	534	184	49.9 bc	1.2 c
2 WAPF (8 Nov 2005)						
De-Crease	50	106	540	197	63.3 a	1.6 a
After fruit drop (9 Dec 2005)						
De-Crease	50	99	531	187	52.9 bc	1.3 c
P-value		0.2290	0.1921	0.5857	0.0237	0.0001

[z]Means in the same column followed by different letters are significantly different at the 5% level

No creasing developed at harvest time on Palmer Navel fruit in Marble Hall (Table 5.2.2.3). Fruit were however, harvested very early (1 month earlier than normal) due to a rapid drop in titratable acidity. Retain® applied at petal fall had no effect on total yield (kg/tree), the number of fruit per tree as well as marketable yield (fruit larger than count 88 (69 mm)) per tree, but significantly reduced average fruit weight per tree compared to the control, due to a greater number of fruit per tree (although not significant). Therefore, Retain® resulted in more fruit of a smaller size per tree.

Table 5.2.2.3. Effect of Retain® applied at petal fall on 7 Oct 2005 on creasing incidence of Palmer Navel fruit in Marble Hall

Treatments	**Concentration**	**Yield**	**Fruit**	**Market. Yield 88 (69 mm)**	**Fruit weight**
	-/100l-	***-kg/tree-***	***--no.--***	***--%--***	***--g/fruit--***
Control		170	589	76 ab[z]	286 a
De-Crease	50 ml	187	719	77 a	260 ab
Goëmar Zinzmax	250 ml 150 ml	160	620	80 a	262 ab
Retain®	83 g	168	721	65 b	234 b
P-value		0.7848	0.4671	0.0565	0.0397

[z]Means in the same column followed by different letters are significantly different at the 5% level

Retain® applied at petal fall on Washington Navel trees in Citrusdal significantly improved percentage fruit set, but had no effect on total yield (kg/tree), number of fruit per tree, total marketable yield per tree (larger than count 88 (69 mm)), or average fruit weight per tree (Table 5.2.2.4). Retain®, applied at petal fall, decreased the creasing severity, but had no effect on the incidence of creasing. There was a weak correlation between creasing severity and fruit diameter, therefore creasing was not restricted to the smaller fruit and even large fruit had creasing (data not shown).

Table 5.2.2.4. Effect of Retain® applied at petal fall (5 Oct 2005) or after fruit drop (5 Dec 2005) on creasing incidence of Washington Navel fruit in Citrusdal

Treatments	Conc.	Set	Yield	Fruit	M. Yield 88 (>69 mm)	Fruit weight	Creasing Incidence	Creasing Severity
	-/100 l-	*--%--*	*-kg/tree-*	*-no.-*	*--%--*	*--g/fruit--*	*---%---*	*--(0-4)--*
Control		9.7 b[z]	197	681	41	163 abc	58	1.4 b
Petal fall (5 Oct 2005)								
De-Crease	50 ml	10.9 b	167	798	34	154 bc	58	1.4 b
Retain®	83 g	46.3 a	174	847	27	149 c	51	1.2 c
1 Goëmar, Zinzmax, 4 Nov 2005	200, 150	12.2 b	203	646	43	169 a	56	1.4 b
After fruit drop (5 Dec 2005								
Retain®	83 g	--	176	685	39	167 ab	63	1.5 a
P-value		0.0001	0.1861	0.6486	0.1651	0.0353	0.0704	0.0001

[z]Means in the same column followed by different letters are significantly different at the 5% level

Conclusion

None of the treatments De-Crease, Retain® or Goëmar resulted in a significant reduction in creasing incidence, but Retain® resulted in an increased set of smaller fruit. Therefore, none of the treatments is a commercial solution for the reduction of creasing. Therefore, the best control measures currently available are pruning to improve light distribution in the tree and harvest as early as possible. GA_3 is also a very good control measure for creasing if its application is properly timed. It should be noted that overall creasing incidence was very high and it is possible that treatment effects would be different with a lower creasing incidence.

Future research

Due to the insignificant results from these products, future research will focus more on other untested products as well as earlier than normal application of GA_3, to eliminate the negative effect of GA_3 on colour development. Future research will also focus more on alternative Ca sources and other combinations of products.

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5.2.3 Relationship of bearing position on a tree and the incidence and severity of creasing/albedo breakdown

Experiment 863 by Stephan Verreynne (CRI at SU)

Opsomming

Die doel van die studie was om: 1) te bepaal of die posisie van vrugte in 'n boom 'n effek op die voorkom en graad van kraakskil het, 2) om te bepaal of kraakskil erger in die binnekant (skadukant) of buitekant (sonkant) van vrugte voorkom en 3) om te bepaal of hierdie verskille in kraakskil verband hou met makro- en mikroelement konsentrasies in die albedo weefsel van vrugte by oestyd. Washington Navel bome in Citrusdal in 'n noord-suid ryrigting is gebruik vir die studie. Vrugte is geoes van vier sub-sektore, die binne en buite boonste dele van die boom, en die binne en buite onderste dele van die boom in die vier sektore, nl. noord, suid, wes en oos. Vir buite sub-sektore is die skil opgedeel in die buite- (sonkant) of binnekant (skadukant) van die vrug. Kraakskil voorkoms (%), skildikte en minerale element konsentrasies in die albedo weefsel van die vrugte is bepaal. In die algemeen was kraakskil voorkoms baie hoog (>55%) in alle bome en kan kraakskil voorkoms in die volgende orde geplaas word: boonste binne (92%) = onderste binne (85%) = boonste buite (65%) = onderste buite (71%). Binne vrugte het betekenisvol meer kraakskil (%) as buite vrugte gehad, wat die belangrikheid van lig in kraakskil ontwikkeling toon. In terme van die kant van die boom, kan kraakskil voorkoms in die volgende orde geplaas word: noord (82%) > oos (81%) > wes (75%) > suid (74%). Wanneer binne - en buitevrugte vergelyk word, het binnevrugte 'n groter kraakskil voorkoms, dunner skille, laer kalsium (Ca) en hoër kalium (K) konsentrasies in die albedo weefsel van vrugte by oestyd. Sterk negatiewe korrelasies tussen skildikte en kraakskil voorkoms (%) (r = -0.42) en tussen kraakskil voorkoms (%) en Ca konsentrasies in die albedo weefsel (r=-0.45) en 'n sterk positiewe korrelasie tussen kraakskil voorkoms (%) en K konsentrasies in die albedo weefsel (r = 0.54) is waargeneem. Ten spyte dat vrugte van buite sub-sektore konstant dikker skille en 'n laer graad van kraakskil op die sonkant (buitekant) as op die skadukant (binnekant) van vrugte gehad het, het geen van die makro- of mikroelement konsentrasies in die albedo weefsel tussen die buite- en binnekant van die vrugte betekenisvol verskil nie. Daar word aangeneem dat lig en/of temperatuur 'n rol speel in die verskille wat waargeneem is, dus, snoei om ligverspreiding in die boom te verbeter word aanbeveel. Met 'n laer voorkoms van kraakskil en groter verskille tussen verskillende posisies in die boom, kan selektiewe oes 'n moontlike opsie wees.

Introduction

Creasing or albedo breakdown is a physiological disorder resulting in cracks in the albedo and showing channels on the surface of the fruit (Treeby et al., 1995). Although the contributing factors have been studied extensively and are known, the exact cause of creasing is still unresolved. Creasing yearly causes tremendous losses in both Navels and Valencias. The objectives of this study are: 1) to determine if the position of fruit on a tree has an effect on the incidence and the severity of creasing, 2) to determine if creasing is more pronounced on the inside (shaded) or outside (exposed) part of the fruit and 3) to determine if these differences in creasing incidence mentioned above do occur, if they are related to differences in macro- and micronutrient concentrations in the albedo tissue of fruit at harvest.

Jones et al. (1967) showed that fruit on the south side of the tree (NH) had more creasing than the north side of the tree and that the south side was the first to show creasing.

Previous studies (Le Roux and Crous, 1938; Fourie and Joubert, 1957; Jones et al., 1967) also showed that the shaded, inside part of the fruit has more creasing than the exposed side of fruit. If there is a difference in creasing incidence between the inside (shaded) or outside (exposed) part of the fruit and if these mineral nutrients are directly involved in creasing development, there should also be differences in mineral nutrient concentrations in shady vs sunny sides of fruit. Storey et al. (2002) and Treeby and Storey (2002) reported that the sunny side of fruit had a greater Ca concentration in albedo tissue than the shady side, whether creased or not. Kruger et al. (2005) concluded that mobile elements (N, P, K) in the rind had higher concentrations in inside than outside fruit and that the shady side of outside fruit had a greater concentration of mobile elements in the rind than the sunny side of the fruit. Immobile elements (Ca, Mg, Zn, Mn, Fe and B) occur at a higher concentration in the rind in outside compared to inside fruit, with a greater concentration in the sunny side than the shady side of outside fruit rinds (Kruger et al., 2005).

Storey et al. (2002) showed variation in nutrient concentrations in the albedo tissue from fruit collected from different positions on the tree, but did not report the creasing incidence in the different positions on the tree. There were, however, larger variations in K than Ca concentrations in albedo tissue in fruit among different positions in the tree. Haas (1950) reported that the accumulation of P in the peel resulted in thinner peels and that the inner portion (shady part) of the peel contained less phosphorus than the outer portion.

Storey et al. (2002) reported that concentrations of Ca in the albedo tissue were negatively correlated with creasing incidence. Creased fruit had a lower Ca in albedo tissue than no-creased fruit (Storey et al., 2002), but there was no difference in leaf Ca concentrations. Creased fruit had a thinner peel and greater concentrations of N, P, and K in the peel and lower concentrations of Ca, Mg and Na in the peel than non-creased fruit (Jones et al., 1967).

Application of K has been reported to reduce creasing incidence (Fourie and Joubert, 1957; Jones et al. (1967), possibly due to increased peel thickness. Therefore, creasing incidence seems to be related to peel thickness. Creasing incidence was negatively correlated with peel thickness and flavedo thickness, but not albedo thickness (Treeby et al., 2000). A combination of soil-and foliar applied K resulted in a decrease in creasing, thicker peels, greater N and K concentration in the leaves, no effect on P and lower Ca and Mg concentrations in the leaves (Embleton et al., 1973).

High phosphate application tends to increase creasing incidence (Le Roux and Crous, 1938; Jones et al., 1967). P (superphosphate) application resulted in thinner peels, no effect on creasing and N concentration in the leaves, greater K, Ca and Mg concentrations and lower K concentrations in the leaves (Embleton et al., 1973). Creasing is normally associated with thin peels and low nitrogen content, which would suggest excess phosphate (Le Roux and Crous, 1938). Soil applied N and P in combination with foliar KNO_3 resulted in a significant reduction in creasing incidence, reduced Ca concentration in the leaves, increased K concentrations in the leaves, but had no effect on N, P, Mg, and Na concentrations in the leaves (Bar-Akiva, 1975).

This study will give insight into the effect of light and temperature on creasing incidence and may help in developing pruning and picking strategies as a control measure of creasing.

Materials and methods

Plant material. Washington Navel trees in Citrusdal planted in a north-south row direction were used for this study.

Treatments. Each tree (replicate) was divided into four sectors, *viz*. north, south, west and east. In each sector, fruit was harvested from four different sub-sectors, the inside top part of the tree, the outside top part of the tree, the inside bottom part and the outside bottom part of the tree. The sixteen positions in each tree were replicated 8 times. For top and bottom outside sub-sectors the peel of fruit collected was divided into sections of the outside (sunny side) and the inside (shady side) of the fruit. Fruit from each sub-sector were used to measure, creasing incidence (%), creasing severity, fruit size and peel thickness. Measurements of mineral nutrients in albedo tissue of fruit from each sub-sector were also done. Creasing severity was scored 0-4 with 0= no creasing, 2=50% creasing and 4=100% on a per fruit basis. Creasing incidence (%) represents the percentage of creased fruit sampled. Pearson's correlation coefficients reported were determined by the SAS statistical program and only r values >0.5 were considered physiologically significant.

Results and discussion

Creasing incidence, severity, and peel thickness. There were no differences in fruit diameter from fruit sampled from the different sub-sectors (Table 5.2.3.1). Therefore, differences in creasing incidence among sub-sectors were not due to fruit diameter, which is also shown by the weak correlations between fruit diameter and creasing incidence (%) (r = -0.16) or creasing severity (r = -0.18), respectively (Table 5.2.3.2). In general, creasing incidence (%) was very high (>55%) in all trees from the block of trees used for the study (Table 5.2.3.1). However, differences in creasing incidence in fruit sampled from the different sub-sectors were observed. Top inside fruit had a greater incidence than top outside fruit (Table 5.2.3.1). Similarly, bottom inside fruit had a greater creasing incidence than bottom outside fruit. Therefore, within the tree, creasing incidence can be ranked as follows based on significant levels of 5%: top inside = bottom inside > top outside = bottom outside. On average, inside fruit had a significantly greater creasing incidence (%) than outside fruit, which shows the importance of light in creasing development. There were no significant differences in creasing incidence between fruit sampled from the north versus fruit sampled from the south side of trees, between fruit sampled from the west and east sides of trees and between fruit sampled from the top versus the bottom part of trees. Creasing incidence can be ranked as follows

regarding the side of the tree with average incidence percentage in brackets: north (82%) = east (81%)> west (75%) = south (74%). A similar trend was observed at a different site in Citrusdal with less overall creasing in the trees (full data not shown) north (52%) > east (44%) > south (40%) > west (29%). This is similar to the results of Jones et al. (1967), but contrary to what was expected since north and east in the southern hemisphere is considered the sunny side of the tree.

A similar trend as described above was observed for creasing severity and will not be discussed in detail (Table 5.2.3.1). The exception was that fruit sampled from the north side of trees had a greater creasing severity than fruit sampled from the south side of trees.

Average peel thickness can be ranked as follows based on significant levels of 5%: top outside = bottom outside > top inside = bottom inside (Table 5.2.3.1), which is the inverse than for creasing incidence (%). This is shown by the strong negative correlations between average peel thickness and creasing severity (r = -0.58) or creasing incidence (%) (r = -0.42), respectively (Table 5.2.3.2). There were no significant differences in average peel thickness between fruit sampled from the north versus fruit sampled from the south side of trees, or between the west and east sides of trees, but outside fruit had a greater average peel thickness than inside fruit and top fruit had a greater average peel thickness than bottom fruit (Table 5.2.3.1).

Table 5.2.3.1. Fruit diameter, creasing severity and incidence, and peel thickness in different positions (sub-sectors) of Washington Navel trees harvested in Citrusdal

Position within tree		**Diameter**	**Creasing severity**	**Creasing incidence**	**Average peel thickness**
		--mm--	*--0-4--*	*--%--*	*--mm--*
North	Top outside	69.6	1.0 efg[z]	66.3 ef`	4.9 ab
	Top inside	68.8	2.6 a	91.3 ab	3.8 efg
	Bottom outside	67.1	1.6 de	78.8 abcde	4.8 ab
	Bottom inside	68.1	2.5 ab	90.0 abc	3.4 fgh
West	Top outside	69.4	0.7 g	57.5 f	4.8 ab
	Top inside	68.6	2.7 a	95.0 a	3.7 efg
	Bottom outside	67.9	1.0 efg	57.5 f	4.4 bcd
	Bottom inside	68.3	2.3 abc	90.0 abc	3.3 gh
South	Top outside	69.3	1.2 efg	70.0 def	4.6 abc
	Top inside	70.7	2.0 bcd	85.0 abcd	3.9 def
	Bottom outside	68.7	1.0 efg	68.8 def	4.5 abc
	Bottom inside	68.7	1.9 cd	73.8 cdef	3.5 fgh
East	Top outside	69.7	0.9 fg	63.8 ef	5.0 a
	Top inside	70.2	2.9 a	95.0 a	3.5 fgh
	Bottom outside	68.7	1.4 edf	77.5 bcde	4.1 cde
	Bottom inside	68.4	2.5 abc	87.5 abc	3.2 h
	P-value	0.3457	0.0001	0.0001	0.0001
Average	Top outside	69.5	1.0	64.4	4.8
	Top inside	69.6	2.5	91.6	3.7
	Bottom outside	68.1	1.3	70.6	4.5
	Bottom inside	68.4	2.3	85.3	3.4
Source:	df				
Treatment	15				
North vs South	1	0.1123	0.0102	0.0929	0.4004
West vs East	1	0.2594	0.1358	0.1642	0.3900
Outside vs Inside	1	0.9906	0.0001	0.0001	0.0001
Top vs Bottom	1	0.0028	0.8456	1.0000	0.0001

[z]Means in each column with the same letter are not significantly different at the 5% level

Table 5.2.3.2. Relationships between creasing severity or creasing incidence (%) and peel thickness, fruit diameter and macro- or micronutrients in the albedo tissue of Washington Navel fruit sampled in Citrusdal

Peel thickness vs. Creasing severity			
		r	***P-value***
Peel thickness	Creasing severity	-0.58	0.0001
Peel thickness vs. Creasing incidence (%)			
Peel thickness	Creasing %	-0.42	0.0001
Fruit diameter vs. Creasing %, Creasing severity and Peel thickness			
Diameter	Creasing %	-0.16	0.0744
Diameter	Creasing severity	-0.18	0.0374
Diameter	Peel thickness	0.03	0.7307
Creasing % vs mineral nutrients in albedo			
Creasing %	N	0.37	0.0003
Creasing %	P	0.21	0.0402
Creasing %	K	0.54	0.0001
Creasing %	Ca	-0.45	0.0001
Creasing %	Mg	-0.21	0.0444
Creasing %	Na	-0.25	0.0163
Creasing %	Mn	-0.25	0.0157
Creasing %	Fe	0.02	0.8650
Creasing %	Cu	-0.05	0.6317
Creasing %	Zn	-0.05	0.6306
Creasing %	B	0.07	0.5258
Creasing severity vs mineral nutrients in albedo			
Creasing severity	N	0.43	0.0001
Creasing severity	P	0.23	0.0220
Creasing severity	K	0.72	0.0001
Creasing severity	Ca	-0.67	0.0001
Creasing severity	Mg	-0.18	0.0741
Creasing severity	Na	-0.09	0.4063
Creasing severity	Mn	-0.35	0.0004
Creasing severity	Fe	-0.08	0.4255
Creasing severity	Cu	-0.03	0.7902
Creasing severity	Zn	-0.12	0.2630
Creasing severity	B	0.09	0.3803
Peel thickness vs mineral nutrients in albedo			
Peel thickness	N	0.01	0.8940
Peel thickness	P	-0.17	0.0994
Peel thickness	K	-0.50	0.0001
Peel thickness	Ca	0.39	0.0001
Peel thickness	Mg	0.33	0.0011
Peel thickness	Na	0.04	0.6763
Peel thickness	Mn	0.07	0.4725
Peel thickness	Fe	0.07	0.4870
Peel thickness	Cu	0.06	0.5953
Peel thickness	Zn	0.06	0.5488
Peel thickness	B	-0.26	0.0107

Macronutrients

Although there were significant differences in nitrogen (N) and phosphorus (P) concentrations in albedo tissue from fruit sampled from different sub-sectors of the tree (Table 5.2.3.3), there were no consistent trends as were shown for creasing incidence (%) (Table 5.2.3.1). The weak correlation between P concentration in albedo tissue and creasing incidence (r = 0.21) or creasing severity (r = 0.23), respectively confirms these findings (Table 5.2.3.2). N concentrations in the albedo tissue showed a stronger, but still weak correlation with creasing incidence (r = 0.37) or creasing severity (r = 0.43), respectively (Table

5.2.3.2). There were no significant differences in Mg concentrations in albedo tissue of fruit sampled from different sub-sectors in the tree (Table 5.2.3.3), which was also shown by the weak correlation between Mg concentration in albedo tissue and creasing incidence (r = -0.21) or creasing severity (r = -0.18), respectively (Table 5.2.3.2).

Potassium (K) concentration in the albedo tissue showed a similar trend to what was observed for creasing incidence (%) and can be ranked as follows: top inside = bottom inside > top outside = bottom outside (Table 5.2.3.3). Interestingly, K concentrations in the albedo tissue showed very strong correlations with both creasing incidence (%) (r = 0.54) and creasing severity (r = 0.72) (Table 5.2.3.2). Of all the macro- and micronutrients measured, only K concentration in the albedo tissue showed a strong correlation (r = -0.50) with average peel thickness (Table 5.2.3.2). This is contrary to what we know about the relationship between leave K concentrations and creasing development (Embleton et al., 1973).

Calcium (Ca) concentration in the albedo tissue, which showed very strong negative correlations with both creasing incidence (%) (r = -0.45) and creasing severity (r = -0.67) (Table 5.2.3.2), can be ranked as follows: top outside = bottom outside > top inside = bottom inside (Table 5.2.3.3). The ranking showed an inverse order to what was observed for creasing incidence. Therefore, inside fruit had lower Ca concentrations in albedo tissue than outside fruit. This negative correlation was previously reported by Storey et al. (2002).

Table 5.2.3.3. Macronutrient concentrations of albedo tissue from fruit sampled from different positions (sub-sectors) in Washington Navel trees in Citrusdal

POSITION WITHIN TREE		**N**	**P**	**K**	**Ca**	**Mg**
		----------percentage----------				
North	Top outside	0.69 abc[z]	0.032 bc	0.28 efgh	0.59 a	0.065
	Top inside	0.71 ab	0.037 a	0.39 ab	0.43 efgh	0.062
	Bottom outside	0.69 abc	0.035 ab	0.31 def	0.53 abcd	0.062
	Bottom inside	0.69 abc	0.032 bc	0.38 abc	0.40 gh	0.055
West	Top outside	0.63 d	0.030 cd	0.24 h	0.57 ab	0.067
	Top inside	0.73 a	0.035 ab	0.42 a	0.38 h	0.058
	Bottom outside	0.68 abc	0.032 bc	0.31 def	0.48 cdef	0.062
	Bottom inside	0.73 a	0.037 a	0.43 a	0.40 gh	0.062
South	Top outside	0.65 cd	0.030 cd	0.25 h	0.58 ab	0.060
	Top inside	0.67 bcd	0.030 cd	0.32 de	0.51 bcde	0.057
	Bottom outside	0.65 cd	0.027 d	0.26 fgh	0.55 abc	0.063
	Bottom inside	0.63 d	0.027 d	0.30 defg	0.46 defg	0.053
East	Top outside	0.67 bcd	0.030 cd	0.25 gh	0.53 abcd	0.062
	Top inside	0.66 bcd	0.030 cd	0.32 cde	0.44 efgh	0.055
	Bottom outside	0.63 d	0.030 cd	0.25 fgh	0.57 ab	0.060
	Bottom inside	0.66 bcd	0.030 cd	0.36 bcd	0.41 fgh	0.053
	P-value	0.0004	0.0001	0.0001	0.0001	0.0731
Average	Top outside	0.66	0.030	0.25	0.56	0.061
	Top inside	0.69	0.033	0.36	0.44	0.058
	Bottom outside	0.66	0.030	0.28	0.53	0.061
	Bottom inside	0.68	0.031	0.37	0.42	0.056
Source:	df					
Treatment	15					
North vs South	1	0.0007	0.0001	0.0001	0.0709	0.2608
West vs East	1	0.0041	0.0041	0.0003	0.1855	0.0412
Outside vs Inside	1	0.0042	0.0377	0.0001	0.0001	0.0045
Top vs Bottom	1	0.5168	0.4348	0.0965	0.0402	0.2333

[z]Means in each column with the same letter are not significantly different at the 5% level

Micronutrients

There were no significant differences in sodium (Na), iron (Fe), copper (Cu) and zinc (Zn) concentrations of albedo tissue sampled from different sub-sectors (Table 5.2.3.4). Fe, Cu and Zn concentrations showed no significant differences in fruit sampled from north vs south, west vs east and outside vs inside, but top fruit had a greater concentration in Na than bottom fruit. Although significant differences in both manganese (Mn) and boron (B) concentration in albedo tissue from fruit sampled from different positions in the tree, respectively, were observed, similar or inverse trends to creasing incidence or severity with regard to position in the tree were not observed as shown by very weak correlations (r = 0.35) with both creasing incidence (%) or creasing severity (Table 5.2.3.2).

Table 5.2.3.4. Micronutrient concentrations of albedo tissue from fruit sampled from different positions (sub-sectors) in Washington Navel trees in Citrusdal

POSITION WITHIN TREE		**Na**	**Mn**	**Fe**	**Cu**	**Zn**	**B**
		----------mg/kg----------					
North	Top outside	50.9	4.3 a[z]	571.1	2.9	28.1	19.2 abc
	Top inside	54.6	2.7 bcd	21.6	2.5	8.5	19.5 a
	Bottom outside	59.3	2.8 bcd	19.4	2.4	9.3	18.8 abcde
	Bottom inside	51.7	2.0 cd	18.3	2.3	7.8	19.0 abcd
West	Top outside	41.3	3.0 bc	23.7	3.2	10.0	18.2 cdef
	Top inside	32.5	2.4 bcd	14.9	2.6	7.1	19.0 abcd
	Bottom outside	82.0	2.3 bcd	20.5	2.3	9.8	17.8 ef
	Bottom inside	67.5	1.8 cd	33.3	2.2	9.4	18.8 abcde
South	Top outside	46.3	2.8 bcd	17.9	2.1	9.7	18.2 bcdef
	Top inside	37.1	2.3 bcd	15.2	3.0	8.7	18.6 abcdef
	Bottom outside	54.7	2.7 bcd	19.8	2.3	10.0	18.1 def
	Bottom inside	45.5	2.2 bcd	22.1	2.2	16.5	17.6 f
East	Top outside	63.1	2.3 bcd	15.4	2.6	8.7	18.5 abcdef
	Top inside	48.1	1.8 cd	14.9	2.4	7.4	18.9 abcd
	Bottom outside	67.5	3.3 ab	152.2	2.2	9.6	19.3 ab
	Bottom inside	59.2	1.6 d	18.4	2.6	8.6	18.3 bcdef
	P-value	0.2038	0.0110	0.5030	0.9000	0.5179	0.0249
Average	Top outside	50.4	3.1	157.0	2.7	14.1	18.5
	Top inside	43.1	2.3	16.7	2.6	7.9	19.0
	Bottom outside	65.9	2.8	53.0	2.3	9.7	18.5
	Bottom inside	56.0	1.9	23.0	2.3	10.6	18.4
Source:	df						
Treatment	15						
North vs South	1	0.2889	0.1617	0.1721	0.6915	0.5544	0.0005
West vs East	1	0.6372	0.7156	0.7887	0.6905	0.8854	0.2763
Outside vs Inside	1	0.1846	0.0003	0.1717	0.5152	0.1470	0.2167
Top vs Bottom	1	0.0112	0.1144	0.4950	0.1236	0.7338	0.0973

[z]Means in each column with the same letter are not significantly different at the 5% level

Differences between the outside (sunny) and inside (shady) part of fruit sampled from outside positions (sub-sectors)

Both peel thickness on the sunny side of the fruit and peel thickness on the shady side of the fruit resulted in similar trends due to position of the sub-sector sampled from (Table 5.2.3.5) as described by average peel thickness above (Table 5.2.3.1). Fruit sampled from outside sub-sectors (top and bottom for north, west, south and east) consistently had a greater peel thickness on the sunny side than on the shady side of the fruit, but in the case of fruit sampled from the inside (top or bottom) the difference in peel thickness between the sunny and shady part of the fruit was not significant as shown by P-values > 0.0500 (only significant for the north top outside fruit) (Table 5.2.3.5).

Table 5.2.3.5. Peel thickness on the sunny or shady side of fruit sampled from different positions (sub-sectors) in Washington Navel trees in Citrusdal

POSITION WITHIN TREE		Peel thickness (sun)	Peel thickness (shade)	*P-value*
		---------- -mm----------		
North	Top outside	5.2 a[z]	4.6 ab	0.0050[y]
	Top inside	3.7 de	3.8 cde	0.1539
	Bottom outside	5.2 a	4.5 ab	0.0013
	Bottom inside	3.5 de	3.3 fg	0.0257
West	Top outside	5.1 a	4.6 ab	0.0435
	Top inside	3.7 de	3.7 def	0.7440
	Bottom outside	4.7 ab	4.2 bcd	0.0051
	Bottom inside	3.3 e	3.4 efg	0.3585
South	Top outside	4.9 ab	4.3 bc	0.0006
	Top inside	4.0 cd	3.8 cde	0.1193
	Bottom outside	4.7 ab	4.2 bc	0.0078
	Bottom inside	3.5 de	3.5 efg	0.6033
East	Top outside	5.2 a	4.8 a	0.0284
	Top inside	3.5 de	3.5 efg	0.6889
	Bottom outside	4.4 bc	3.8 cde	0.0005
	Bottom inside	3.2 e	3.1 g	0.3741
	P-value	0.0001	0.0001	-
Average	Top outside	5.1	4.6	-
	Top inside	3.7	3.7	-
	Bottom outside	4.8	4.2	-
	Bottom inside	3.4	3.3	-
Source:	df			
Treatment	15			
North vs South	1	0.3995	0.4447	-
West vs East	1	0.6267	0.2159	-
Outside vs Inside	1	0.0001	0.0001	-
Top vs Bottom	1	0.0016	0.0001	-

[z]Means in each column with the same letter are not significantly different at the 5% level

[y]Means for a parameter in each horizontal row are significantly different if P <0.0500

Similarly, creasing severity was greater on the shady side than on the sunny side of fruit in all but one sub-sector (Table 5.2.3.6). Therefore, the shady side with thin peels had a greater creasing severity than the sunny side with thicker peels.

Table 5.2.3.6. Differences in creasing severity between the outside (sunny) and inside (shady) part of fruit sampled from different positions from the outside sub-sectors of Washington navel trees in Citrusdal

POSITION WITHIN TREE		Creasing		
		sun	shade	*P-value*
		-------(0-2)-------		
North	Top outside	0.21	0.75	0.0006[z]
	Bottom outside	0.39	1.18	0.0001
West	Top outside	0.11	0.61	0.0002

	Bottom outside	0.39	0.65	0.1234
South	Top outside	0.44	0.75	0.0091
	Bottom outside	0.31	0.68	0.0190
East	Top outside	0.09	0.85	0.0014
	Bottom outside	0.30	1.11	0.0001

[z]Means for a parameter in each horizontal row are significantly different if P<0.0500

Although some sub-sectors had significant differences for some parameters between the shady and sunny side of fruit sampled from outside sub-sectors, no general trends similar to peel thickness and creasing severity mentioned above were observed for nitrogen (N) and phosphorus (P) (Table 5.2.3.7), potassium (K) and calcium (Ca) (Table 5.2.3.8), magnesium (Mg) and sodium (K) (Table 5.2.3.9), manganese (Mn) and iron (Fe)(Table 5.2.3.10), copper (Cu) and zinc (Zn) (Table 5.2.3.11), or boron (B) (Table 5.2.3.12).

Table 5.2.3.7. Differences in N and P concentrations between the outside (sunny) and inside (shady) part of fruit sampled from different positions from the outside sub-sectors of Washington navel trees in Citrusdal

POSITION WITHIN TREE		**Nitrogen (N)**			**Phosphorus (P)**		
		sun	**shade**	***P-value***	**sun**	**shade**	***P-value***
		---percentage---			*---percentage---*		
North	Top outside	0.68	0.70	0.2431	0.032	0.030	0.3632[z]
	Bottom outside	0.67	0.70	0.0874	0.033	0.033	1.0000
West	Top outside	0.61	0.65	0.1604	0.028	0.030	0.3632
	Bottom outside	0.67	0.70	0.3050	0.028	0.030	0.6109
South	Top outside	0.63	0.66	0.4500	0.028	0.030	0.3632
	Bottom outside	0.65	0.65	0.8040	0.027	0.027	ns[y]
East	Top outside	0.67	0.68	0.4112	0.028	0.030	0.3632
	Bottom outside	0.61	0.64	0.0395	0.030	0.030	ns

[z]Means for a parameter in each horizontal row are significantly different if P<0.0500
[y]ns is not significant

Table 5.2.3.8. Differences in K and Ca concentrations between the outside (sunny) and inside (shady) part of fruit sampled from different positions from the outside sub-sectors of Washington navel trees in Citrusdal

POSITION WITHIN TREE		**Potassium (K)**			**Calcium (Ca)**		
		sun	**shade**	***P-value***	**sun**	**shade**	***P-value***
		---percentage---			*---percentage---*		
North	Top outside	0.26	0.30	0.1128	0.63	0.54	0.0200
	Bottom outside	0.30	0.31	0.6373	0.56	0.50	0.0628
West	Top outside	0.21	0.26	0.0149	0.60	0.53	0.2407
	Bottom outside	0.27	0.34	0.1518	0.51	0.45	0.0666
South	Top outside	0.22	0.27	0.1051	0.60	0.54	0.1844
	Bottom outside	0.24	0.27	0.0834	0.58	0.50	0.0333
East	Top outside	0.24	0.26	0.1068	0.55	0.51	0.2492
	Bottom outside	0.24	0.27	0.0446	0.60	0.53	0.0282

[z]Means for a parameter in each horizontal row are significantly different at P<0.0500

Table 5.2.3.9. Differences in Mg and Na concentrations between the outside (sunny) and inside (shady) part of fruit sampled from different positions from the outside sub-sectors of Washington navel trees in Citrusdal

POSITION WITHIN TREE		Magnesium (Mg)			*Sodium (Na)*		
		sun	shade	*P-value*	sun	shade	*P-value*
		---percentage---			*-------mg/kg-------*		
North	Top outside	0.067	0.058	0.0422[z]	55.8	45.9	0.0892
	Bottom outside	0.063	0.060	0.3632	55.8	62.8	0.4928
West	Top outside	0.063	0.065	0.7412	39.9	42.6	0.5005
	Bottom outside	0.062	0.062	ns[y]	115.9	48.2	0.2900
South	Top outside	0.057	0.058	0.6109	49.5	43.1	0.2197
	Bottom outside	0.058	0.063	0.0756	53.8	55.5	0.7522
East	Top outside	0.060	0.058	0.6109	66.8	59.4	0.2076
	Bottom outside	0.057	0.070	0.4650	45.4	89.6	0.1381

[z]Means for a parameter in each horizontal row are significantly different at P<0.0500
[y]ns is not significant

Table 5.2.3.10. Differences in Mn and Fe concentrations between the outside (sunny) and inside (shady) part of fruit sampled from different positions from the outside sub-sectors of Washington navel trees in Citrusdal

POSITION WITHIN TREE		Manganese (Mn)			Iron (Fe)		
		sun	shade	*P-value*	sun	shade	*P-value*
		------mg/kg------			*---- ---mg/kg-------*		
North	Top outside	5.2	3.3	0.3975[z]	1113.5	28.6	0.3633
	Bottom outside	2.9	2.8	0.5355	19.9	19.0	0.7647
West	Top outside	2.8	3.2	0.5058	27.9	19.6	0.4511
	Bottom outside	2.3	2.3	0.9631	21.4	19.5	0.5335
South	Top outside	3.0	2.6	0.3596	20.1	15.7	0.0595
	Bottom outside	3.2	2.3	0.0665	17.2	22.5	0.3868
East	Top outside	2.2	2.4	0.1460	15.8	14.9	0.3786
	Bottom outside	2.9	3.7	0.5844	16.8	287.5	0.3548

[z]Means for a parameter in each horizontal row are significantly different if P<0.0500

Table 5.2.3.11. Differences in Cu and Zn concentrations between the outside (sunny) and inside (shady) part of fruit sampled from different positions from the outside sub-sectors of Washington navel trees in Citrusdal

POSITION WITHIN TREE		Copper (Cu)			Zinc (Zn)		
		sun	shade	*P-value*	sun	shade	*P-value*
		------mg/kg------			*------mg/kg------*		
North	Top outside	3.2	2.5	0.4345[z]	46.4	9.7	0.3652
	Bottom outside	2.5	2.3	0.7587	9.1	9.6	0.5372
West	Top outside	2.1	4.3	0.2809	9.5	10.4	0.4242
	Bottom outside	2.3	2.2	0.4161	10.2	9.5	0.1982
South	Top outside	2.0	2.2	0.4946	10.2	9.1	0.1003
	Bottom outside	2.1	2.4	0.3873	10.3	9.7	0.3815
East	Top outside	3.0	2.2	0.3592	8.6	8.8	0.5146

	Bottom outside	2.0	2.3	0.2137	9.1	10.0	0.1129

[z]Means for a parameter in each horizontal row are significantly different if P<0.0500

Table 5.2.3.12. Differences in B concentrations between the outside (sunny) and inside (shady) part of fruit sampled from different positions from the outside sub-sectors of Washington navel trees in Citrusdal

POSITION WITHIN TREE		**Boron (B)**		
		sun	**shade**	***P-value***
		-------mg/kg-------		
North	Top outside	19.8	18.6	0.0214[z]
	Bottom outside	19.0	18.6	0.4085
West	Top outside	17.7	18.7	0.0866
	Bottom outside	18.1	17.6	0.4893
South	Top outside	18.3	18.2	0.7377
	Bottom outside	17.9	18.4	0.2649
East	Top outside	18.5	18.5	0.8951
	Bottom outside	19.0	19.5	0.1369

[z]Means for a parameter in each horizontal row are significantly different if P<0.0500

Therefore, even though there were differences in creasing severity and peel thickness between the sunny (outside) and shady (inside) side of fruit sampled from outside sub-sectors none of the macro- or micronutrient concentrations differed consistently between the inside and outside of fruit sampled from outside sub-sectors. Also, only N concentrations in the albedo tissue from the shady side showed a positive correlation with r > 0.5 with creasing severity (r = 0.52) (Table 5.2.3.13). The relationship between creasing severity and nutrients in the albedo tissue in the outside (sunny) side of fruit sampled from outside sub-sectors did not show any strong correlations with r > 0.5 (Table 5.2.3.13).

Table 5.2.3.13. Relationships between creasing severity and macro- or micronutrients in the albedo tissue of Washington Navel fruit in the outside (sunny) and inside (shady) part of fruit sampled from different positions from the outside sub-sectors of Washington navel trees in Citrusdal

Creasing severity vs mineral nutrients in albedo in the sunny side of fruit			
		r	***P-value***
Peel thickness (sun)	Creasing severity (0-2)	-0.17	0.1797
Creasing severity (0-2)	N	0.25	0.0862
Creasing severity	P	0.03	0.8615
Creasing severity	K	0.18	0.2125
Creasing severity	Ca	-0.15	0.3150
Creasing severity	Mg	-0.32	0.0273
Creasing severity	Na	-0.13	0.3802
Creasing severity	Mn	-0.10	0.4994
Creasing severity	Fe	-0.04	0.7696
Creasing severity	Cu	-0.17	0.2466
Creasing severity	Zn	-0.04	0.7721
Creasing severity	B	-0.08	0.6044
Creasing severity vs mineral nutrients in albedo in the shady side of fruit			
Peel thickness (shade)	Creasing severity (0-2)	-0.05	0.7192
Creasing severity (0-2)	N	0.52	0.0001
Creasing severity	P	0.07	0.6633
Creasing severity	K	0.33	0.0239
Creasing severity	Ca	-0.22	0.1252
Creasing severity	Mg	0.03	0.8500

Creasing severity	Na	0.09	0.5474
Creasing severity	Mn	-0.37	0.0109
Creasing severity	Fe	-0.13	0.3661
Creasing severity	Cu	-0.09	0.5599
Creasing severity	Zn	-0.14	0.3539
Creasing severity	B	-0.13	0.3946

Conclusion

It can be concluded that position of fruit on a tree plays a role in creasing incidence, which can be ranked as follows: top inside (92%) = bottom inside (85%) > top outside (65%) = bottom outside (71%). In general, creasing incidence (%) was consistently greater than 55% in all trees which made comparisons and conclusions difficult. With a lower creasing incidence and larger differences among the different positions in the tree, selective harvesting may be an option. Inside fruit had a greater creasing incidence, thinner peels, lower Ca and greater K concentrations in the albedo tissue of fruit at harvest than outside fruit. It is assumed that light and temperature plays a role in the differences observed, therefore, pruning to improve light distribution in the tree is recommended. The north and east sides of trees had a greater creasing incidence than west and south sides, which is contrary to what was expected. Even though fruit sampled from outside sub-sectors had a greater peel thickness and lower creasing severity on the sunny side (outside) than on the shady side (inside) of the fruit, none of the macro- or micronutrient concentrations in the albedo tissue of fruit differed between the outside and inside of fruit sampled. The possible involvement of light and or temperature in early cell division in albedo tissue of fruit cannot be excluded as a factor influencing creasing development.

Future research

From the literature there are some discrepancies regarding creasing incidence, leaf mineral concentrations and albedo tissue mineral concentrations. Also, in this study, K concentrations in the albedo tissue showed a very strong positive correlation with creasing incidence (%), but the inverse is true for leaf K concentrations. For future research it would be worth looking at the relationship between leaf nutrient concentrations and albedo tissue nutrient concentrations and how both relate to creasing incidence. Light and or temperature may be involved in early cell division of albedo tissue. Therefore, light measurements in the different positions of the tree should be included in future studies.

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5.2.4 Effect of manipulation of carbohydrate and mineral nutrient allocation in the tree on creasing incidence

Experiment 864 by Stephan Verreynne (CRI at SU)

Opsomming

Kraakskil is 'n fisiologiese abnormaliteit wat krake in die albedo veroorsaak en lei tot kanale op die oppervlak van die vrug. Die middellamella, wat die "sement" tussen selle van die albedo uitmaak, bestaan uit pektien wat polisakkariedes is wat komplekse met sekere minerale elemente vorm. Die minerale elemente wat moontlik betrokke is by hierdie pektien komplekse is Mo, B, Zn Ca, S en Mg. Dus, koolhidrate en minerale elemente is nodig vir pektienvorming om sterk verbindings tussen die selwande van albedo selle te vorm. Die doel van die studie is om die effek van die manipulasie van koolhidraat sintese en allokasie van koolhidrate en minerale elemente op die voorkoms en intensiteit van kraakskil te evalueer. Dit word gedoen om die belangrikheid van koolhidraat en minerale element allokasie in die struktuur en sterkte van selwandkonneksies van die albedo selle te evalueer. Behandelings sluit in die plasing van skadunet oor blare, toemaak van vrugte met sakkies, ringelering van raamtakke, snoei van vensters om ligverspreiding te verbeter, verwydering van blare agter vruggies en handuitdunning van vrugte, net na die fisiologiese vrugvalperiode en in laat Januarie. Washington Navel bome in Citrusdal is gebruik vir die studie. Die studie is begin en die eerste manipulasies is uitgevoer in November 2006 en die bome sal eers in Junie 2007 geoes word. By oestyd sal persentasie kraakskil bepaal word op vrugte wat verskillende behandelings ontvang het. Resultate sal in die volgende jaarlikse navorsingsverslag verskyn.

Summary

Creasing or albedo breakdown is a physiological disorder resulting in cracks in the albedo and showing channels on the surface of the fruit. The middle lamella, cementing the cell walls in the albedo together, is composed of pectins, which are polysaccharides that form complexes with mineral elements. The mineral elements suggested to be involved in these pectin complexes are Mo, B, Zn, Ca, S and Mg. Therefore, carbohydrates and mineral nutrients are required for pectin formation to form strong connections between the cell walls of cells of the albedo. The objective of this study is to evaluate the incidence and severity of creasing after manipulation of carbohydrate synthesis and allocation of carbohydrates and mineral nutrients. This is done to evaluate the importance of carbohydrate and mineral nutrient allocation in the structure and strength of cell wall connections of the albedo cells. Treatments include covering leaves behind fruit with shade cloth, covering fruit with bags, girdling scaffold branches, pruning windows to improve light distribution in the trees, removing leaves from behind fruit and hand thinning fruit-bearing shoots, just after physiological fruit drop and in late January. Washington Navel trees in Citrusdal were used for this study. The trial was started and the first manipulations were done in November 2006 and will only be harvested in June 2007. Fruit will be collected at harvest from trees from each treatment and percentage creasing incidence will be determined on these fruit. Results will be presented in the next annual research report.

5.2.5 Effect of elevated ethylene and CO_2 levels on rind condition of Clementine mandarin

Experiment 780 by Paul Cronje, Graham Barry (CRI at SU) and Marius Huysamer (SU)

Opsomming

Gedurende 2006 is 'n proef gedoen waar hoë CO_2 (5%) en etileen by twee konsentrasies (1 en 5 dpm) toegedien is asook tydens opberging van Nules Clementine mandaryne (–0.5°C vir 32 dae). 'n Aparte behandeling van vrugte toegemaak in plastiek houers vir dieselfde periode was ook ingesluit. Die vrugte is ontleed vir kleurverlies, powwerigheid en die interne kwaliteit. Daar was baie min powwerigheid gesien en geen betekenisvolle verskille tussen behandelings soos gemeet in wegtrek tussen pulp en flavedo nie. Daar was wel 'nverlaging in skeiding van die segmente van mekaar in die vrugkern met al die behandelings. Die meetings van chroma en "lightness" het wel betekenisvolle verkille aangetoon maar die belangrike Hue° het geen verskille tussen behandelings gelewer nie. Sleg die sap inhoud het betekenvol verskille tussen behandelings getoon, met al die behandelings wat 'n hoër vlak gelewer het as die kontrole. Die projek sal in 2007 verder gevoer word deur kombinasies tussen etileen en CO_2 gasse te gebruik om die effek te bepaal op ontwikkeling van powwerigheid.

Introduction

The loss in fruit quality during shipment of citrus fruit under cold sterilization protocol necessitates an investigation of the variables that could play a role. Two main problems often experienced after the approximately 22 days shipment at -0.6°C are a loss in colour and the development of puffiness. Puffiness, a postharvest physiological disorder, is seen as a bulging of the rind as it pulls away from the pulp.

The first factor studied was high CO_2 levels and the results were reported previously by Cronje, Barry and Huysamer (2004, 2005). The effect of elevated CO_2 levels on rind condition of Nules Clementine mandarins was not negative in either increasing the puffiness or loss of colour. However due to the importance of CO_2, as it influences fruit respiration and quality (Kay & Paull, 2004), it was necessary to do a follow-up experiment. The aim of the experiment was to determine the influence of elevated ethylene levels during cold storage of Nules Clementine mandarins and compare it with 5% CO_2.

Material and methods

The fruit used were Nules Clementine mandarins harvested on 10 May 2006 from the Paarl area and were degreened (3 days) and packed according to normal commercial practices.

Six replicates consisting of 20 fruit each were used per treatment. Fruit were placed in a bucket with a connection to a flow board and an outlet from the cold room. Premixed gas from Afrox was used and the treatments were as follows: 0.03% (normal air), 5% CO_2, 1, 5 ppm ethylene and fruit closed for the experiment's duration in a plastic bucket. The gas bottles were connected to flow boards from which tubes fed into the buckets in the cold room. In all treatments air made up the balance of the gas mixture (i.e. 21% O_2 plus nitrogen). The buckets were kept in a cold room at –0.5°C for 32 days (to simulate the maximum commercial period at these conditions). The flow rate of the treatment gases was high enough to prevent a build up of additional ethylene and CO_2 inside the bucket.

After the storage period the fruit were kept at ambient temperature (15 to 20°C) for one week, with the buckets open to prevent CO_2 and ethylene build-up. The fruit colour was evaluated with a chromameter (Minolta NR 4000, Osaka, Japan) after cold storage. The symptoms of rind disorders were scored and the fruit were cut open to evaluate the degree of puffiness as well as the internal colour change of the pulp. The degree of puffiness was measured with a calliper according to the distance between the pulp and the peel and the separation of the centre of the fruit as the segments detached (Fig. 5.2.5.1). The data were analysed with GLM procedures of SAS 2.

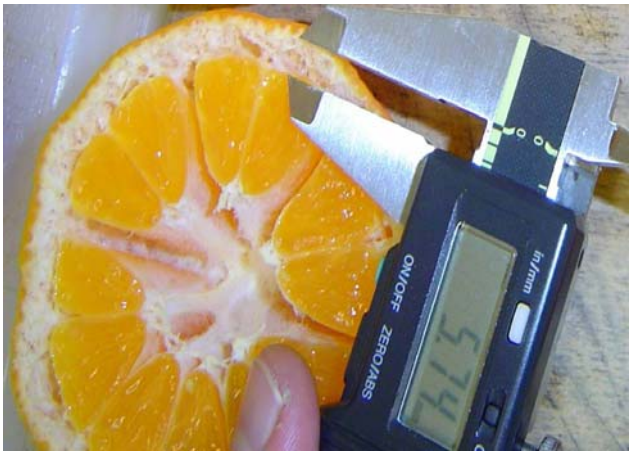

Fig. 5.2.5.1. Measurement of degree of puffiness.

Results and discussion

The experiment yielded low levels of puffiness, even in the treatment consisting of a closed bucket for 32 days. Against expectations the control treatment had significantly higher (at 99% significance) level of core separation compared with 1 ppm ethylene and the closed bucket treatment (Fig.5.2.5.2a). The same trend, but not significantly, was seen in the separation of the rind and pulp (Fig. 5.2.5.2b).

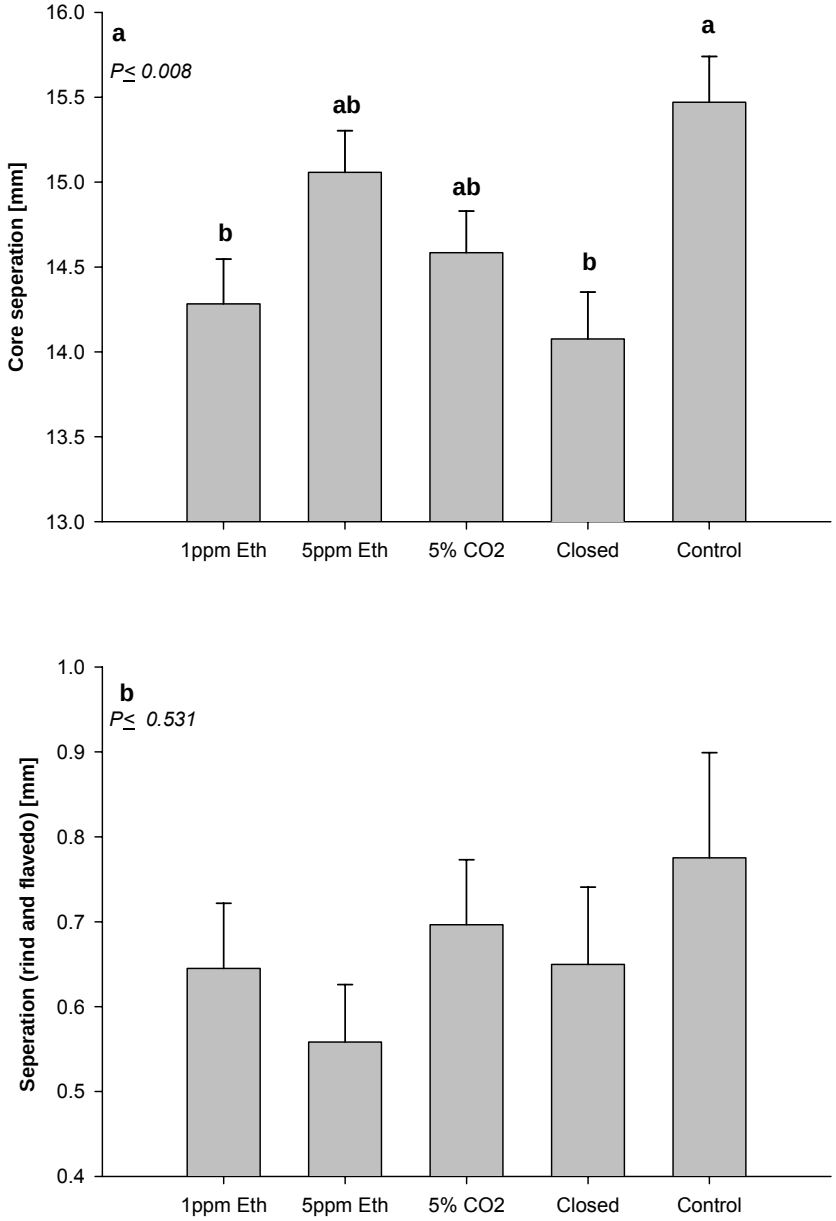

Fig. 5.2.5.2ab. The effect of ethylene (1 and 5ppm), 5% CO_2, closed buckets and air had on separation of the flavedo from pulp and at the core of Nules Clementine mandarins during storage of 32 days at -0.5°C. Bars with different letters indicate a significant difference at 99% significance. If no letters occur on the bars treatment differences were non significant.

It is thought that loss of rind colour and puffiness go hand-in-hand but the results of Chroma, Lightness and Hue° measurements do not illustrate this (Fig. 5.2.5.3abc). Chroma and lightness measurements did show significant differences between treatments but no clear indication of a pattern was evident and the most important value of Hue° (high value = more orange/red and low value = more yellow) did not show any significant differences. The significantly lower chroma value of the closed treatment did indicte a less vivid colour of orange. The significant value of lightness in the control indicted the fruit were somewhat darker than the other treatments.

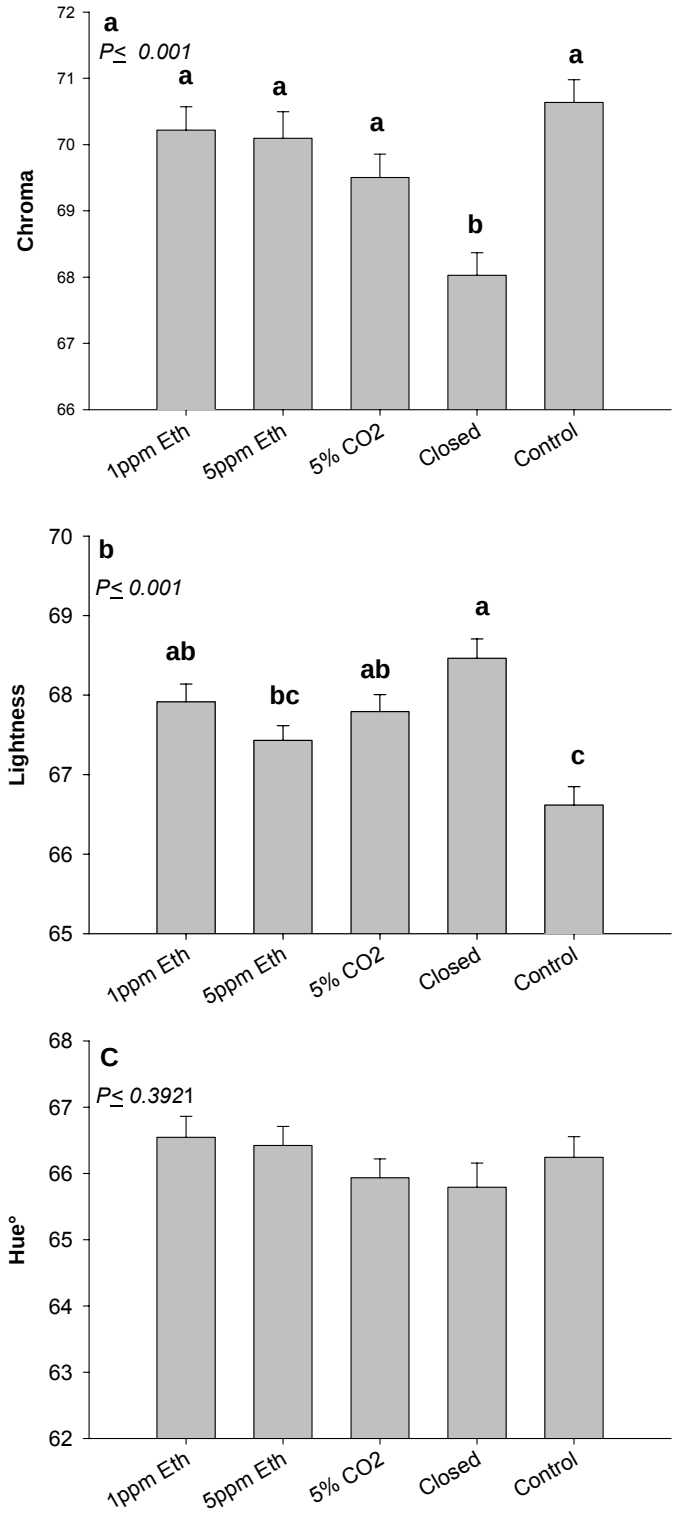

Fig. 5.2.5.3abc. The effect of ethylene (1 and 5 ppm), 5% CO_2, closed buckets and air on colour measurements of Nules Clementine mandarins during storage of 32 days at -0.5°C. Bars with different letters indicate a significant difference at 99% significance. Where no letters occur on the bars treatment differences were non-significant.

Measurements of the internal quality aspects after storage, e.g. juice content, °Brix, TSS and Ratio (°Brix/TSSS) did not yield significant differences except the juice content. The control fruit had significantly lower juice content than CO_2 treatments (Fig. 5.2.5.4. abcd). No bleaching effect on the fruit pulp was seen.

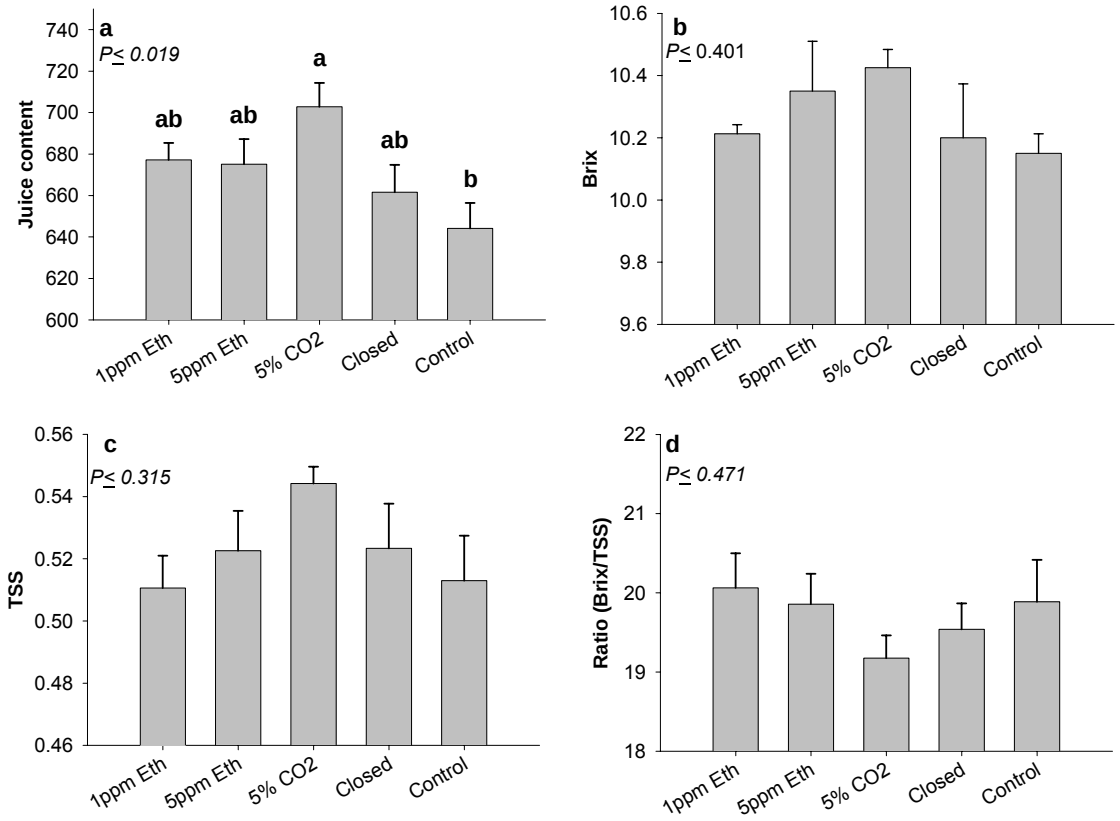

Fig. 5.2.5.4 abcd. The effect of ethylene (1 and 5 ppm), 5% CO_2, closed buckets and air on internal quality of Nules Clementine mandarins during storage of 32 days at -0.5°C. Bars with different letters indicate a significant difference at 99% significance. Where no letters occur on the bars treatment differences were non significant.

Conclusion

During the previous seasons of 2004/5 the effect of CO_2 on puffiness development was studied and showed no significant detrimental effect (CRI annual report: 2004, 2005). It was therefore decided to do the same study on ethylene at elevated levels during 2006. It is concluded that the results show that as an individual gas it is not detrimental to Nules Clementine mandarins during storage at -0.5°C. The postharvest physiology of the non-climacteric citrus fruit is complicated, and the effect of these two gasses normally associated with increased rate of senescence in fruit highlight this fact. The logical reaction of fruit to these two gases would be a very rapid rate of senescence with physiological defects and decay developing in a short period and not the reported results. It could be that the interaction with the -0.5°C cold storage plays a critical role in determining the fruit physiological response. The very small difference found in colour measurements of the ethylene treatments also reinforces this idea.

Future research

At the start of the project no knowledge of fruit behaviour during shipment at -0.5°C was known and individual gases were applied. This year and the previous season's results point the research in the direction of a probable combination effect. During 2007 Nules Clementine mandarins will be subjected to high levels of CO_2 and ethylene at the same time.

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5.2.6 **Ontwikkeling van voor en na-oes strategië wat die voorkoms van koueskade kan verminder in verskeie sitrus kultivars**

Experiment 832 deur Paul Cronje (CRI) en Pieter de Jongh (SU)

Summary

Various experiments consisting of pre- and postharvest treatments were done with lemons Clementines and Valencias for efficacy of prevention of chilling injury. The chemicals were chosen for their wide range of mode of actions in the plant biochemical system. Gibberellic acid, LPE, Raynox, TBZ, AVG, 1-MCP, jasmonic acid, salicylic acid, spermidien, Ca-nitrate as well as citrus waxes of solid contents from 10 to 24%, were used. Two warm water treatments of 40 and 50°C were also tested. LPE was the only preharvest treatment resulting in a commercially significant reduction in chilling injury of lemons. Jasmonic acid reduced the amount of pitting whereas the high solid wax (24%) reduced pitting as well as scalding of the Valencia oranges. The most dramatic result was the 50°C warm water treatment which had 40% less CI than the control or 40°C. The treatments used in 2006 will be repeated in 2007 to confirm the results and the promising treatments will be used in expanded experiments.

Inleiding

Koueskade word deur lae, bo-vriespunt temperature geïnduseer in vrugte gedurende die na-oes ketting. Dit is ’n fisiologiese afwyking wat veral by tropiese en sub-tropiese vrugte en plante voorkom wat uiteraard nie aangepas is om by sulke lae temperature normaal te funksioneer nie. Die hoeveelheid koueskade wat voorkom hang af van die temperatuur, die tydsduur wat die vrug aan hierdie temperatuur blootgestel is, sowel as die sensitiwiteit van die vrug teenoor lae temperature. ’n Verdere faktor wat die mate van koueskade beïnvloed is die ouderdom van die vrug, oftewel die fisiologiese stadium waarin die vrug op die tydstip van verkoeling verkeer, asook die kultivar of spesie (Kays en Paull, 2004).

Koueskade-simptome ontwikkel dikwels baie stadig tydens verkoeling, maar word vinnig sigbaar wanneer hierdie vrugte uit die verkoelingstoestande verwyder word. Die simptome kan, net soos die mate van koueskade, verskil van kultivar tot kultivar. Sommige kultivars toon interne simptome, terwyl ander kultivars weer eksterne simptome toon. Die algemeenste simptome is verkleuring en verbruining, abnormale rypwording, ingesonke letsels van die flavedo, asook verhoogde afbraak en verrotting van die vrug (Lafuente et al., 2005).

Volgens navorsing wat tot dusver gedoen is, blyk koueskade die resultaat te wees van ’n afname in membraan-integriteit (Sanchez-Ballesta *et al*., 2006). Hierdie afname in membraan-integriteit sal dan lei tot ’n verswakte regulatoriese werking van die membrane, metaboliese wanbalans, asook die outolise en afsterwing van selle (Lyons, 1973). Gevolglik ontstaan simptome soos die insinking van die flavedo en die uitdroging van die skil aan die stempel-end van die vrug (Sanchez-Ballesta *et al*., 2006).

Met die koue sterilisasie protokol wat ’n vereiste is vir die uitvoer van sitrus na verskeie lande, is koueskade ’n wesenlike probleem. Die rede hiervoor is dat die vrugte vir lang tydperke aan verkoelingstoestande onder 0°C blootgestel word wat die voorkoms van koueskade vergroot. Verskeie strategieë is al ontwikkel en gebruik om koueskade te verminder bv. verwelking van pomelos. In hierdie proef word die effek van verskeie voor-oes behandelings, na-oes behandelings, warmbad-behandelings, asook waks-behandelings op die persentasie koueskade en vogverlies bepaal. Die persentasie vogverlies word bepaal as sekondêre aanduiding van die graad van koueskade as gevolg van die uitdroging wat voorkom tydens koueskade. Daar was meestal op suurlemoene gefokus onrede die baie hoër koue sensitiwiteit van die kultivar, maar van die behandelings is wel op Clementines en Valencia lemoen herhaal.

Materiale en metodes

<u>Voor-oes behandelings van Eureka suurlemoen</u>

Die voor-oes proef bestaan uit vyf behandelings. Die vrugte is op 6 April 2006 onderskeidelik met gibberelliene (GA) 10 dpm, lysophatidylethanolamine (LPE) 200dpm, Raynox (2.5 ℓ/100 ℓ H_2O) en StopitCaCl (450 ml/100 ℓ) water bespuit. LPE is ’n natuurlike lipied wat kommersieel van eiers en sojabone verkry word. Daar is gevind dat hierdie lipied die kleurontwikkeling en houvermoë van cranberries en tamaties verbeter deur die antosianien-konsentrasies te verhoog (Ozgen et al., 2005). Raynox is ’n carnuba-waks en word by appels gebruik om sonbrand te voorkom. Tien bome per behandeling is gespuit met elk van die bogenoemde middels, terwyl daar ’n oop ry tussen elke behandeling gelos is. Die vyfde behandeling dien as kontrole en die bome is met water bespuit. Die Eureka suurlemoene is op 20 April 2006 ge-oes (week voor kommersiële oes van boord). Die vrugte is dieselfde dag gewaks met 10% Carnuba Tropical en teen kamertemperatuur gestoor vir tien dae waarna die vrugte by -0.5ºC geplaas vir 30 dae. Gewigsverlies en koue skade insidensie is bepaal en aangeteken na 7 dae opberging teen kamer temperatuur.

Na-oes behandelings van Eureka suurlemoene

Tydens die na-oes proef is daar van agt behandelings gebruik gemaak. Die suurlemoene is op 20 April 2006 ge-oes van bogenoemde boord. Vir elke behandeling is daar van tien herhalings met vyf vrugte elk, gebruik gemaak. Die vrugte is direk na-oes gemerk en geweeg. Die vrugte is in emmers geplaas vir ’n periode van tien minute, met die verskeie behandelings daarin. Die verskillende behandelings het uit CaCl (8 ℓ/100 ℓ water), CalciMax, Jasmoonsuur (25 ml/ℓ water = 500 dpm), Aminoethoxvinyl (Retain) teen 83 g/100 ℓ water, Salisielsuur (4000 mg/ℓ), Spermidien (100 mg/ℓ water) en thiabendazole (TBZ) teen 1 ml/5 ℓ water, bestaan. Retain (AVG) is ’n middel wat aangewend word om etileen-sintese te inhibeer. Kalsium speel vermoedelik ’n rol in seker vrugte se koue bestandheid, heel moontlik agv stabilisering van selmembrane. In die stres respons tydens koue skade speel spermadien, salisiel- en jasmoonsuur ’n rol in koue weerstandbiedende in seker plantspesie, alhoewel nog geen duidelikheid verkry is van die fisiologies werking in sitrus vrugte nie. TBZ is gebruik in die eksperiment a.g.v verslae wat meld dat TBZ aangewend as ’n na-oes swammiddel, vermoedelik koue-bestandheid verhoog (Cohen *et al*., 2000). Die agste behandeling was die kontrole en ’n water doop. Na die dompeling van die vrugte in die verskeie middels is dit vir tien minute laat staan om droog te word, waarna daar waks toegedien is. Die suurlemoene is in koelopberging by -0.5ºC geplaas vir 30 dae.

Warmbad-behandelings van Eureka suurlemoene

Die vrugte wat in die warmbad-proef gebruik is op 20 April 2006 ge-oes. Die warmbad-proef bestaan uit drie behandelings. Die verskil in die behandelings is bloot die temperatuur van die water en die proef bestaan dus uit ’n warmwater-behandeling by 40ºC, sowel as by 50ºC. Die derde behandeling dien as die kontrole waartydens die vrugte vir tien minute in water by kamertemperatuur (~20°C) geplaas is. Vir elke behandeling bestaan daar tien herhalings met ’n totaal van vyf vrugte per herhaling. Die suurlemoene is vir ’n tydperk van tien minute in die warmwater gedompel. Na die dompeling van die vrugte in die verskeie middels is dit vir tien minute laat staan om droog te word, waarna daar waks toegedien is. Die suurlemoene is toe in koelopberging by -0.5ºC geplaas vir 30 dae.

Waks-behandelings van Eureka suurlemoene

Die waks-proef bestaan uit vier behandelings, naamlik Carnuba Tropical (18%) wat as kommersiëlekontrole gedien het, CT(10%), CT(14%) en SF890HS (24%). Elke behandeling het uit tien herhalings met vyf vrugte bestaan. Die verskil tussen die waksbehandelings is die hoeveelheid vaste stowwe (in hakkies agter elke waks) wat in die betrokke waks voorkom. Hoe hoër die vastestof-inhoud, hoe beter word die vrug teen koueskade beskerm asook die voorkoms a.g.v. ’n beter glans. ’n Hoër vastestof-inhoud kan egter lei tot ’n hoër voorkoms van skildefekte bv. gepokteskil van Nawels. Die suurlemoene is in koelopberging by -0.5ºC geplaas vir 30 dae.

Na-oes behandelings van Nules Clementine mandaryne

Die Clementines is gedurende die week 15-18 Mei geoes in die Paarl omgewing en die selfde na-oes chemikalie behandelings is gebruik as met die Eureka suurlemoene. Die vrugte is teen -0.5°C opgeberg vir 30 dae waarna dit vir een week by kamertemperatuur opgeberg is voor die vrugte ondersoek is vir koue skade simptome.

Na-oes behandelings van Valencia lemoene

Die Valencia lemoene is gedurende 28-31 Augustus ge-oes in Citrusdal en vervoer na Stellenbosch. Dit is die volgende dag behandel met twee wakse nl. Carnuba tropical 10% en HS SF980 (24%). Jasmoonsuur en TBZ is ook weer gebruik teen dieselfde konsentrasies as genoem met die suurlemoene.

Daar was ook drie nuwe behandelings met 1-MCP teen 1000, 100 en 10 dpb. Die chemikalie was as ’n doop behandeling gedoen en vrugte was vir vyf minute blootgestel. Die chemikalie bind met die molekules in die selwand waar etileen gewoonlik sou bind. Dit het dus ’nuitwerking op die verouderings asook die stres fisiologie van die vrug.

Resultate

Voor-oes behandelings van Eureka suurlemoene (Tabel 5.2.6.1)

Tydens die voor-oes proef is die meeste vogverlies aangeteken by die vrugte wat met GA bespuit is. Die gemiddelde persentasie vogverlies by die GA-behandelde vrugte was 4,9%. Hierdie persentasie vogverlies is betekenisvol hoër as wat die LGE, Raynox en kontrole. Die suurlemoene wat met Stopit-CaCl behandel is het die tweede meeste vogverlies (3.2%) getoon. Maar verskil nie betekenisvol van enige behandeling nie. Die Raynox behandelings het die vogverlies nie betekenisvol beïnvloed nie alhoewel dit juis ingesluit is oor dit vermoedelik ’nbeperkende effek op vog verlies kan teweeg bring a.g.v. die waks samestelling daarvan. Die gemiddelde persentasie koueskade was die hoogste by die water-kontrole. Die vrugte wat met die ander middels behandel is, het almal ’n verlaaging in koueskade getoon in vergelyking met die water-kontrole maar slegs LGE was betekenisvol laer. Die Stopit-CaCl behandeling het kou eskade die minste beperk en het ’n

gemiddelde persentasie koueskade van 50% getoon, terwyl die gemiddelde persentasie koueskade by die water-kontrole 52,5% was. Die GA- en Raynox-behandelde vrugte het ’n gemiddelde persentasie koueskade van 36,7% en 40%, onderskeidelik, getoon. LGE het ’n betekenisvolle vermindering in koueskade van 16,7% gehad.

Tabel 5.2.6.1. Die insidensie van koueskade en persentasie vogverlies van Eureka suurlemoen soos beïnvloed deur voor-oes spuit behandelings

Suurlemoene Behandeling: *Voor oes*	**Vog verlies**		**Koue skade**	
	%	**Std fout**	**%**	**Std fout**
Gibberellien suur (10dpm)	4.90 **a**	1.66	36.67	9.18
LGE	2.29 **b**	0.46	16.67	4.45
Raynox®	2.27 **b**	0.06	40.00	6.55
Stopit-Ca®	3.19 **ab**	0.19	50.00	5.46
Kontrole (H_2O)	2.28 **b**	0.32	52.50	8.39
F-toets	*0.014******		*0. 84^{ns}*	
	P≤ 0.01		*P≤ 0.01*	

Na-oes behandelings van Eureka suurlemoene (Tabel 5.2.6.2)
Soos in die voor-oes behandelings het die kontrole weereens ’n gemiddelde persentasie koueskade van ongeveer 52,5% getoon. Jasmoonsuur was oneffektief in die beperking van koueskade en het met ’n gemiddelde persentasie van 55% die simptoom verhoog. CaCl, Salisielsuur, Spermidien en TBZ het koueskade tot ’n meerdere mate as die kontrole beperk. CalciMax het koueskade tot ’n groot mate beperk met ’n gemiddelde persentasie koueskade van 33,3%. Retain het die minste koueskade tot gevolg gehad, met ’n gemiddelde persentasie van 22,5% -alhoewel nie betekenisvol nie-. Dit is meer as helfte van die indeks van die kontrole. Die verlaging in % vog verlies van die Retain en die lae koue skade insidensie stem ooreen alhoewel geen betekenisvolle verskille in die koue skade simptome nie.

Tabel 5.2.6.2. Persentasie vogverlies en koueskade simptome van Eureka suurlemoene na-oes behandeling deur ’nverskeidenheid chemikalie en opberging vir 30 dae teen -0.5°C

Suurlemoene Behandeling: *Na-oes*	**Vogverlies**		**Koueskade**	
	%	**Std fout**	**%**	**Std fout**
Calsiumchloried	3.13	0.18	47.50	6.48
CalciMax®	2.36	0.19	33.33	5.46
Retain®	1.8	1.49	22.50	7.32
Jasmoon suur	2.77	0.36	55.00	8.81
Salisiel suur	3.85	0.33	40.00	6.88
Spermadien	2.9	0.16	45.00	10.52
TBZ	4.20	0.51	40.00	6.55
Kontrole(H_2O)	2.65	0.16	52.50	8.39
F-toets	*0.21^{ns}*		*0.18^{ns}*	
	P≤ 0.01		*P≤ 0.01*	

Warmbad-behandelings van Eureka suurlemoene (Tabel 5.2.6.3)

Geen betekenisvolle verskille het vorendag gekom tussen behandelings se gemiddelde persentasie vogverlies teen 99% waarskynlikheid nie, maar daarteen oor is hoog betekenisvolle verskille in koue skade gevind. By die bepaling van die gemiddelde persentasie koueskade is gevind tussen die 50°C (6.25%) en die kontrole (45.83%) en 40ºC-behandeling (49.50%) gemiddelde persentasies van 45,8% en 49,5%, onderskeidelik getoon het.

Tabel 5.2.6.3. Die voorkoms van % vogverlies asook koueskade simptome van Eureka suurlemoene na drie water bad behandelings en opberging teen -0.5°C vir 30 dae

Suurlemoene	**Vog verlies**			**Koue skade**	
Behandeling: *Warm bad*	**%**	**Std fout**		**%**	**Std fout**
Warm bad 40°C	2.77	0.40		49.50 **a**	11.09
Warm bad 50°C	2.61	0.09		6.25 **b**	3.05
Kontrole 20°C	2.65	0.06		45.83 **a**	7.55
F-toets	*0.280*^{ns}			*0.009****	
	P≤0.01			*P≤ 0.01*	

Waks-behandelings van eureka suurlemoene (Tabel 5.2.6.4)

By die waks-behandeling het slegs die C T10%-waks ’n laer persentasie vogverlies as die CT-kontrole getoon, alhoewel nie betekenisvol nie. Die SF890HS-waks het in teenstelling van wat verwag sou word die meeste vogverlies tot gevolg gehad met ’n waarde van 3.1%. By die interpretasie van die syfers moet egter onthou word die waks is as ’n doop behandeling aangewend en nie as ’n normale pakhuis behandeling nie. Geen betekenisvolle verskille in koue skade was gevind nie al was ’n 85% waarskynlikheid vlak gebruik. Die groot variasie in koue skade voorkoms kon hiervoor verantwoordelik wees.

Tabel 5.2.6.4. Die voorkoms van persentasie vogverlies en koueskade van Eureka suurlemoene na aanwending van vier wakse met verskillende vaste stowwe inhou en opberging teen -0.5°C vir 30 dae.

Suurlemoene	**Vogverlies**		**Koueskade**	
Behandeling: *Waks*	**%**	**Std fout**	**%**	**Std fout**
Carnuba Tropical 10%	2.29	0.30	45.00	10.52
Carnuba Tropical 14%	2.60	0.19	35.00	6.27
Carnuba Tropical (18%)	2.58	0.18	52.50	6.48
HSSF890 (24%)	3.12	0.32	45.00	7.23
F-toets	*0.168*^{ns}		*0.482*^{ns}	
	P≤0.01		*P≤ 0.0 1*	

Na-oes behandelings van Nules Clementine mandaryne (Tabel 5.2.6.5)

Daar is van dieselfde na-oes behandelings as met die Eureka suurlemoen gebruik gemaak. Die Clementines was ge-oes gedurende 9-12 Mei in die Paarl omgewing. Daar was egter baie lae vlakke van koue skade wat voorgekom het en geen betekenisvolle verskille was gevind nie.

Tabel 5.2.6.5. Voorkoms van koueskade soos beïnvloed deur na-oes behandelings van Nules Clementine mandaryne na opberging teen -0.5°C vir 30 dae.

Nules Clementine mandaryne Behandeling: *Na-oes behandelings*	**Koueskade**	
	%	Std fout
Carnuba Tropical10%	0	0
Carnuba Tropical14%	0.38	0.18
Carnuba Tropical 18%	0.75	0.31
Glukose	0.13	0.13
Jasmoon suur	0.25	0.25
Stopit-Ca®	0	0
Salisiel suur	0.5	0.27
TBZ	0	0
Kontrole (H_2O)	0.25	0.16
F-toets	0.07^{ns}	
	P= 0.01	

Na-oes behandelings van Valencia lemoene (Tabel 5.2.6.6)

Daar was geen betekenisvolle verskille gevind tussen behandelings se persentasie vogverlies nie en die data word nie aangetoon nie. Daar was egter betekenisvolle verskille in albei klasse waarin koue skade gemeet was nl. skilverbruining en gepokteskil (P< *0.01)*. Die waks HSFS890 (24%) het die voorkoms van beide skilverbruining (8.75%) en gepokteskil (1.25%) betekenisvol verminder i.v.m. die kontrole se koueskade van 27% en 23% onderskeidelik. Jasmoonsuur het die voorkoms van gepokteskil ook betekenisvol verminder na 2.5%. Carnuba Tropical waks het beide die koueskade simptome verlaag alhoewel nie betekenisvol nie. TBZ asook 1-MCP het nie enige betekenisvol verskil in koue skade teweeg gebring nie.

Tabel 5.2.6.6. Koueskade, soos bepaal deur skilverbruining en gepokte skil van Valencia lemoene na na-oes behandeling en opberging teen -0.5°C vir 30 dae

Valencia lemoen Behandeling: Na-oes doop behandelings	**Koueskade**				
	Skilverbruining	**Std fout**		**Gepokteskil**	**Std fout**
Carnuba Tropical (10%)	18.75 **ab**	3.98		13.75 **ab**	3.75
HS SF890 (24%)	8.75 **b**	3.50		1.25 **b**	1.25
1000dpb 1-MCP	18.57 **ab**	5.22		30.00 **a**	5.46
100dpb 1-MCP	22.50 **ab**	3.66		30.00 **a**	3.78
10dpb 1-MCP	36.25 **a**	8.79		30.00 **a**	5.35
TBZ	31.25 **a**	5.80		27.50 **a**	5.26
Jasmoon suur	32.50 **a**	4.12		2.50 **b**	1.64
Geen behandeling	38.75 **a**	22.50		25.49 **a**	2.50

Kontrole (H_2O)	27.50 **ab**	4.53		23.75 **a**	6.25
F-toets	*0.008***			*0.001***	
	P≤ 0.01			*P≤ 0.01*	

Gevolgtrekkings

Die oogmerk van die projek is om koueskade volgens twee strategie te beperk nl. voor-oes manipulasie van die vrug aan die boom asook die na-oes desensitisering en beheer van simptome. Die chemikalie wat gebruik was word gekies a.g.v. verwysings daarna in die vakliteratuur oor die vermoedelike invloed op koueskade in ander plantspesies en vrugtipes.

Die voor-oes behandelings het teleurstellende resultate opgelewer in die sin dat gibberelien suur en kalsium behandelings nie positiewe resultate gelewer nie. Die natuurlike lipied, LPE het egter 'n verlaging in koueskade simptome gehad en pas in met die literatuur waar 'n verbetering in rakleeftyd van tamaties gerapporteer is (Ozgen *et al*., 2005).

Die variasie in resultate van waks aanwending tussen kultivars dui die problematiek aan van die fisiologiese defek: waar waks aanwending koueskade verlaag in Valencia lemoene het dit nie die verlangde effek met suurlemoene nie. Dit bly egter een van die betroubaarste voorsorgmaatreëls as indirekte verlaging a.g.v. die feit dat die waks vog verlies beperk, en al was daar koueskade, die simptome minder sigbaar maak. Die gebruik van 'n swaarder waks (hoër vastestowe of "solids") het al getoon dat dit koueskade simptome beter beperk, soos gesien in die Valencia vrugte (Tabel 5.2.6.7) maar kan weer lei tot ander fisiologies defekte soos gepokteskil van Nawels.

Die resultate van die TBZ behandeling in al drie kultivars was teleurstellend in en daar was geen betekenisvolle verlaging in koueskade voorgekoms nie. Daar word algemeen aanvaar in die internasionale sitrus bedryf dat TBZ wel koueskade voorkoms kan verlaag. Geen verduideliking kon gevind word vir die resultate nie.

Die insluiting van Salisiel-, Jasmoonsuur, die poli-amien spermidien, AGV en 1-MCP as na-oes behandelings is daarop gemik om die stresreaksie wat koueskade teweegbring sodoende te manipuleer om die selle in die flavedo te de-sensitiseer/versterk. Die variasie in resultate dui egter op die kompleksiteit van die fisiologiese effek wat koueskade teweeg bring. Dit is die tweede jaar wat Jasmoonsuur gebruik is en die verlaging op gepokteskil van die Valencia lemoene was die eerste positiewe resultaat. Hierdie resultaat sal deur 'n konsentrasie reeks behandeling opgevolg word. Die uiterse lae konsentrasie waar die nuwe planthormoon plantgroei beïnvloed noodsaak so 'n strategie.

Die twee chemikalie, 1-MCP en AVG, wat direk op die etileen sisteem inwerk (maar op verskillende vlakke) het geen betekenisvolle positiewe resultaat gelewer nie. Die kennis van die etileen sisteem in die nie-klimakteriese vrugsoorte soos sitrus is nog baie onduidelik. Maar wat wel bekend is die feit dat die etileen sisteem kritiese is in enige stres situasie en dien meestal as tussen sel boodskapper. Daar sal dus verder na die chemikalie gekyk moet word maar alternatiewe toedienings en tyd van toedienings sal ook oorweeg word veral as in ag geneem word dat Retain (AVG) wel 'n verlaging in koueskade teweeg gebring het al was dit nie betekenisvol nie (Ben-Amor *et a*l., 1999).

Vanuit die resultate is dit duidelik dat die warmbad-behandelings die beste resultate getoon het, in terme van die beheer en beperking van koueskade. Deur die vrugte in 'n warmbad by 50ºC te dompel blyk die effektiefste te wees in die beperking van koueskade. In die algemeen word Suid Afrikaanse sitrus verpak deur van swamdoder aanwending in warmwater gebruik te maak. Die resultate dui daarop dat indien die temperatuur hiervan beter beheer kan word om naby die 50°C merk te bly, 'n voordeel verkry kan word in koueskade beheer. Dit is egter baie belangrik dat daar vir elke kultivar in 'n pakhuis se omgewing 'n sensitiwiteit vir die warm bad behandeling vasgestel moed word voor so 'n maatreël geïmplementeer kan word.

Toekomstige navorsing

Daar sal in die volgende jaar meeste van die behandelings herhaal word maar aanwendings tye en konsentrasie sal verander word. Suksesse soos die LPE, Retain, Jasmoonsuur sal veral op gekonsentreer word.

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5.2.7 Preharvest conditions influencing rind condition: determining the role of preharvest carbohydrate levels on rind breakdown of Nules Clementine mandarin

Experiment 758 by Paul Cronje, Graham Barry (CRI at SU) and Marius Huysamer (SU)

Opsomming

Nie-klimakteriese vrugte soos sitrus besit nie die vermoë om stysel te stoor en om te skale gedurende na-oes opberging vir vrugrespirasie nie. Daarom is dit belangrik dat die vlakke van koolhidrate sodanig is gedurende vruggroei dat daar nie 'n tekort kan ontstaan nie. Suiker vlakke (sukrose, glukose en fruktose) van Nules Clementine mandaryn flavedo, was bepaal deur 'n HPLC gedurende vrugontwikkeling van die seisoene 2004 en 2005. Daar was betekenisvolle verskille tussen binne en buitevrugte gevind in beide die seisoene met buitevrugte wat meer koolhidrate besit. Die hoër vlakke van skilafbraak van binne vrugte gedurende opberging kan verband hou met die laer suikervlakke. Die laer suikervlak sal vermoedelik lei tot 'n laer respirasietempo wat die ontwikkeling van die fisiologies defek kan teweeg bring. Die data toon dat die binnevrugte inderdaad van swakker kwaliteit is (hoër skilafbraak) en was vermoedelik a.g.v. die gebrek aan die nodige koolhidraat reserwes om die verlangde na-oes periode te voltooi.

Introduction

Non-climacteric fruit such as citrus lack the ability to store carbohydrate reserves such as starch and depend on continued import of sugars while being attached to the plant. Although the fruit itself is able to photosynthesize carbohydrates during the early stage of development, it is mainly dependent on the supply of photosynthates from the leaves (Tzur *et al*., 1991; Kadyi and Tanaka, 1972).

Arguably the main contributor to internal quality of citrus fruit is expressed as total soluble solids and constitutes 10-20% of fruit fresh weight (Erickson, 1968) of which carbohydrates account for 70-80% in the pulp (Davis and Albrigo, 1994). The major groups of carbohydrates include alcohol soluble monosaccharide sugars (mainly glucose and fructose), disaccharides sugars (mainly sucrose) and the alcohol insoluble structural polysaccharides, mainly starch, cellulose, hemi-cellulose and pectins. The total alcohol soluble solids in citrus juice and rind include carbohydrates, organic acids, free amino acids, lipids, flavonoids, volatiles, inorganic ions, vitamins and carotenoids and are dissolved in the cell sap (USDA-ARS, 1960; Nagy and Attaway, 1980; le Roux, 1996).

Sucrose, glucose and fructose are the main sugars in juice of most commercial *Citrus* species (Ting and Attaway, 1971). Sucrose is the main non-reducing and major translocation sugar in citrus fruit (Davis and Albrigo, 1994). Even though the rind and pulp are two vastly different physiological structures, the rind accumulates comparable basic sugars during the growing season to that of the juice. Due to the importance of sugars in internal quality, most research on carbohydrates has focused on the levels in the pulp. It is, therefore, necessary to extrapolate the available information to the situation in the rind/flavedo.
The catabolism of sucrose provides the necessary carbon required for anabolic processes during fruit growth and development. At the early stages of fruit development the highest rate of sucrose catabolism occurs, corresponding with the highest respiration rate, dry weight accumulation and the highest demands for

carbon. At later stages of fruit development where sucrose catabolism is lower due to less acid hydrolysis, it may limit the availability of carbon and result in slower growth rates of the fruit (Echeverria and Burns, 1989).

The reducing sugar concentrations have been reported to be more than double the sucrose levels during early growth of Marsh grapefruit, but sucrose levels at full maturity exceed the reducing sugar levels (Ting and Deszyck, 1961). The same occurrence has been reported in Clementine and oranges (Tadeo *et al*., 1987). It is known that the main metabolic event which influences internal fruit quality at the harvest maturity stage is connected to build-up and breakdown of sucrose in the fruit. However, the build up and hydrolysis of carbohydrates in the flavedo prior to harvest is not well understood from a quality perspective and especially regarding a possible role in the development of physiological disorders during storage. Carbohydrates have been shown to play a role in susceptibility to physiological disorders such as chilling injury (higher levels decrease CI) and rind staining (no apparent effect) (Holland *et al*., 2005; Purvis and Grierson, 1982).

The aim of this study was to determine the effect of pre-harvest conditions on the sucrose, glucose and fructose levels prior to harvest of Nules Clementine mandarin. The levels of sugars in the flavedo of fruit growing inside the canopy (low sunlight) and outside of the canopy (full sunlight) were compared during the 2004 and 2005 seasons. The inside fruit are known to be more susceptible to the physiological disorder rind breakdown that develops after 3-4 weeks of storage at non-chilling temperatures.

Materials and methods

Fruit samples

Fruit were sampled monthly during the 2004 and 2005 seasons, starting in January until harvest in May. Sampling occurred according to fruit position within the canopy. Fruit developing in full sunlight were sampled as “outside fruit” whereas fruit developing in the canopy with no direct sunlight were sampled as “inside fruit”. Eight replicates of 30 fruit each were harvested for both treatments from the Nules Clementine mandarin orchard on Welgevallen Experimental Farm, University of Stellenbosch. Fruit size was measured and the flavedo removed, frozen in liquid nitrogen and stored at -80°C. This sampling was continued every month (about every 4 weeks) until harvest.

On the determined commercial harvest date (~16 May) fruit were harvested, according to their position during their development, e.g. inside or outside of the canopy. The fruit were picked into wooden bins and subjected to a 72 hour degreening treatment wherafter they were separately packed according to either the inside or outside classification. These fruit were stored at 7.5°C for the duration of the experiment (14 and 16 weeks respectively for 2004 and 2005).

Extraction and purification for sugar analysis

For the sugar analysis, 0.1 g of dry flavedo was weighed into a 10 ml glass tube. A stock solution of extraction medium consisting of methanol (99% pure), $ChCl_3$ (chloroform) and distilled H_2O, was made up to the ratio of 60:25:15. From the stock solution 5 ml was added to the flavedo sample and vortexed for 30 seconds. The samples were left for 16 hours for extraction, wherafter they was centrifuged for 10 minutes at 3000 rpm. The clear supernatant was pipetted into a new tube. 1 ml of the extraction solution was added to the residue of the first tube, vortexed for 30 seconds and centrifuged at 3000 rpm for 10 minutes. The clear supernatant was pipetted and added to the corresponding sample tube. To complete the liquid extraction 1 ml of $CHCl_3$ was added to the supernatant and shaken before adding 2 ml deionised H_2O. The solution was vortexed for 30 seconds before centrifuging at 3000 rpm for 10 minutes to separate the layers. The top aqueous layer was quantitatively (6 ml) pipetted into a new glass tube and evaporated to dryness on a vacuum centrifuge (model SVC 200H, Savant, Farmingdale, NY). After complete evaporation of the solvent, 5 ml of deionised H_2O was added to dissolve the dried residue. One ml of the aqueous solution was loaded onto a C18 cartridge. The C18 (5 mg) cartridges were conditioned by soaking for 16 hours in MeOH before use. Before use the MeOH was washed out with four portions of 5 ml deionised water under vacuum. After loading the 1 ml of the sample to the C18 cartridge a vacuum pump was used to suck the sample into the C18 cartridges. The sample was washed through with 4 x 2 ml deionised water under vacuum. The sample was collected in a 10 ml volumetric flask which was filled up to exactly 10 ml. The solution was shaken and filtered through a 0.45 µm filter directly into a marked HPLC vial. The C18 cartridge was washed with MeOH for re-use. The C18 and liquid extraction were necessary to remove the pigments and the phenolics from the flavedo as these compounds could interfere with the efficiency of the HPLC column.

Sugars were separated using an HPLC system (1100 Series; Hewlett Packard, Waldbronn, Germany) with an autosampler (1100 Series; Hewlett Packard) operated by HP ChemStation software (LC Rev.A.06.03 [509]; Hewlett Packard). A Transgenomic-™ ion exchange stainless steel column (300 x 7.8 mm) (model ICSep ICE-ION-300; Transgenomic, Omaha, NE) with a guard column (model GC-801; Transgenomic) was

maintained at 29°C. Sugars were separated using 17 mM H_2SO_4 at a flow rate of 0.4 mL.min^{-1}. A differential refractive index monitor (model R401; Water) was used to detect separated sugars. An injection volume of 20 µL per sample was used. Eight replications per treatment were used.

Results

The incidence of rind breakdown in 2004 and 2005 seasons differed markedly between the inside and outside fruit during storage (Fig. 5.2.7.1). The increase over storage time illustrates the progressive nature of this postharvest disorder. The lower rind breakdown incidence in 2004 compared to 2005 demonstrates the effect of macroclimate on this disorder.

During the 2004 and 2005 seasons similar differences were found between inside and outside fruit in terms of sucrose, glucose and fructose contents in the flavedo (Figs. 5.2.7.2 and 5.2.7.3). These three major sugars are known in plants to be part of the major respiratory substrates necessary for plant development and growth. The higher levels of sucrose than the two reducing sugars (fructose and glucose) concur with reports in the literature. From both the seasons' data it is evident that the dramatic increase in carbohydrate levels only starts after February even though there were already significant differences between treatments in all of the sugars in January and February.

Discussion and conclusion

As most citrus fruit ripen, sugars, especially sucrose, increase (Ting and Attaway, 1971; Koch *et al*., 1986). This sugar accumulation in the pulp was found to be affected by temperature and light intensity thereby influencing the rate of maturation of the fruit (Kimball, 1984; Baldwin, 1993). In contradiction to these reports and the data presented here, Purvis (1980) reported no differences found in the levels of total soluble carbohydrates, reducing sugars or sucrose in the rind of Marsh grapefruit sampled from the exterior and interior of the tree canopy.

The supply of carbon skeletons for the energy needs and maintenance of physiological and biochemical activities in maturing and harvested citrus fruit is likely to be a highly regulated process of synthesis and degradation. The data presented here show that two aspects will need to be considered in the carbohydrate ratios of the flavedo: firstly the pre-harvest phase of development and secondly the postharvest storage phase.

The data were collected during the stage III of fruit growth when the rind has a renewed growth and thickening phase (Spiegel-Roy and Goldschmidt, 1996) and would therefore need carbohydrates. The significantly lower levels of all three sugars in the flavedo of the inside fruit during January through harvest in May, could translate into the development of a weaker flavedo at this stage of maturation. This same trend has also been found in the fruit pulp where the internal quality (TSS) of inside heavily shaded fruit is lower than outside fruit due to shaded leaves being unable to supply the necessary carbohydrates (Rouse and Zekri, 2006).

The second aspect to consider is the effect of lower carbohydrate levels in the flavedo on the postharvest quality of the fruit. Transpiration and respiratory activity are the main physiological processes involved in postharvest deterioration of citrus fruit and can lead to a loss in aesthetic appeal (le Roux, 1997) and probably induction of physiological disorders. Not only are organic acids used in the pulp as respiratory substrate but soluble sugars can also serve as glycolytic substrates in citrus pulp during the postharvest period (Goren *et a*l., 2000). A significant positive correlation exists between respiratory rates and the sugar content in plant tissue (Saglio and Pradet, 1980). It stands to reason that if the flavedo tissue is lacking in carbohydrate substrates for respiration, as seen in the inside fruit, these fruit may be more susceptible to development of a postharvest physiological disorder such as rind breakdown at non-chilling temperatures and a possible accompanying lower respiration rate.

Since the first South African industry reports of this disorder in the 1990s, yellow and inside fruit have been labelled as "weaker/more prone" than well coloured fruit developing on the outside of the canopy (van Rensburg *et al*., 2003). The data presented here as well as those reported in 2005 (Cronje *et al*., 2005) successfully identified two major imbalances between susceptible and non-susceptible fruit, *viz*. mineral imbalances (Ca, Mg and K levels) and carbohydrates in the flavedo tissue.

Future research

The ongoing research projects into rind condition will aim to manipulate these aspects in order to lessen the impact of rind disorders. From these results it is clear that cultural aspects such as nutrition and crop load management via pruning and thinning should not be neglected in this cultivar.

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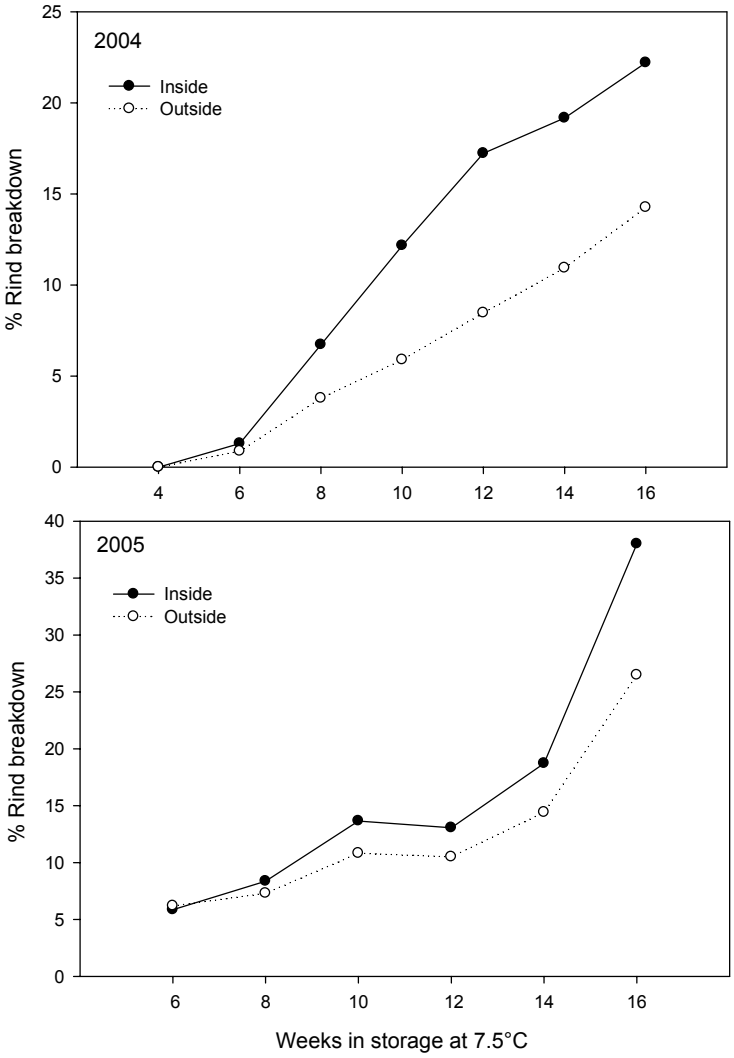


Figure 5.2.7.1. The cumulative incidence of rind breakdown of Nules Clementine mandarin during storage at 7.5°C for the 2004 and 2005 seasons. The solid line represents the inside fruit (low sunlight levels) and the broken line the outside fruit (developed in full sunlight).

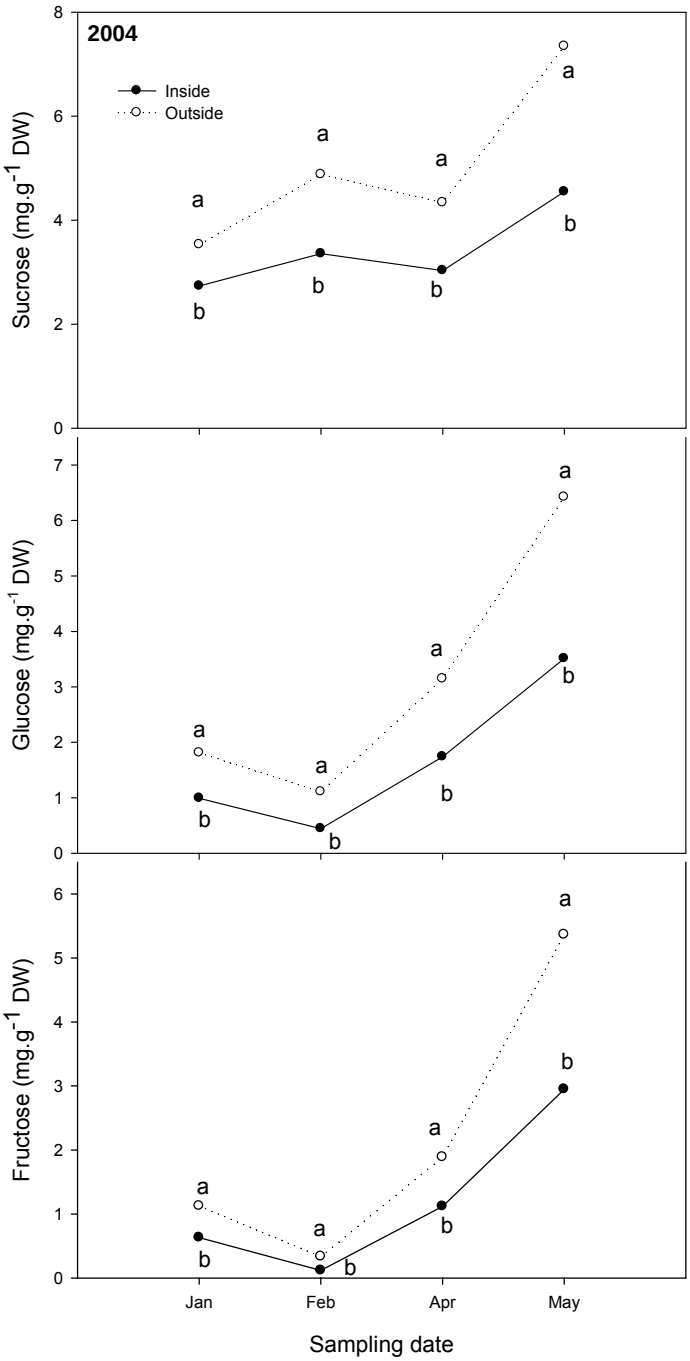


Figure 5.2.7.2. The levels of three sugars (sucrose, glucose and fructose [mg.g^{-1} dry weight]) measured in the developing flavedo during the 2004 season. Significant differences (P< 0.01) between treatments per sampling date are denoted by different letters.

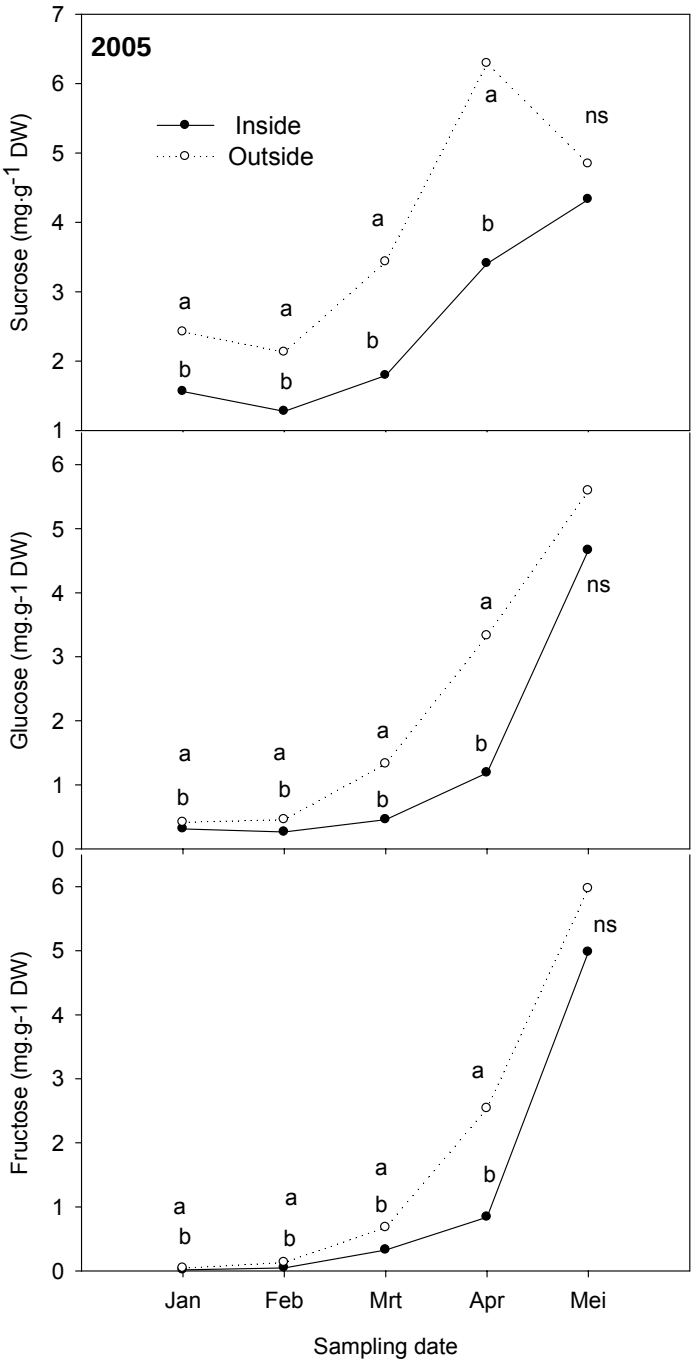


Fig. 5.2.7.3. The levels of three sugars (sucrose, glucose and fructose [mg.g^{-1} Dry Weight]) measured in the developing flavedo during the 2005 season. Significant differences (P< 0.01) between treatments per sampling date are denoted by different letters and ns represents non significant.

5.2.8 **The influence of cold disinfestation and duration of storage on the condition of Oroblancos/Sweeties exported to Japan**

Experiment 874 by K.H.Lesar (CRI)

Opsomming

Die Oroblanco is koue sensitive, maar daar is nogtans 'n mark in Japan. Dit beteken dat kouesterilasie sal moet gedoen word. Die doel van die werk was dus on te sien wat die efek van so 'n behandeling op vrug gehalte sal wees. Die voorkoms van die swam Diplodia en 'n oleo-simtoom was betekenisvol groter as koueskade. Die werk sal in 2007 herhaal word, maar die vlak van patogene is as 'n problem beskou.

Introduction

At some stage there was uncertainty as to whether the Oroblanco was a grapefruit or an orange. The trade, including the Japanese trade, now recognises the Oroblanco as a low acid white grapefruit and it has gained a good reputation of being a high quality fruit in Japan. The USA has been exporting Oroblancos to Japan for some time, and now the South African citrus industry has received a request to export Oroblancos to Japan. However, cold sterilization will be necessary.

Simulated cold disinfestation trials conducted in 1992 by Burdette demonstrated Oroblancos were particularly sensitive to chilling injury (CI). These results were reported by Barry in the 1992 Outspan Research Progress Report. However, details of the condition of fruit after cold sterilization were not provided. Therefore it is necessary to determine the condition of the Oroblanco after cold disinfestation and simulated shipping to Japan**.**

Materials and methods

80x16 kg standard packhouse treated export cartons (count 32) of Oroblancos were received from TSB Hectorspruit on the 20 March 2006.

The fruit was divided up into 4 replicates x 5 cartons (count 32 i.e. 160 fruit) per treatment.

The cartons were marked and stored under cold sterilisation and shipping conditions as follows:

Treatment 1	Treatment 2	Treatment 3	Treatment 4
12 days @ -0.6°C	12 days @ -0.6°C	12 days @ 11°C	12 days @ 4.5°C
8 days @ 11°C	8 days @ 4.5°C	7 days @ 20°C	7 days @ 20°C
7 days @ 20°C	7 days @ 20°C		

After storage the following results were recorded.

Results

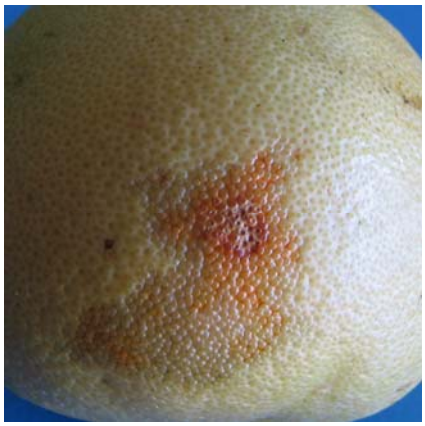
The results indicated that the incidence of the pathogen Diplodia, lying latent under the calyx of the fruit was high, leading to a high incidence of Diplodia stem end rot after the cold disinfestation treatment, compared to the fruit shipped at 4.5°C and 11°C.The incidence of typical CI symptoms was low compared to the increased manifestation of “oleo” type damage after shipping (see figures below).

Table 5.2.8.1. The incidence of waste and rind damage on Oroblancos after cold disinfestation and shipping to Japan.

Treatments	% Decay Diplodia stem-end rot	% Chilling injury symptoms Typical	% Chilling injury Symptoms “Oleo” like damage
1	11.7 b	0.3 a	8.9 a
2	12.2 b	0.3 a	8.8 a
3	3.7 a	0.2 a	20.6 c
4	7.3 a	0.3 a	14.8 b

Means in the same column followed by the same letter are not significantly different (Fisher’s Unprotected LSD; P= 0.05)

Typical CI damage



“Oleo” type damage

Discussion and conclusions

The above results indicate firstly that the incidence of the pathogen Diplodia, lying latent under the calyx of the fruit was high, leading to a high incidence of Diplodia stem end rot after the cold disinfestation treatment.

Previous years’ research results have indicated that fruit stored at low temperatures, with a high degree of this quiescent pathogen, have a higher risk of infection by this pathogen than fruit stored at higher temperatures. This is because the fruit stored at the low temperatures is stressed somewhat and this promotes the development of the infection.

This places citrus cultivars that are exported to markets where cold disinfestation is a requirement, at a greater risk of high infection by this pathogen, if present. The last 2-3 citrus seasons have indicated a higher than normal presence of the pathogens Diplodia and Anthracnose due to inconsistent and late season rainfall.

Future research

This trial work will be repeated in the 2007 production season.

5.2.9 **Hot water dip treatments to prevent chilling injury on early and late season harvested lemons exported to Japan**
Experiment 869 by K.H.Lesar (CRI)

Opsomming

Warm water behandeling vir die voorkoming van koue skade is al in verskeie sitrus kultivars gebruik, maar daar is nog nie baie navorsing op suurlemoene gedoen nie. Die doel van die werk was om die behandeling te ondersoek. 'n Warm water behandeling van 53°C is gebruik. Vrugte het heelwat verwelk, en as gevolg is the proef beëindig, maar sal in 2007 oorgedoen word.

Introduction

Producers and exporters alike, lose millions of Rands every year due to chilling injury (CI) on lemons and grapefruit exported to Japan. Citrus fruit can incur rind damage due to CI if stored for extended periods at sub-optimal temperatures as occurs with lemons and grapefruit exported to Japan, during the cold disinfestation (sterilisation) of the fruit against fruit fly. Research conducted by CRI in 2001 on the conditioning of grapefruit at 16 and 20°C and the heat shock treatment (35°C for 3 days in a hot room) of lemons and grapefruit, prior to the cold disinfestation treatment, demonstrated promising results in the reduction of CI. Research conducted by other researchers on hot water dip treatments, with and without fungicides (specifically thiabendazole), prior to sub-optimal temperature storage, have also demonstrated promising reduction of CI on lemons and grapefruit. Factors that cause CI are still largely unknown. Methods to reduce CI, especially during cold disinfestation, must be afforded high priority.

Materials and methods

Early (green) and late (fully coloured) lemons were harvested at Larten Estates in the Karino area for the purpose of this trial.

The green and fully coloured lemons were then separately divided up into 6 replicates of 20 fruit per treatment. The lemons were treated on the packline at CRI in Nelspruit. The fruit was washed in the high pressure spray with a suitable sanitising agent (Prasin). After washing, the lemons were treated in the hot water bath for 2 minutes at ambient (18°C) and at 53°C and thereafter dried in the drying tunnel.

The waxed treatments were done by means of a dip treatment and the fruit were allowed to dry overnight before storage.

The following treatments were conducted:

1. Untreated Control
2. Treated Control Ambient dip at 18°C for 2 minutes
3. Hot water dip at 53°C for 2 minutes unwaxed
4. Hot water dip at 53°C for 2 minutes waxed (Carnauba wax)
5. 1000 ppm TBZ dip at 18°C for 2 minutes
6. 1000 ppm TBZ dip at 53°C for 2 minutes
7. Condition for 7 days + hot water dip at 53°C for 2 minutes

After treatment the fruit was stored as follows.

3 reps x 20 fruit 4 weeks at 10°C + 7 days at 20 °C

3 reps x 20 fruit 14days at -0.6°C + 2 weeks at 10°C + 7 days at 20°C

After cold disinfestation and shipping of the lemons no CI symptoms were evident on the treated lemons.

These lemons (green and coloured) were then stored, after 'shipping' for 4 weeks, for a further 4 weeks at both 10°C and 2°C to possibly induce CI. The lemons were evaluated after 2 weeks extended storage and then stored a further 2 weeks and evaluated again.

After a total period of 8 weeks the lemons were looking a bit withered because of moisture loss (especially the unwaxed fruit). The fruit would have been stored for 2 weeks longer if all the fruit was waxed, but due to overall condition the trial was terminated.

Discussion and conclusions

No CI symptoms occurred, even after extended storage. The lemons used were obtained from peteca prone orchards at Larten Estate, from the same orchards where a high incidence of peteca spot was experienced in the 2004 and 2005 citrus seasons. These same lemons were used for the 2006 peteca spot/wax trials and this CI trial.

Good peteca spot results were recorded in the 2005 trials with lemons from the same source after shipping and 6 weeks extended storage at 2°C, but not in 2006, even after 6 weeks extended storage.

It is suggested that the sensitivity of the rinds of lemons is established on the tree before harvest, while the fruit is still green and fairly immature. The environmental conditions appear to play a very big role in the potential for disorder development. These then predispose the fruit to peteca, CI and other rind conditions during the harvesting of the fruit and the further handling and treatments. This could also explain why petaca spot varies considerably from time to time, as conditions in the orchard change.

Prior to picking and packing in 2006, a few cartons of untreated Marsh, S/Ruby and Rose were picked at Tecklenburg. This fruit was stored at CRI under cold sterilisation conditions and then "shipped" for 2 weeks longer at 2°C. No injury was found, unlike that on fruit from the same source in 2005. It therefore seems that the sensitivity of the rinds had changed from one season to the next.

Future research

This CI trial will be repeated during the 2007 season. A few different hot water bath temperatures and one or two different waxes will be included in this trial, and extended storage, if necessary. In addition, preliminary work indicated that the micro-element molybdenum may play a role in chilling injury, and will be tested.

5.2.10 Eureka lemon physiological profile: Storage temperature and storage duration response curves for Eureka lemon harvested at different physiological maturities, with special reference to Peteca spot

Experiment EX 01-06 by Parton Khumalo, Arrie de Kock and Jerome Davids (Experico)

Opsomming

Eureka suurlemoene is geoes by drie verskillende ryphede, gewaks by 'n kommersiële pakhuis, en opgeberg by -0.5, 4.5, 7.5 en 10°C vir 4, 20, 40, 60 en 80 dae onderskeidelik. Na koelopberging is die vrugte blootgestel aan 'nraklewe periode van 7 dae by ±20°C. Die vrugte is ge-evalueer voor, sowel as na raklewe. Die effek van opbergingstemperatuur en -tyd op die algemene kwaliteit van Eureka suurlemoene was konsekwent by vrugte van verskillende oes ryphede. Oor die algemeen was daar lae vlakke van Peteka, ongeag vrugrypheid. In die meeste gevalle was daar 'n interaksie tussen die opbergingstemperatuur en die -tyd in terme van die ontwikkling van Peteka. Daar kon egter nie 'n definitiewe patroon vasgestel word nie. Dit beteken waarskynlik dat opbergingstemperatuur nie die hoof oorsaak van Peteka is nie, aangesien die defek ontwikkel het op vrugte by al die verskeie opbergingstemperature. Peteka het ook reeds op 'n vroeë stadium gedurende koelopberging ontwikkel en het nie vermeerder gedurende opberging nie. Die eksperiment het ook die sensitiwiteit van Eureka suurlemoene ten opsigte van lae koelopbergings temperature uitgelig. Koelopbergingstemperature onder 7.5°C het hoë vlakke van koue-skade tot gevolg gehad, veral met verlengde opberging. Hierdie patroon was duidelik in vrugte van verskillende oesryphede. Verlengde opberging by al die opbergingstemperature het aanleiding gegee tot hoër vlakke van bederf. In vrugte wat by –0.5 en 4.5°C gestoor is, was die bederfvlakke 100% in die meeste gevalle, as gevolg van koue-skade. Swart-pit, 'n bakteriese siekte wat veroorsaak word deur *Pseudomonas syringae* en manifesteer as swart-bruin versonke merke op die skil is waargeneem na verlengde opberging. Dit wil voorkom of swart-pit vlakke hoër was by vrugte wat by 4.5°C opgeberg is teenoor ander koelopbergingstemperature. Die effek van koelopbergingstemperatuur en -tyd op Eureka suurlemoene was gedemonstreer. Die gevolgtrekking is dus dat opbergingstemperatuur en -tyd nie die hoof oorsake vir die ontwikkeling van Peteka is nie en gevolglik ook nie gebruik kan word om die defek te bestuur nie. Weens die sensitiwiteit van Eureka suurlemoene ten opsigte van lae koelopbergings temperature, blyk dit dat optimum kwaliteit verkry sal word deur koelopberging by temperature hoër as 7.5°C vir tot 60 dae. Die effek van oesrypheid op die ontwikkeling van Peteka kon nie in hierdie ondersoek bepaal word nie, maar kan nie uitgesluit word as moontlik oorsaak van die defek in Eureka suurlemoene nie. Die proef in die huidinge formaat kan beëindig word, maar die effek van oesrypheid op die ontwikkeling van Peteka moet egter deel vorm van toekomstige proewe met die doel om die probleem op te los.

Introduction

Eureka lemon develops various disorders in storage, among which is peteca spot. This disorder manifests as depressions on the fruit rind with normal colour, and then oil glands begin to darken (Murata 1997). Peteca spot reduces the market value of the fruit due to rind deformation, resulting in huge economic losses to the South African citrus industry and also to the citrus industry worldwide. Therefore, research aimed at reducing peteca spot is necessary.

Some research has been conducted on peteca spot and largely focused on the effect of fruit waxing. However, progress in solving the disorder has been slow, as peteca spot still occurs from time to time, suggesting that the underlying causes for peteca spot are still not understood.

To contribute to our understanding of peteca spot this experiment was conducted with the objective to determine the effect of storage temperature and storage duration on the quality of Eureka lemon fruit harvested at different maturities.

Materials and methods

Trial site detail
Fruit were harvested from healthy 15 year old Eureka lemon trees, grafted on rough lemon, in Franschoek (Imibala).

Treatments
Fruit were harvested at three maturity stages, namely early harvest (19 June 2006), mid harvest (28 June 2006) and late harvest (28 July 2006). At each harvest date ~600 kg of fruit were harvested into lug boxes. The fruit were wilted for ~24 hours in the orchard and then transported to the packhouse for waxing and packing. Fruit for this experiment were not degreened but packed immediately on arrival at the packhouse. During packing the fruit were moved through a commercial pack-line and a light natural wax was applied. Waxed fruit was then packed into the MO7T telescopic pear carton (7 kg). A total of 80 cartons were packed at each harvest date. The 80 cartons of fruit packed at each harvest date were divided into four groups of 20 cartons each and stored at -0.5, 4.5, 7.5 and 11°C. The 20 cartons of fruit at each storage temperature were further divided into 4 groups of five cartons each (replicates) corresponding to storage durations of 20, 40, 60 and 80 days. After each storage duration a shelf-life period of 7 days at 20°C was implemented. Evaluations were conducted before and after shelf-life.

Evaluation stages and parameters

At harvest (10 fruit per replicate)
Rind colour rating (scale of 1-8), soluble solids content (SSC) [°Brix] and titratable acidity (TA) [%].

Before and after shelf life (50 fruit per replicate)
Peteca spot incidence (%), chilling injury incidence (%), decay (%), black pit (%) and rind colour rating (scale of 1-8)

Statistical detail
The trial was laid out a randomised complete block design with three harvest maturities replicated 5 times. Three tree plots were used in the orchard. After storage data were analysed as a two-way ANOVA, using STATISTICA, where Factor A = storage temperature and Factor B = storage duration. Results from each harvest maturity were analysed separately.

Results and discussion

Early harvest (19 June 2006)
At harvest
Eureka lemon fruit were harvested on the 19 June 2006 at a rind colour rating of 4.0, and an SSC of 7.2°brix (Table 5.2.10.1).

After cold storage
A significant interaction occurred between storage temperature and storage duration on the development of peteca spot (Table 5.2.10.2). Generally, the levels of peteca spot were low across treatments and did not show a definite trend of increasing or decreasing over time and between storage temperatures. This suggests that neither time nor temperature is the main cause for the development of peteca spot. However, it has been observed for two seasons that, with extended storage for up to 60 and 80 days the incidence of

peteca spot is reduced. This is probably due to the manifestation, in greater severity, of other disorders like decay, chilling injury and black pit, which then mask the peteca spot. It is also possible that the reduction of peteca spot over time could be due to the fact that the disorder is not progressive. A significant interaction occurred between storage temperature and storage duration on the development of chilling injury. This disorder did not develop on fruit stored at the different temperatures for up to 40 days, afterwhich low levels developed on fruit in fruit stored at 4.5°C. After 80 days storage all fruit at –0.5°C developed chilling injury, while low levels (9.2%) were evident in fruit stored at 4.5°C. A significant interaction occurred between storage temperature and storage duration on the rind colour rating. However, no definite trend could be established. For up to 40 days storage, at the respective temperatures, no decay developed on fruit. Thereafter, the disorder appeared and was higher in fruit stored at 4.5 and 7.5 compared to fruit stored at the other temperatures. A significant interaction occurred between storage temperature and storage duration on the development of black pit, a bacterial disease caused by *Pseudomonas syringae*, which manifests as brown-black sunken spots on the fruit rind. This disorder developed after 40 days in fruit stored at 4.5 and 7.5°C. With increasing storage duration, the levels of this disorder increased and remained higher in fruit stored at 4.5°C than at the other temperatures.

After shelf-life

Storage duration was a significant factor in the development of peteca spot in fruit stored at different temperatures (Table 5.2.10.3). The disorder appeared on fruit after storage for 10 days, increased significantly at 40 days and decreased thereafter. The reason for this trend could be that development of other disorders masked the presence of peteca spot, after 60 and 80 days. Storage temperature did not significantly affect the development of peteca spot. No chilling injury occurred in fruit stored at the different temperatures for up to 40 days. However, after 60 and 80 days storage, all the fruit stored at –0.5°C and 4.5°C (only after 80 days) developed chilling injury with little or none developing in the fruit stored at the other temperatures. A significant interaction occurred between storage temperature and storage duration on the rind colour rating of fruit. Fruit stored at –0.5°C for 10 days had a higher colour rating than fruit stored at the other temperatures. Fruit stored at 7.5 and 10°C for up to 80 days had the lowest rind colour rating. No decay developed in fruit stored at the different temperatures up to 10 days. Thereafter, the disorder developed and increased significantly in fruit stored at 4.5 and –0.5°C, to 100% after 80 days. The presence of chilling injury on fruit was the main cause of the high levels of decay that developed after shelf life in fruit stored at low temperatures. Black pit developed and was high after 60 days in fruit stored at 4.5°C, and only appeared after 80 days in fruit stored at 7.5 and 10°C.

Mid harvest (28 June 2006)

At harvest

Fruit were harvested on the 28 June 2006 at a rind colour rating of 1.8 and SSC of 7.0°brix (Table 5.2.10.4).

After cold storage

The incidence of peteca spot increased significantly over 40 days storage. Thereafter, levels decreased, possibly due to the reasons previously stated (Table 5.2.10.5). Storage temperature was a significant factor in the development of peteca spot, but did not show a definite trend with increasing storage temperature. Chilling injury developed on fruit stored at –0.5°C at 20 days, and increased significantly over time to 100% after 60 and 80 days storage at this temperature. No or low levels of the disorder occurred on fruit of the other treatments. A significant interaction occurred between storage temperature and storage duration on the rind colour rating of fruit. However, no definite trend could be established. Decay development was significantly affected by storage duration. The disorder increased significantly after 60 and 80 days storage. Storage temperature significantly affected the development of decay, with none occurring in fruit stored at –0.5°C, and an increase at higher temperatures. Across the different storage temperatures no black pit appeared for up to 40 days. Thereafter, the disorder developed in fruit stored at 4.5, 7.5 and 10°C, with higher levels occurring in fruit at 4.5°C.

After shelf-life

The after shelf life evaluation was not conducted in fruit stored for 10 days at the respective temperatures (Table 5.2.10.6). Peteca spot only occurred on fruit after 40 days storage and the levels were higher in fruit stored at 4.5 and 7.5°C compared to the other storage temperatures. With extended storage peteca spot was greatly reduced, as previously explained. Chilling injury occurred in fruit stored at low temperature and levels increased significantly to 100% after 60 and 80 days in fruit stored at –0.5°C. A significant interaction occurred between storage temperature and storage duration on the rind colour rating. Extended storage of 80 days at 4.5, 7.5 and 10 °C resulted in a significantly lower rind colour rating than the other treatments, indicating that this fruit was more yellow in colour. Decay was evident after 40 days storage. The levels were significantly higher in fruit stored at –0.5°C and increased to 100% after 60 and 80 days storage, while levels remained lower in fruit of the other treatments. Black pit appeared in fruit stored at 4.5, 7.5 and 10°C after 60

days. The levels of this disorder increased after 80 days and remained high in fruit stored at 4.5 and 7.5°C compared to the other treatments.

Late harvest (28 July 2006)
At harvest
Fruit were harvested on the 28 July 2006 at a rind colour rating of 1.5 and SSC of 6.4°brix (Table 5.2.10.7).

After cold storage
The levels of peteca spot seemed to increase across storage temperatures for up to 40 days storage (Table 5.2.10.8). Thereafter the levels again decreased. Significant levels of chilling injury developed after 40 days in fruit stored at –0.5°C, with levels increasing over time to 100% after 80 days storage. No or low levels of chilling injury developed in fruit of the other treatments. A significant interaction occurred between storage temperature and storage duration on the rind colour rating of fruit. Extended storage at high temperatures, 4.5, 7.5 and 10°C, resulted in a lower rind colour rating than the other treatments. A significant interaction occurred between storage temperature and storage duration on the development of decay. The disorder developed with extended storage on fruit of most temperatures. Black pit also developed with extended storage in fruit stored at the different temperatures. The levels of this disorder were higher in fruit stored at 4.5°C compared to the other treatments.

After shelf-life
Peteca spot appeared early in the storage life of fruit and seemed to increase up to 40 days in fruit stored at the 4.5, 7.5 and 10°C (Table 5.2.10.9). Thereafter, the levels greatly decreased. Chilling injury was evident at 40 days in fruit stored at –0.5°C, with levels increasing to 100% with extended storage. Lower or no chilling injury developed in fruit stored according to the other treatments. A significant interaction occurred between storage temperature and storage duration on the rind colour rating of fruit. Extended storage at high temperatures, 4.5, 7.5 and 10°C, resulted in a lower rind colour rating than the other treatments. In fruit stored at –0.5°C, 100% decay developed after 60 and 80 days, whereas lower or no decay developed in fruit of the other treatments. Black pit developed in fruit stored at 4.5, 7.5, and 10°C after 60 and 80 days. The levels of this disorder were significantly higher in fruit stored at 4.5°C.

Table 5.2.10.1. Harvest maturity of Eureka lemon fruit sampled on 19 June 2006

Rind colour rating [1]	TSS (°Brix)	Titratable acidity
4.0	7.2	5.6

Rind colour rating determined using the CRI rind colour chart set number 37, where 8 = poor coloured, dark green with no colour break, and 1= yellow and fully coloured fruit (CRI, 2004).

Discussion and conclusions

Generally, low levels of peteca spot were observed in this experiment. Low susceptibility of fruit to peteca spot was possibly due to the maturity of the fruit at harvest. It was shown in previous experiments that fruit harvested early during the picking window of Eureka lemon fruit were more susceptible to peteca spot. In this experiment, fruit were harvested between 19 June and 28 July at a rind colour rating between 4 and 1.5, which may be late in the season and therefore fruit may have been at optimum and post-optimum maturity. Consequently, fruit harvested at this maturity may not have been as susceptible to peteca spot as fruit harvested earlier, when less mature. The objective of this experiment was also to establish the effect of storage temperature and storage duration on the development of peteca spot. The results showed that the development of peteca spot did not consistently increase or decrease with increasing storage temperature and duration. This trend has been observed over two seasons. It would therefore seem that storage temperature is not the main cause for peteca spot, and as such cannot be used to control the development of the disorder in Eureka lemon fruit. Furthermore, peteca spot develops early during the storage life of the fruit and is not progressive in storage. Evident in this experiment was the sensitivity of Eureka lemon fruit to low temperature storage. Chilling injury was high in fruit stored at –0.5 and 4.5°C, and as a consequence, opportunistic infection by *Penicilium digitatum* occurred, resulting in high decay levels. Optimum quality of Eureka lemon fruit is achieved by storage at temperatures above 7.5°C for up to 60 days.

It can be concluded that:
Storage temperature was not the main cause for peteca spot. Therefore, storage temperature cannot provide a solution for peteca spot in Eureka lemon fruit. Peteca spot is a disorder that develops early during the

storage life of a fruit and it is not progressive. Harvest maturity and the effect thereof on the development of peteca spot has to be investigated further and should therefore form part of future experiments aimed at solving the disorder. The sensitivity of Eureka lemon fruit to low temperature storage was demonstrated. Therefore, optimum fruit quality can be achieved in fruit stored at temperatures above 7.5°C for up to 60 days. The experiment in its current form can terminate.

Future research

There is a need to better elucidate the effect of harvest maturity on the development of peteca spot. Therefore, this factor should form part of future experiments aimed at reducing or solving peteca spot. Information has been generated on the effect of storage temperature and storage duration. The experiment in its present form can terminate, with certain factors to be incorporated into future trials.

Reference cited

Murata T., 1997. Citrus. In: S., Mtira, (editor), Postharvest Physiology and Storage of Tropical and Subtropical Fruits. CAB International. Oxon, UK, pp. 21-46.

Table 5.2.10.2. Quality of Eureka lemon fruit harvested on 19 June 2006 and evaluated before shelf-life

Response variable	Interaction[1]	Storage duration (days) [A][2]						Storage temperature (°C) [B]				Prob>F[3]		
		4	10	20	40	60	80	-0.5	4.5	7.5	10	A	B	AxB
Peteca spot	-0.5	1.6bcd	1.3abdc	0.0a	2.7d	0.0a	0.0a					**	NS	*
(%)	4.5	0.3a	0.5ab	0.0a	0.0a	0.0a	0.0a							
	7.5	0.7abc	2.1cd	1.1abc	0.0a	0.0a	0.0a							
	10	0.0a	0.5ab	1.0abc	1.4abcd	0.5ab	0.0a							
Chilling injury	-0.5	0.0a	0.0a	0.0a	0.0a	0.0a	100.0d							
(%)	4.5	0.0a	0.0a	0.0a	0.0a	1.8b	9.2c							
	7.5	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a							
	10	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a							
Rind colour	-0.5	3.0cdefgh	3.2fghi	2.5ab	3.3ghi	3.5i	3.1fghi					***	***	***
rating[4]	4.5	3.0cdefgh	3.3ghi	3.0cdefgh	2.3a	2.9bcdefg	3.0cdefgh							
	7.5	2.6abcd	2.9bcdefg	2.8bcdef	2.2a	2.5ab	2.9bcdefg							
	10	2.6abcd	3.3ghi	2.7bcdef	2.6abcd	2.8bcdef	2.6abcd							
Decay (%)	-0.5	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a					***	***	***
	4.5	0.0a	0.0a	0.0a	0.0a	3.1bc	10.0d							
	7.5	0.0a	0.0a	0.0a	0.0a	5.0c	8.6d							
	10	0.0a	0.0a	0.0a	0.0a	1.3ab	1.7ab							
Black pit (%)	-0.5	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a					***	***	***
	4.5	0.0a	0.0a	0.0a	0.9ab	15.1d	11.8c							
	7.5	0.0a	0.0a	0.0a	3.7b	0.5a	1.2ab							
	10	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a							

[1]If itemised interaction occurred between factor A and B
[2]Values in the same row or column followed by different letters indicate significant differences (P<0.05) according to the LSD test.
[3]Two-way ANOVA with factor A being storage temperature and factor B being storage duration
[44]Rind colour rating determined using the CRI rind colour chart set number 37, where 8 = poor coloured, dark green with no colour break, and 1= yellow and fully coloured fruit (CRI, 2004)

Table 5.2.10.3. Quality of Eureka lemon fruit harvested on 19 June 2006 and evaluated after shelf-life. Rind colour rating determined using the CRI rind colour chart set number 37, where 8 = poor coloured, dark green with no colour break, and 1= yellow and fully coloured fruit (CRI, 2004)

Response variable	Interaction[1]	Storage duration (days) [A][2]				Storage temperature (°C) [B]				Prob>F[3]		
		10	40	60	80	-0.5	4.5	7.5	10	A	B	AxB
Peteca spot (%)		1.1a	2.8b	0.1a	0.0a	1.2	0.7	1.6	0.6	***	NS	NS
Chilling injury (%)	-0.5	0.0a	0.0a	100c	100.0c					***	***	***
	4.5	0.0a	0.0a	0.6a	100.0c							
	7.5	0.0a	0.0a	0.5a	2.7b							
	10	0.0a	0.0a	0.0a	0.0a							
Rind colour rating[4]	-0.5	4.0e	3.0d	3.0d	-[5]					***	***	***
	4.5	3.0d	2.3bc	2.4bc	-							
	7.5	2.9d	2.4bc	2.5c	1.0a							
	10	3.1d	2.4bc	2.2b	1.0a							
Decay (%)	-0.5	0.0a	9.9c	0.0a	100.0e							
	4.5	0.0a	4.6b	15.6d	100.0e							
	7.5	0.0a	1.0a	4.2b	0.7a							
	10	0.0a	0.0a	0.4a	0.5a							
Black pit (%)	-0.5	0.0a	0.0a	0.0a	0.0a					***	***	***
	4.5	0.0a	0.0a	14.5c	0.0a							
	7.5	0.0a	0.0a	0.0a	6.0b							
	10	0.0a	0.0a	0.0a	1.4a							

[1]If itemised interaction occurred between factor A and B
[2]Values in the same row or column followed by different letters indicate significant differences (P<0.05) according to the LSD test.
[3]Two-way ANOVA with factor A being storage temperature and factor B being storage duration
[4]Rind colour rating determined using the CRI rind colour chart set number 37, where 8 = poor coloured, dark green with no colour break, and 1= yellow and fully coloured fruit (CRI, 2004)
[5]Rind colour could not be measured because of bleached rind colour due to chilling injury

Table 5.2.10.4. Harvest maturity of Eureka lemon fruit sampled on 28 June 2006

Rind colour rating[1]	TSS (°Brix)	Titratable acidity
1.8	7.0	4.5

Rind colour rating determined using the CRI rind colour chart set number 37, where 8 = poor coloured, dark green with no colour break, and 1= yellow and fully coloured fruit (CRI, 2004)

Table 5.2.10.5. Quality of Eureka lemon fruit harvested on 28 June 2006 and evaluated before shelf-life. Rind colour rating determined using the CRI rind colour chart set number 37, where 8 = poor coloured, dark green with no colour break, and 1= yellow and fully coloured fruit (CRI, 2004)

Response variable		Inter-action[1]		Storage duration (days) [A][2]							Storage temperature (°C) [B]					Prob>F[3]		
				4	10	20	40	60	80		-0.5	4.5	7.5	10		A	B	AxB
Peteca spot (%)				0.5a	1.6b	3.0c	4.8d	0.0a	0.0a		2.6b	0.8a	1.4a	1.8ab		***	*	NS
Chilling injury		-0.5		0.0a	0.0a	6.4b	33.7c	100.0d	100.0d							***	***	***
(%)		4.5		0.0a	0.0a	0.0a	0.4a	0.0a	0.9a									
		7.5		0.0a	0.0a	0.0a	0.0a	0.0a	0.4a									
		10		0.0a	0.0a	0.0a	0.0a	0.0a	0.0a									
Rind colour		-0.5		3.0cdefgh	3.9j	3.1efghi	3.5hi	2.8bcdef	2.6bc									
rating[4]		4.5		2.9bcdefg	3.1efghi	2.9bcdefg	3.1efghi	2.5ab	3.0cdefgh									
		7.5		3.0cdefgh	3.0cdefgh	3.0cdefgh	2.6bcd	2.5ab	2.6bcd									
		10		2.8bcdef	2.9bcdefg	2.7bcde	2.7bcde	2.5ab	2.1a									
Decay (%)				0.4ab	0.3a	0.2a	0.6ab	1.8b	3.4c		0.0a	1.5b	1.8b	1.2ab		***	*	NS
Black pit (%)		-0.5		0.0a	0.0a	0.0a	0.0a	0.0a	0.0a							***	***	***
		4.5		0.0a	0.0a	0.0a	0.0a	12.8cd	15.0d									
		7.5		0.0a	0.0a	0.0a	0.0a	11.2c	4.8b									
		10		0.0a	0.0a	0.0a	0.0a	4.2b	1.5a									

1 - 5 see Table 5.2.10.3 for definitions

Table 5.2.10.6. Quality of Eureka lemon fruit harvested on 28 June 2006 and evaluated after shelf-life.

Response variable	Interaction[1]	Storage duration (days) [A][2]				Storage temperature (°C) [B]				Prob>F[3]		
		10	40	60	80	-0.5	4.5	7.5	10	A	B	AxB
Peteca spot (%)	-0.5	-	4.8a	0.0a	0.0a					***	NS	*
	4.5	-	9.9c	0.0a	0.0a							
	7.5	-	9.2b	0.0a	0.0a							
	10	-	3.3a	0.0a	0.0a							
Chilling injury (%)	-0.5	-	25.4c	100.0d	100.0d					***	***	***
	4.5	-	0.7a	2.6a	11.8b							
	7.5	-	0.0a	0.0a	0.0a							
	10	-	0.0a	0.0a	0.0a							
Rind colour rating[4]	-0.5	-	3.0d	-[5]	-					***	***	***
	4.5	-	2.4c	2.6cd	1.1ab							
	7.5	-	1.6b	2.6cd	1.0a							
	10	-	2.5cd	2.4c	1.0a							
Decay (%)	-0.5	-	49.2d	100.0e	100.0e					***	***	***
	4.5	-	6.4ab	16.4c	10.7bc							
	7.5	-	1.5a	12.8bc	2.9a							
	10	-	0.6a	3.0a	1.5a							
Black pit (%)	-0.5	-	0.0a	0.0a	0.0a					***	***	***
	4.5	-	0.0a	12.8cd	15.9de							
	7.5	-	0.0a	8.4bc	19.3e							
	10	-	0.0a	2.7ab	2.0a							

1 – 5 see Table 5.2.10.3 for definitions

Table 5.2.10.7. Harvest maturity of Eureka lemon fruit sampled on 28 July 2006

Rind colour rating[1]	TSS (°Brix)	Titratable acidity
1.5	6.4	5.8

Rind colour rating determined using the CRI rind colour chart set number 37, where 8 = poor coloured, dark green with no colour break, and 1= yellow and fully coloured fruit (CRI, 2004)

Table 5.2.10.8. Quality of Eureka lemon fruit harvested on 28 July 2006 and evaluated before shelf-life

Response variable	Inter-action[1]	Storage duration (days) [A][2]						Storage temperature (°C) [B]				Prob>F[3]		
		4	10	20	40	60	80	-0.5	4.5	7.5	10	A	B	AxB
Peteca spot (%)	-0.5	0.6a	0.8a	0.6a	2.3a	0.0a	0.0a					***	**	***
	4.5	0.0a	0.0a	0.0a	0.6a	0.0a	0.0a							
	7.5	0.0a	1.5a	7.2b	10.9c	0.0a	0.0a							
	10	0.0a	0.7a	0.0a	7.7bc	0.0a	0.0a							
Chilling injury (%)	-0.5	0.0a	1.7a	0.0a	32.2c	48.5d	100.0e					***	***	***
	4.5	0.0a	0.0a	0.0a	0.0a	1.7a	9.4b							
	7.5	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a							
	10	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a							
Rind colour rating[4]	-0.5	1.7b	2.9f	3.1h	3.1h	2.2cd	2.3cde					***	***	***
	4.5	2.0bc	2.4de	2.5def	2.5def	1.0a	1.0a							
	7.5	1.7b	2.4cde	3.0gh	2.7ef	1.0a	1.0a							
	10	2.0bc	2.2cd	2.6def	2.2cd	1.0a	1.0a							
Decay (%)	-0.5	0.0a	0.0a	0.0a	0.0a	0.5a	0.0a					***	NS	*
	4.5	0.0a	0.0a	0.0a	1.6a	0.7a	20.4d							
	7.5	0.0a	0.0a	0.0a	4.9ab	5.5abc	6.4abc							
	10	0.0a	0.0a	0.5a	1.8a	10.9bc	13.2cd							
Black pit (%)	-0.5	0.0a	0.0a	0.0a	0.0a	3.0ab	0.0a					***	***	***
	4.5	0.0a	0.0a	0.0a	0.0a	16.5f	12.9ef							
	7.5	0.0a	0.0a	0.0a	0.0a	9.4de	8.8cde							
	10	0.0a	0.0a	0.0a	0.0a	4.6bcd	6.2bcde							

Table 5.2.10.9. Quality of Eureka lemon fruit harvested on 28 July 2006 and evaluated after shelf-life.

Response variable	Interaction[1]	Storage duration (days) [A][2]				Storage temperature (°C) [B]				Prob>F[3]		
		10	40	60	80	-0.5	4.5	7.5	10	A	B	AxB
Peteca spot (%)	-0.5	3.8a	2.3a	0.0a	0.0a					***	NS	*
	4.5	1.5a	8.8b	0.0a	0.0a							
	7.5	1.2a	13.4b	0.0a	0.0a							
	10	1.9a	9.0b	0.0a	0.0a							
Chilling injury (%)	-0.5	0.0a	40.8b	100.0e	100.0e					***	***	***
	4.5	0.0a	0.6a	25.7b	55.0d							
	7.5	0.0a	0.0a	0.0a	0.0a							
	10	0.0a	0.0a	0.0a	0.0a							
Rind colour rating[4]	-0.5	2.4cd	3.2f	-[5]	-					***	***	***
	4.5	2.7de	2.6de	1.0a	2.0c							
	7.5	1.6b	2.5d	1.0a	2.0c							
	10	3.0ef	2.8def	1.0a	1.0a							
Decay (%)	-0.5	0.0a	0.0a	100.0e	100.0e					***	***	***
	4.5	0.0a	13.5c	13.0c	55.6d							
	7.5	0.0a	5.3abc	10.6bc	2.6ab							
	10	0.7a	0.0a	7.7abc	4.0ab							
Black pit (%)	-0.5	0.0a	0.0a	0.0a	0.0a					***	***	***
	4.5	0.0a	0.0a	22.3c	20.6c							
	7.5	0.0a	0.0a	9.9b	10.2b							
	10	0.0a	0.0a	8.2b	7.8b							

1 – 5 see Table 5.2.10.3 for definitions

5.2.11 Effect of changes in relative humidity and rind water status during handling of Eureka lemon on the development of peteca spot

Experiment EX02-06 by Parton Khumalo, Arrie de Kock and Jerome Davids (Experico)

Opsomming

Eureka suurlemoene met 'n gevordere skilkleur (kleur plaat 4.6). is laat in die seisoen geoes Die vrugte is blootgestel aan verskeie ontgroeningsbehandelings, met en sonder etileen, waarna dit gewaks en by 10°C gestoor is. Die vrugte is na 4, 10, 20 en 60 dae onderskeidelik ge-evalueer. Oor die algemeen was daar lae vlakke van Peteka waargeneem, waarskynlik as gevolg van die laat seisoen vrugte teen optimum oesrypheid. Die ontwikkeling van Peteka was nie betekenisvol beïnvloed deur die verskillende na-oes-behandelings nie. Dit dui daarop dat 'n kort blootstellingstyd aan 'n lae humiditeit na oes, waarskynlik nie genoeg is om Peteka te induseer in vrugte wat by optimum rypheid geoes is nie. Daar was marginale verskille in vogverlies tussen die behandelings.alhoewel, hoe langer die opberging, hoe groter die vogverlies. Die voorkoms van Peteka het egter nie konsekwent verhoog met langer opberging van die onderskeie behandelings nie. Die skilkleurgradering en bederf was nie betekenisvol beïnvloed deur die behandelings nie. Die ekperiment kon nie bevestig dat 'n 24 uur vertraging by 'n lae humiditeit na ontgroening, die risko vir die ontwikkling van Peteka verhoog nie. Daar word voorgestel dat die eksperiment herhaal word in 2007 met vrugte wat in terme van rypheid, vroeg. (April-Mei) geoes word. Met die doelwit om Peteka te veminder, word aanbeveel dat behandelings met addisionele humiditeits-skommelings, ingesluit word in die komende seisoen.

Introduction

In previous experiments where the development of peteca spot was evaluated in Eureka lemon fruit, it was observed that degreened fruit were more susceptible to this disorder than non-degreened fruit. The higher susceptibility to peteca spot of degreened fruit could be attributed to fluctuations in relative humidity during handling, as it has been shown that fluctuations in relative humidity during postharvest handling of Navelina Navel orange increased fruit susceptibility to rind staining (Alferez et al., 2003). Rind staining is a disorder morphologically similar to peteca spot. Hopefully, by reducing fluctuations in relative humidity during postharvest handling of Eureka lemon fruit, the development of peteca spot will be reduced.

The objective of this experiment was to establish the effect of changes in relative humidity, with or without ethylene degreening, on the development of peteca spot during storage of Eureka lemon fruit.

Materials and methods

Trial site detail
Fruit were harvested from healthy 15 year old Eureka lemon trees, grafted on rough lemon, in Franschoek (Imibala).

Treatments
Harvested fruit were wilted in harvesting bins for 24 hours and then transported to the degreening chambers where they were drenched with a fungicide mixture and again delayed for 24 hours at ambient temperature and RH before degreening. The fruit were again delayed for 24 hours after degreening. Treatments are shown in Figure 5.2.11.1. After treatment fruit were waxed at a commercial packhouse in Franshhoek and transported to Stellenbosch for storage at 10°C. Non-destructive assessment of peteca spot was conducted after 4, 10, 20 and 60 days storage. Complete evaluations were conducted on fruit stored for 10 and 60 days at 10°C, and then subjected to a shelf life period of 7 days at 20°C.

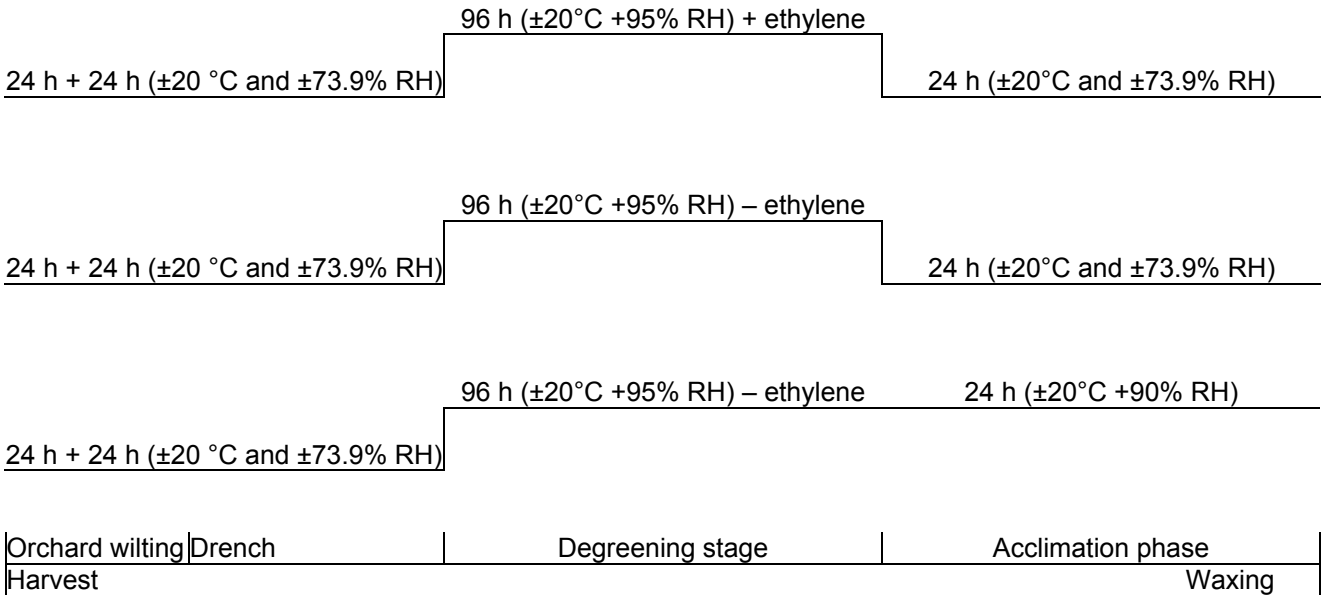


Figure 5.2.11.1: Treatment set showing the different fluctuations in humidity after degreening

Evaluation stages and parameters

At harvest (conducted on 10 fruit per replicate)
Rind colour rating, soluble solids content (SSC) [°Brix], TA (%), and rind moisture content (%).

Before and after shelf life
Peteca spot incidence (%), moisture loss (%), decay (%), black pit (%) and rind colour rating (scale of 1-8).

Statistical detail
The trial was laid out a complete randomised design with 5 replicates. A single carton containing ±50 fruit comprised a replicate. Data collected from the non-destructive evaluations are presented without any statistical analysis. Data collected after 10 and 60 days storage were analysed as a one-way ANOVA using STATISTICA. Data from each storage duration were analysed separately.

Results and discussion

At harvest
Fruit used in this experiment were harvested late in the season (June), with rind colour rating of 4.6 (Table 5.2.11.1).

Peteca spot incidence and moisture loss over time in cold storage
Generally, the levels of peteca spot were low and did not show major differences between treatments (Table 5.2.11.2). The reason for this could be that fruit were harvested late in the season, when the colour was advanced and at optimum maturity, and consequently were not inherently susceptible to peteca spot. Peteca spot incidence did not show a consistent trend of increasing or decreasing over time. There was a definite increase in moisture loss over time across treatments (Table 5.2.11.3). Marginal differences in moisture loss occurred between treatments. Overall, the results suggest that degreening with or without ethylene and 24 hours exposure of fruit to low relative humidity during handling may not accentuate the development of peteca spot in Eureka lemon fruit.

Quality for fruit stored for 10 days plus a shelf-life period,
No significant differences were observed between treatments in the development of peteca spot, decay and rind colour (Table 5.2.11.4).

Quality for fruit stored for 60 days plus a shelf-life period
No significant differences were observed between treatments in the development of peteca spot, black pit, decay and rind colour (Table 5.2.11.5).

Overall, It could not be demonstrated that fluctuations in relative humidity during postharvest handling and storage of Eureka lemon fruit accentuate the development of peteca spot. The reasons for this result could be that, inherently the fruit used in this experiment was not susceptible to peteca spot because it was at optimum maturity, thereby confounding treatments differences. It is also possible that the humidity fluctuation, of 24 hours, employed in this experiment was not long enough to induce peteca spot. This result may suggest that a short fluctuation in relative humidity during postharvest handling of Eureka lemon fruit harvested at optimum maturity may not aggravate the development of peteca spot. Therefore, the current postharvest handling protocols, if adhered too, may not be the cause for peteca spot. There is, however, a need to verify this result and further investigate the underlying causes for peteca spot.

Table 5.2.11.1. Harvest maturity of Eureka lemon fruit

Rind colour rating[1]	TSS (°Brix)	Titratable acidity	Rind moisture content (%)
4.6	6.8	5.3	85.4

[1]Rind colour rating determined using the CRI rind colour chart set number 37, where 8 = poor coloured, dark green with no colour break, and 1= yellow and fully coloured fruit (CRI, 2004)

Table 5.2.11.2. Peteca spot development over time in Eureka lemon fruit exposed to different degreening treatments with or without ethylene and evaluated after different cold storage periods

Treatment[1]	Peteca spot (%) at different storage durations (days)			
	4	10	20	60
T1 (+ethylene)	0.0	3.6	1.3	0.0
T2 (-ethylene)	0.0	0.0	1.7	0.0
T3 (- ethylene & no RH change)	0.0	1.6	1.0	0.0

[1]T1 = 96 hours degreening with ethylene at high RH followed by 24 hours delay at ambient temperature and RH, T2 = 96 hour degreening without ethylene followed by 24 hours delay at ambient temperature and RH, T3 = 96 hours degreening without ethylene followed by waxing and storage without delay at low humidity

Table 5.2.11.3. Moisture loss over time in Eureka lemon fruit exposed to different degreening treatments with or without ethylene and evaluated after different cold storage periods

Treatment[1]	Moisture loss (%) at different storage durations (days)			
	4	10	20	60
T1 (+ethylene)	0.9	1.9	2.9	6.5
T2 (-ethylene)	0.9	1.0	2.5	5.8
T3 (- ethylene & no RH change)	1.1	1.2	1.9	4.5

[1]See Table 5.2.11.2 for definition

Table 5.2.11.4. Quality of Eureka lemon fruit exposed to different degreening treatments with or without ethylene, and stored for 10 days at 10°C plus an additional 7 days at 20°C

Response variable	Treatment[1]			Prob>F[2]
	T1 (+ethylene)	T2 (-ethylene)	T3 (-ethylene & no RH change)	
Peteca spot (%)	3.6	0.0	1.6	NS
Decay (%)	0.0	0.0	1.1	NS

Rind colour rating[3]	2.6	2.7	2.5	NS

[1]See Table 5.2.11.2 for definition
[2]One-way ANOVA where NS = no significant differences P=0.05
[3]See Table 5.2.11.1 for definition

Table 5.2.11.5. Quality of Eureka lemon fruit to different degreening treatments with or without ethylene and stored for 60 days at 10°C plus an additional 7 days at 20°C

Response variable	**Treatment[1]**			**Prob>F[2]**
	T1 (+ethylene)	**T2 (-ethylene)**	**T3 (-ethylene & no RH change)**	
Peteca spot (%)	0.0	0.0	0.0	NS
Black pit (%)	3.4	2.8	2.1	NS
Decay (%)	2.8	1.2	1.9	NS
Rind colour rating[3]	2.5	2.4	2.3	NS

[1 - 3]See Table 5.2.11.4 for definitions

Conclusions

The presence or absence of ethylene during degreening was not a significant factor in the development of peteca spot. Fruit exposure to a low humidity (±73.9%) for 24 hours after degreening may not have been long enough to accentuate the development of peteca spot compared to fruit exposed to a low humidity for a shorter duration, only during waxing. Moisture loss on fruit increased over time in storage, but the incidence of peteca spot did not always show a corresponding increase across treatments. No commercial recommendation can be made at this stage. However, it is suggested that the experiment be repeated in 2007 to verify results. Furthermore, the experiment should be conducted on early harvested fruit and there should be minor adjustments in the treatment set, to reduce humidity fluctuations and reduce the development of peteca spot.

Reference cited

Alférez, F., Agusti, M., Zacarías, L., 2003. Postharvest rind staining in Navel oranges is aggravated by changes in the storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143-152.

5.2.12 **Effect of fruit wilting and rind water status on the development of Peteca spot in Eureka lemon fruit**

Experiment EX03-06 by Parton Khumalo, Arrie de Kock and Jerome Davids (Experico)

Opsomming

Eureka suurlemoene is laat in die seisoen in ’n boord in Franschhoek ge-oes. Die vrugte is verwelk vir 24, 48 en 96 uur voor ontgroening. Die vrugte was na ontgroening weer verwelk vir 24 of 96 uur. Hierna is die vrugte gewaks en opgeberg by 10°C vir 4, 10, 20 en 60 dae onderskeidelik. Dit wil voorkom asof die risiko vir Peteka hoër is by vrugte wat langer as 24 uur verwelk is voor ontgroening. Die verwelkingsperiode na ontgroening was egter nie ’n betekenisvolle faktor in die ontwikkeling van Peteka nie. Die ontwikkeling van Peteka oor opbergingstyd was nie konstant meer of minder nie. Skilkleur was in sommige gevalle betekenisvol beïnvloed deur die behandelings maar hierdie effek het nie ’n vaste patroon gevolg nie. Bederfvlakke was laag in hierdie populasie vrugte en was nie betekenisvol beïnvloed deur verwelking voor of na ontgroening nie. Die gevolgtrekking is dus dat suurlemoene nie vir langer as 24 uur verwelk moet word voor ontgroening nie aangesien dit die risiko vir Peteka ontwikkeling verhoog. Die eksperiment behoort in 2007 herhaal te word om die resultate te bevestig. Dit is ook belangrik dat die vrugte in 2007 vroeg ge-oes moet word in terme van rypheid (April-Mei). Geen kommersieële aanbeveling kan in hierdie stadium gemaak word nie.

Introduction

In previous experiments where the development of peteca spot was evaluated in Eureka lemon fruit, it was observed that degreened fruit were more susceptible to this disorder than non degreened fruit. The reason for the higher susceptibility to peteca spot of degreened fruit could be attributed to fluctuations in relative humidity during handling, as it has been shown that fluctuations in relative humidity during postharvest handling of Navelina Navel orange increased fruit susceptibility to rind staining (Alferez et al., 2003). Rind staining is a disorder morphologically similar to peteca spot. However, Ben-Yehoshua (1987) showed that citrus fruit dehydration reduced rind water and turgor, which resulted in fruit softening, but no rind staining, suggesting that fruit wilting may "cure" the fruit, resulting in reduced susceptibility to certain rind disorders. However, this concept has not been tested on Eureka lemon fruit for the development of peteca spot.

This objective of this experiment was to establish the effect of fruit wilting on the development of peteca spot on Eureka lemon fruit.

Materials and methods

Trial site detail
Fruit were harvested from healthy 15 year old Eureka lemon trees, grafted on rough lemon, in Franschoek (Imibala).

Treatments
Fruit were harvested into 15 kg lug bins. The harvested fruit were wilted at ambient temperature and RH for 24, 48, and 96 hours before degreening, and again wilted for 24 or 96 h after degreening (Figure 5.2.12.1).

Fruit were then waxed at a commercial packhouse in Franshhoek and stored in Stellenbosch at 10°C. Non-destructive assessment of peteca spot was conducted after 4, 10, 20 and 60 days storage. These data are presented without any statistical analysis. Complete evaluations were conducted on fruit stored for 10 and 60 days at 10°C, and then subjected to a shelf life period of 7 days at 20°C.

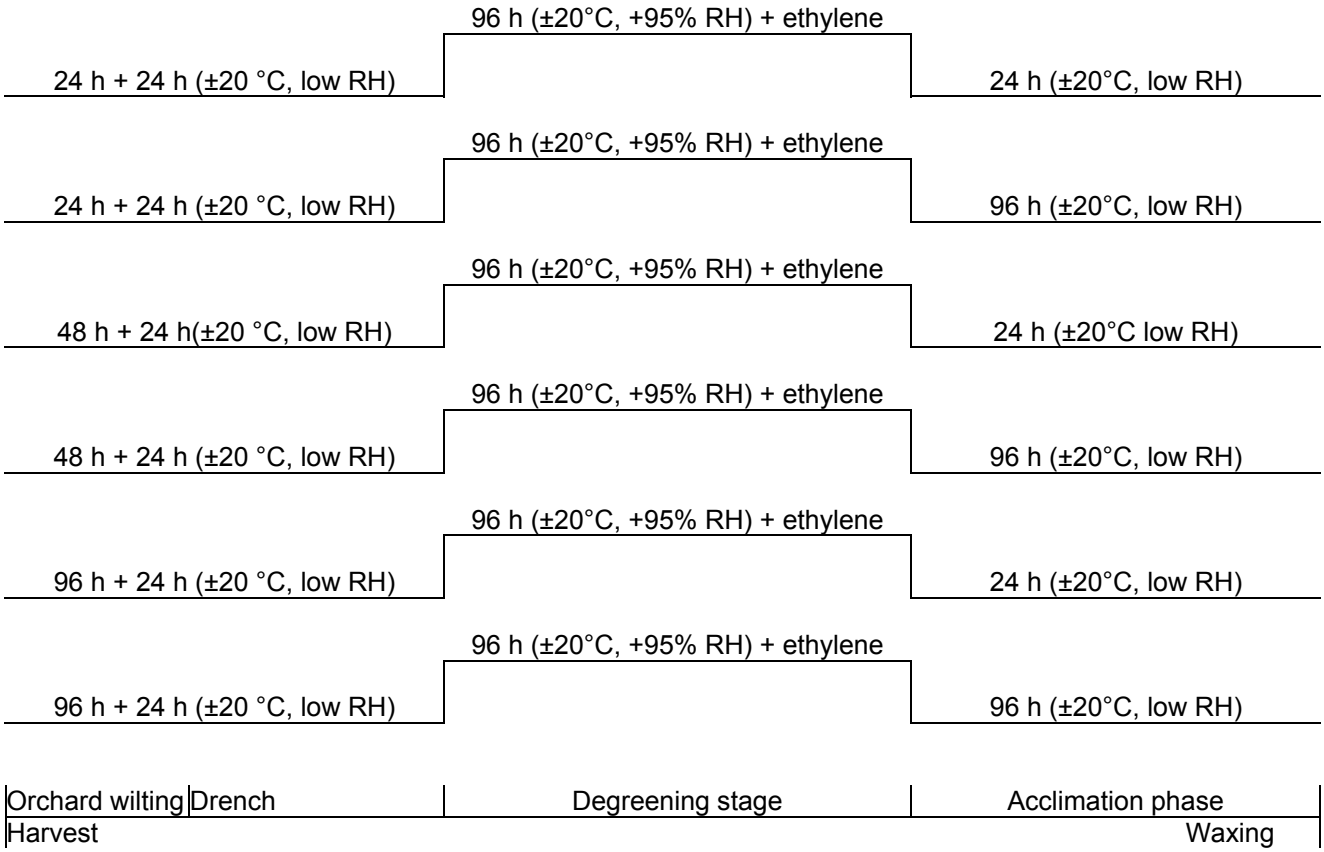


Figure 5.2.12.1: Treatment set showing the different wilting durations before and after degreening

Evaluation stages and parameters

At harvest (conducted on 10 fruit per replicate)
Rind colour, soluble solids content (SSC) [°Brix], TA (%), and rind moisture content (%).

Before and after shelf life
Peteca spot incidence (%), decay (%), black pit (%) and rind colour rating (scale 1-8).

Statistical detail
The trial was laid out a complete randomised design with 5 replicates. Data collected from the non-destructive evaluations is presented without any statistical analysis. Data collected after 10 and 60 days storage was analysed as a two-way ANOVA using STATISTICA, where Factor A = wilting duration before degreening and Factor B = wilting duration after degreening. Data from each storage duration were analysed separately.

Results and discussion

At harvest
Fruit used in this experiment were harvested late in the season (June), with rind colour rating of 4.6 (Table 5.2.12.1).

Peteca spot evaluations over time in cold storage
The peteca spot results over time were purely observational. The observed trend across treatments was that fruit wilted for 24 h before degreening (T1 and T2), irrespective of the wilting time after degreening, tended to exhibit lower levels of peteca spot compared to fruit wilted for longer durations before degreening (Table 5.2.12.2). This trend was consistent over 4, 10 and 20 days storage. The incidence of peteca spot did not show a consistent trend of increasing or decreasing over time in storage. However, in fruit stored for 60 days no peteca spot was observed after cold storage. This suggested disorder may not be progressive in storage, as shown in previous research on peteca spot. It also remains possible that in fruit stored for 60 days, peteca spot may have developed earlier during the storage life of the fruit, but was over time masked, consequently not seen, by the presence of other disorders (decay and black pit), which are reported elsewhere.

Quality after shelf-life, for fruit stored for 10 days plus a shelf life period
The incidence of peteca spot was significantly affected by wilting duration before degreening (Table 5.2.12.3). Fruit wilted for 24 h before degreening had significantly lower levels of peteca spot than fruit wilted for a longer period of 96 h (Table 5.2.12.3). Wilting duration after degreening did not have a significant effect on the development of peteca spot. Wilting duration before degreening did not have a significant effect on rind colour, however, wilting for 96 h after degreening resulted in a marginal, but significantly lower rind colour rating than fruit wilted for a shorter duration. Decay was not affected by wilting duration before or after degreening.

Quality after shelf-life, for fruit stored for 60 days plus a shelf life period
No peteca spot was observed on fruit after extended storage and shelf life (Table 5.2.12.4). This was possibly because the disorder is not progressive in storage. Therefore, if it did not develop earlier in the storage life of the fruit, it is unlikely to develop at a later stage during storage. It is also possible that some peteca spot may have developed earlier during the storage life, but due to the presence of black pit and decay, the peteca spot symptom was masked. Wilting duration before degreening was a significant factor in the development of black pit, a bacterial disease caused by *Pseudomonas syringae* and manifests as brown-black sunken spots on the fruit rind (Figure 5.2.12.2). The incidence of black pit increased with increasing wilting duration before degreening. The highest levels of the disorder occurred in fruit wilted for 96 hours, compared to the other treatments. The wilting duration after degreening did not have a significant effect on the development of black pit. A significant interaction occurred between wilting duration before degreening and wilting duration after degreening on the rind colour rating of fruit. However, no definite trend could be established. Decay was not affected by wilting duration before or after degreening.

Research conducted on rind staining of ‘Navelina Navel’ orange showed that fruit exposure to dehydrating conditions results in a reduced water potential of flavedo and albedo cells (Alferez et al., 2003). Upon exposure to a high relative humidity, flavedo cells were able to recover, but albedo cells could not, resulting in rind staining. It seems that peteca spot develops in a similar pattern. It cannot be confirmed, but remains a possibility that extended wilting for up to and exceeding 48 h before degreening, reduces water potential of the flavedo and albedo cells. Upon fruit exposure to high humidity during degreening, albedo cells in fruit wilted for longer duration are unable to recover, resulting in higher levels of peteca spot in fruit than fruit wilted for a shorter duration, where the abledo and flavedo cells are able to recover. There is a need to

confirm these results using early harvested fruit, which is more susceptible to peteca spot. Furthermore, in-depth attention should be given to rind water and changes thereof, prior to and after degreening.

Table 5.2.12.1. Harvest maturity of Eureka lemon fruit

Rind colour rating[1]	TSS (°Brix)	Titratable acidity	Rind moisture content (%)
4.6	6.8	5.3	85.4

[1]Rind colour rating determined using the CRI rind colour chart set number 37, where 8 = poor coloured, dark green with no colour break, and 1= yellow and fully coloured fruit (CRI, 2004)

Table 5.2.12.2. Peteca spot development over time in Eureka lemon fruit wilted for different durations before and after degreening and evaluated after different cold storage periods

Treatment	Peteca spot (%) at different storage durations (days)			
	4	10	20	60
T1 (24h + 24h)[1]	0.6	0.6	1.7	0.0
T2 (24h + 96h)	1.0	0.0	0.0	0.0
T3 (48h + 24h)	2.4	1.4	3.6	0.0
T4 (48h + 96h)	2.5	4.5	3.6	0.0
T5 (96h + 24h)	3.6	3.5	5.0	0.0
T6 (96h + 96h)	2.5	5.2	5.0	0.0

[1]The wilting duration before and after degreening is shown in parentheses

Table 5.2.12.3. Quality of Eureka lemon fruit, wilted for different durations before and after degreening and stored for 10 days at 10°C plus an additional 7 days at 20°C

Parameter	Wilting duration before degreening[1] (h) Factor [A]			Wilting duration after degreening (h) Factor [B]		Prob>F[2]		
	24	48	96	24	96	A	B	AxB
Peteca (%)	1.2a	4.1b	4.4b	2.6	3.9	*	NS	NS
Rind colour rating[3]	2.4	2.4	2.2	2.4b	2.2a	NS	**	NS
Decay (%)	0.3	0.0	0.0	0.2	0.0	NS	NS	NS

[1]Values in the same row followed by different letters indicate significant differences P<0.05.
[2]wo-way ANOVA with Factor A = wilting duration before degreening and Factor B = wilting duration after degreening
[3]Rind colour rating determined using the CRI rind colour chart set number 37, where 8 = poor coloured, dark green with no colour break, and 1= yellow and fully coloured fruit (CRI, 2004)

Table 5.2.12.4. Quality of Eureka lemon fruit, wilted for different durations before and after degreening and stored for 60 days at 10°C plus an additional 7 days at 20°C

Parameter	Interaction (h)[1]	Wilting duration before degreening[2] (h) Factor [A]			Wilting duration after degreening (h) Factor [B]		Prob>F[3]		
		24	48	96	24	96	A	B	AxB
Peteca (%)		0.0	0.0	0.0	0.0	0.0	NS	NS	NS
Black pit (%)		1.7a	5.9ab	10.4b	5.8	6.1	*	NS	NS
Rind colour rating[4]	24	2.0a	2.9bc	2.2a			**	***	**
	96	2.6b	2.9bc	3.1c					
Decay (%)		2.2	0.7	2.0	1.4	1.9	NS	NS	NS

[1]If itemised interaction occurred between Factor A and Factor B
[2]Values in the same row followed by different letters indicate significant differences P<0.05.
[3]Two-way ANOVA with Factor A = wilting duration before degreening and Factor B = wilting duration after degreening
[4]Rind colour rating determined using the CRI rind colour chart set number 37, where 8 = poor coloured, dark green with no colour break, and 1= yellow and fully coloured fruit (CRI, 2004).

Fig. 5.2.12.2. Black pit symptom observed in fruit evaluated after storage for 60 days

Conclusions

Harvested Eureka lemon fruit should not be wilted for longer than 24 hours before degreening, as this aggravates the incidence of peteca spot and black pit after storage. Fruit wilting after degreening does not seem to be a major factor in the development of peteca spot. The levels of peteca spot do not seem to increase over time in storage, as shown in previous trials. No commercial recommendation can be made at this stage. It is suggested that the experiment be repeated in 2007.

Reference cited

Alférez, F., Agusti, M., Zacarías, L., 2003. Postharvest rind staining in Navel oranges is aggravated by changes in the storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143-152.

5.2.13 Effect of different preharvest chemical treatments and wax applications on development of Peteca spot in lemons

Experiment 833 by Paul Cronje (CRI at SU)

Opsomming

Peteka kol van suurlemoen maak elke jaar in al die suider Afrikaanse sitrus produksie areas 'n groot negatiewe impak. Daar is ongelukkig nie net 'n tekort aan die basiese fisiologiese inligting van die defek nie maar ook in maatreëls waarmee dit beperk kan word. In die 2006 seisoen is daar voor-oes chemikalie op die bome gespuit asook na-oes waks toedienings gedoen om die effek op peteka te bepaal. Die wakse het gewissel van 10 tot 24% vaste stowwe. Ongelukkig het daar in die hele proef geen peteka ontwikkel nie. Dieselfde studie asook nuwe chemikalie en strategie sal in 2007 verder ondersoek word.

Introduction

Peteca spot, the rind pitting of lemons, also called "rumple" (Knorr, 1963) was identified as early as 1917 in the USA lemon industry (Coit, 1917). This disorder can affect over mature fruit on the tree but mostly develops 3 to 4 days after fruit has been packed (Oberbacher and Knorr, 1965; Khalidy et al., 1969; Offers, 1987). In Italy, fruit hanging on the east side of trees, as well as large, mature fruit were found to be more susceptible than small, immature fruit (Salerno, 1963; Knorr and Koo, 1969; Grierson, 1981). Mineral nutrition, particularly relating to Ca and B metabolism, and insufficient irrigation during the critical growth period have been identified by Khalidy et al. (1969) to play a role in the development of the disorder. Peteca spot is thought of as a postharvest disorder but is most probably the result of sub-optimal production and environmental factors that could result in high occurrence.

Due to the impact of this disorder every year in all southern African production areas, research to investigate the effect of different aspects of preharvest conditions and postharvest handling is being conducted. In the first part of the project the aim was to test a variety of agro-chemicals with different modes of action for efficacy in decreasing peteca spot incidence. Secondly, the effect of high and low solid content in waxes will be studied.

Materials and methods

Ten year old Eureka lemon trees in the Stellenbosch area received various whole tree spray treatments on 6 April, two weeks prior to commercial harvest. Fruit were harvested on 20 April. Each treatment consisted of ten replications of ten fruit each. Fruit were waxed with a high 24% solid wax due to reports that high solid wax aggravates peteca spot. The fruit were not cooled and were stored at ambient temperature (20°C) for two weeks. Evaluation for peteca spot was done after each week.

The spray treatments were calcium nitrate at 675 ml/100 ℓ H_2O, Rynox (2.5 ℓ/100 ℓ H_2O), LGE (200 ppm) and Gibberellic acid (10 ppm) and water as a control. The ten trees per treatment were sprayed with 10 litres each. The calcium nitrate was applied to increase the Ca-content in the flavedo as early reports suggested a Ca-deficiency could be responsible and calcium nitrate is the most widely used foliar applied source of calcium. Raynox is a new carnauba based spray used in deciduous fruit to prevent sunburn and was applied to decrease the water loss from the flavedo during the harvest process which could cause tensional stress on the cellular structure. LPE (lysophatidylethanolamine) is a natural lipid reported to enhance colour development and shelf life of cranberries and tomato (Ozgen *et al.*, 2005). The Gibberellic acid was applied because the plant growth regulator is known to decrease the tempo of citrus rind maturity.

Results and discussion

No peteca spots developed in any of the treatments or in the control. The orchard was selected due to very high incidence of peteca spot in the previous two seasons. The treatments will be repeated in 2007 as well as new treatments in a different production area.

Conclusions

It is very important that the fruit do not stand in an area with inadequate ventilation e.g. in a degreening room or shed without air flow between bins and fruit. To test peteca spot sensitivity, sample fruit from orchards 10-7 days before planned harvest date. Store fruit in closed, transparent polyethylene bags at ambient temperature and inspect regularly for spot development (inspect through the bag without opening) and delay the harvest if high incidence is seen.

The variable incidence of this disorder complicates the research and has led to the lack of preventative strategies, but the use of high CO_2 concentrations may indicate whether work on the outlined treatments will be useful in finding solutions. These results once agian illustrate that immature lemon fruit are highly prone to development of peteca spot and should not be harvested.

Future research

During a visit to the Simondium Stellenpack packhouse the technical managers brought to CRI's attention that if they kept their lemon retention samples in plastic bags they developed peteca spot more frequently and at a much quicker rate. Following this information the researcher did a pilot study (not statistically laid out) with fruit from the Simondium area that was picked and put under constant high CO_2 (5%) and ethylene (5 ppm) conditions with a flow through special gas mixture at the Horticultural department of SU, for 14 days. The high CO_2 treatment resulted in very high incidence and fruit in the treatment had the most severe peteca spot development that the researcher has ever seen. The fruit from ethylene treatments had highly developed yellow colour and loose calyces but not no peteca spot. The control fruit also did not have any peteca spot. The experiment was repeated 16 days later with fruit from the same orchard. A statistical layout was done but after 14 days of gas application not one fruit showed a peteca spot. Resulting from this a more extensive statistical trial is envisaged.

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5.2.14 **The effect of different citrus wax applications on the development of peteca spot on lemons**
Experiment 795 by K.H.Lesar (CRI)

Opsomming

Waks met verskeie vastestowwe inhoud is op suurlemoene aangewend, on vas te stel of die behandelings Peteca kan beinvloed. Vrugte is ook rof behandel, en in warm water gedoop. Ongelukkig, het geen simtome van Peteca voorgekom, en dus kan geen gevolgtrekkings gemaak word.

Introduction

Peteca spot is a physiological disorder that appears as deep pits on the peel surface of lemons, usually after packing. Peteca is particularly prevalent when the trees undergo water stress alternating with periods of freely available water in the period two to three months prior to harvest. Other cultural practices, that have been reported to increase the incidence of peteca spot, are late heavy pruning practices, late application of Nitrogen and late oil sprays.

Erratic environmental conditions appear to play a major role in predisposing lemons to the development of peteca, i.e. sudden changes from long periods of hot dry conditions followed by colder weather.

Peteca seems to be more prevalent after the harvesting of lemons during cold, moist or wet conditions.

The rough handling of lemons, especially the more sensitive greener fruit, during picking, transport to the packhouse and operational processes in the packhouse, also seem to predispose the fruit to the development of peteca. The operational processes in the packhouse that have triggered the development of peteca spot on lemons are the rough handling of the fruit, as already mentioned, over brushing and too high brush speeds, too high a temperature in the hot water fungicide bath and in the drying tunnel, and most importantly the waxing of the fruit.

Waxing of the fruit is one of the major critical control processes in the packhouse. Choosing the right wax for lemons, specifically peteca-prone lemons, is critical. It has been reported that the use of heavy waxes should be avoided.

Polyethylene waxes with high solid levels (18% and higher) and/or increased shellac or wood resin levels are classified as heavy waxes. The natural waxes i.e. Carnauba waxes with lower solid levels (16% and lower) and with not too high shellac levels or without shellac are classified as lighter waxes and are reported to be the preferred waxes for peteca-prone lemons.

The application rate of the wax used for lemons is also vitally important. Even though a light wax may be used for peteca-prone lemons, the over application thereof could also induce the development of peteca. The slight under application, but a good uniform coverage of a light wax is by far the desired application of a light wax to lemons to reduce the risk of peteca development. However the slight under application, but erratic non-uniform application of a light wax could also predispose the fruit to loss of quality and cold damage during shipping.

In this trial early season peteca-prone green lemons from Larten (Karino) were harvested early in the morning during cold moist conditions, which are typical conditions conducive to the development of peteca spot. These lemons were treated with Carnauba waxes with different solid levels to determine the effect of these waxes on the possible development of peteca spot after the cold disinfestation (sterilisation) treatment.

Materials and methods

Fifteen lug boxes of green to colour break (T7-T6) lemons were harvested at Larten, Karino and transported to CRI Nelspruit during the last week in March 2006. These lemons were harvested from the same orchards where a high incidence of peteca spot was experienced in the 2004 and to a lesser extent during the 2005 season.

The lemons were treated on the packline by first washing the fruit in the high pressure spray with the quaternary ammonium compound Prasin. The lemons were then exposed to a temperature of 40°C in the hot water bath for 1-2 minutes and then dried in the packline drying unit. A temperature of 40°C is not recommended as being ideal for green to colour break lemons with sensitive rinds. All the packline treated lemons were roughly handled during dumping prior to washing and also after drying of the fruit.

After drying the lemons were divided up into 6 replicates x 20 fruit each per treatment.

The lemons were then waxed, by means of a dip treatment, with the following FMC citrus waxes.

1. PC control Full Strength 17-18% total solids
2. Carnuba Tropical 14% total solids
3. Carnauba Tropical 10% total solids
4. Carnauba Tropical 23% total solids

The waxed fruit was left at ambient temperature to dry overnight. The fruit was stored the following day under simulated cold disinfestation conditions at -0.6° C for 22 days + 7 days at at 20°C. After this storage regime no peteca spot symptoms were detected on the lemons.

The treatments were then stored for a further 6 weeks at 2°C to possibly induce the development of peteca spot symptoms. After simulated cold disinlifestation the fruit stood at ambient (20°C) for 7 days and was then evaluated for any peteca spot symptoms.

Results

No lesion development was observed on the lemons after cold disinfestation.

The lemons were stored for a further 6 weeks thereafter at 2°C to possibly induce peteca spot/CI development.

Still no symptoms were observed after extended storage and thus there were no results in this trial.

Conclusion

There are still far too many unknowns, both pre- and post-harvest, that influence the development of peteca spot on lemons. The occurrence of peteca spot on lemons over the last 10 years has been very erratic and this has resulted in inconsistent research being conducted on this disorder.

Future research

While the erratic prevalence of the disorder creates difficulties, research will continue and these trials will be repeated.

5.2.15 Measuring CO_2 and temperature during a citrus shipment to the USA

Experiment 759 by Paul Cronjè (CRI at SU)

Summary

On the 12 and 13 of June 2006 a data logger to measure CO_2 concentration and temperature sensors were installed in a hold of a ship sailing to the USA. The fruit that were loaded into the hold were the last 'Nules' Clementines of the season and therefore thought to be more prone to physiological defects. The installation and retrieval of the equipment from the ship went according to plan but due to an unforeseen problem no data were recovered. This disappointing result resulted in a critical evaluation of the equipment and the lack of an independent power supply was identified as a critical aspect. This problem has received attention but no satisfactory solution has been found. Once the problem has been solved this line of research will continue.

Opsomming

Gedurende 12 en 13 Junie 2006 is daar verskeie temperatuur sensors asook 'n CO_2 analiseerder geïnstalleer in die vrag van 'n skip oppad na die VSA. Die vrugte wat in die vragruim gelaai was, was die laaste versending 'Nules' Clementine mandaryne en dus vermoedelik meer sensitief vir fisiologiese defekte. Die installering en terugbring van die toerusting uit die VSA het volgens plan verloop maar ongelukkig kon geen data van die datalogger ontrek word nie. Die teleurstellende resultaat het gelei na 'n kritiese her-evaluasie van die toerusting en die gebrek aan 'n onafhanklike kragtoevoer was as problematies geïdentifiseer. Daar is aandag aan die aspek gegee maar geen permanente oplossing kon gevind word nie. Na die probleem suksesvol aangespreek word sal die navorsing rigting hervat.

5.3 PROJECT: FRUIT QUALITY ENHANCEMENT

Project Co-ordinator: Stephan Verreynne (CRI at SU)

5.3.1 Project summary

The fruit quality enhancement project consists of three studies on fruit colour development and a study on the reduction of fruit acidity.

In Section 5.3.2, a study was conducted to determine the concentration of various gibberellin biosynthesis inhibitors required to get a biological response in citrus trees. Repeated applications of Regalis® (10% v/v Prohexadione-calcium) on Eureka lemon at various concentrations (1, 2, 4 and 8 $g \cdot L^{-1}$) as well as Sunny® (5% v/v uniconazole) (at 10 and 20 $mL \cdot L^{-1}$) and Cultar® (25% v/v paclobutrazol) (at 10 $mL \cdot L^{-1}$) had no effect on the rootstock or scion diameters 8 months after the first application. Both the 4 and 8 $g \cdot L^{-1}$ Regalis® treatments, both Sunny® treatments and the Cultar® treatment significantly reduced shoot growth, by reducing internode length.

Methods to improve preharvest rind colour by manipulating vegetative vigour were investigated by applying Prohexadione-calcium (ProCa; Regalis®) to different cultivars (5.3.3). It was shown that foliar application of ProCa at a concentration of 400 $mg \cdot L^{-1}$ applied 6 plus 3 weeks before anticipated harvest has the potential to increase preharvest rind colour of early-maturing citrus cultivars.

In order to improve colour of physiologically mature citrus fruit, a preharvest as well as a postharvest approach was taken in Section 5.3.4. As preharvest approach the effect of foliar applications of molybdenum (Mo) and tungsten (W) on rind colour of early maturing Valencia, Turkey and Navel fruit was investigated, while, as postharvest approach, fruit were exposed to cold and hot temperatures in order to evaluate the effect of cold and heat-shock on rind colour development. Results show that exposing these three citrus types to cold followed by heat-shock significantly enhances degreening of fruit compared to untreated fruit and those subjected to cold-shock alone, but that the physiological response is variety-dependent. Hot and cold water treatment accelerate colour change of Valencia, Turkey and Navel fruit, probably due to the "stressfulness" of the treatments, which is most likely the trigger for an increase in carotenoid concentration in the rind.

The aim of the research in Section 5.3.5 was to reduce fruit acidity of high acid citrus cultivars using alternatives to calcium arsenate. A single application of 1 or 2% MAP applied 6 weeks after fullbloom to Delta and Midknight Valencia orange resulted in significantly lower acidity than the untreated control by ≈0.3% acidity, i.e. an intermediate effect between that of Ca-arsenate and the untreated control. Juice and Brix contents were not affected by the treatments.

Projekopsomming

Die vrugkwaliteit verbeterings projek bestaan uit drie studies op vrugkleur verbetering en 'n studie op suurverlaging van vrugte.

In Seksie 5.3.2 is 'n studie uitgevoer om die konsentrasie te bepaal van verskeie gibberellienbiosintese inhibeerders benodig om 'n biologiese reaksie in sitrusbome te verkry. Herhaalde toedienings van Regalis® (10% v/v Proheksadioon-kalsium) op Eureka suurlemoen teen verskilllende konsentrasies (1, 2, 4 and 8 $g \cdot L^{-1}$) en Sunny® (5% v/v uniconazole) (teen 10 en 20 $mL \cdot L^{-1}$) en Cultar® (25% v/v paclobutrazol) (teen 10 $mL \cdot L^{-1}$) het geen effek op die onderstam of bostam deursneë 8 maande na die eerste toediening gehad nie. Beide die 4 en 8 $g \cdot L^{-1}$ Regalis® behandelings, beide Sunny® behandelings en die Cultar® behandeling het lootgroei betekenisvol verminder deur die internode lengte te verkort.

Metodes om vrugkleur vooroes te verbeter deur vegetatiewe groeikrag te manipuleer, is ondersoek in Seksie 5.3.3. Proheksadioon-kalsium (ProCa; Regalis®) is toegedien op verskillende kultivars. Blaartoediening van ProCa teen 'n konsentrasie van 400 $mg \cdot L^{-1}$ toegedien 6 en 3 weke voor verwagte oesdatum het die potensiaal om vooroes vrugkleur van vroeë sitruskultivars te verbeter.

Om vrugkleur van fisiologiese ryp sitrusvrugte te verbeter, is 'n vooroes en 'n na-oes benadering gevolg in Seksie 5.3.4. As vooroes benadering is die effek van blaartoediening van molibdeen (Mo) en tungsten (W) op vrugkleur van vroeë Valencia, Turkey en Navel vrugte ondersoek. As deel van die na-oes benadering, is vrugte blootgestel aan koue en warm temperature om die effek van koue en hitte-skok op vrugkleurontwikkeling te ondersoek. Die blootstelling van die 3 sitrustipes aan koue gevolg deur hitte-skok het ontgroening betekenisvol verbeter in vergelyking met die kontrole of met vrugte wat net koue skok ontvang het, maar die fisiologiese respons is kultivar afhanklik. Warm en koue water behandeling versnel kleurverandering van Valencia, Turkey en Navel vrugte moontlik a.g.v. "stres" van die behandelings. Hierdie stres is heel moontlik die oorsaak vir die toename in karotenoiëd konsentrasie in die skil.

Die doel van die navorsing in Seksie 5.3.5 was om suur te verlaag in hoë suur sitrus kultivars deur alternatiewe tot kalsiumarsenaat te gebruik. 'n Enkele toediening van 1 of 2% MAP toegedien 6 weke na volblom op Delta en Midknight Valencia lemoene het suur betekenisvol verlaag teenoor die kontrole met ≈0.3%, d.w.s. 'n intermediêre effek tussen die effek van Ca-arsenaat en die kontrole. Sapinhoud en Brix was nie deur die behandelings geaffekteer nie.

5.3.2 **Vegetative growth responses of citrus nursery trees to various growth retardants**
Experiment COL 01/02 by Graham Barry (CRI at SU) and Smit le Roux (SU)

Opsomming

As deel van 'n groter studie om vrugkleur by sitrus te verbeter, is 'n aanvanklike studie uitgevoer om die konsentrasie te bepaal van verskeie gibberellienbiosintese inhibeerders benodig om 'n biologiese reaksie in sitrusbome te verkry. Dit is gedoen deur vegetatiewe groei te meet. Herhaalde blaartoedienings van ProGibb® (4% v/v GA_3) het vegetatiewe groei op lote van Eureka suurlemoen [*Citrus limon* (L.) Burm. f.] kwekerybome met 63% verhoog, met geen betekenisvolle effek op onderstam en bostam deursnit. Herhaalde toedienings van Regalis® (10% v/v Proheksadioon-kalsium) teen verskilllende konsentrasies (1,

2, 4 and 8 g·L^{-1}) en Sunny® (5% v/v uniconazole) (teen 10 en 20 mL·L^{-1}) en Cultar® (25% v/v paclobutrazol) (teen 10 mL·L^{-1}) het geen effek op die onderstam of bostam deursneë 8 maande na die eerste toediening gehad nie. Beide die 4 en 8 g·L^{-1} Regalis® behandelings, beide Sunny® behandelings en die Cultar® behandeling het lootgroei betekenisvol verminder. Sunny® teen 20 mL·L^{-1} het die meeste lootgroei vertraging veroorsaak, d.w.s. 34% korter lote as die kontrole. Alhoewel die aantal nodes op die langste loot nie verskil het van die kontrole nie, het internode lengte betekenisvol verskil tussen die behandelings. Regalis® teen 4 en 8 g·L^{-1}, Sunny® teen 20 mL·L^{-1} en Cultar® teen 10 mL·L^{-1} het internode lengte in vergelyking met die kontrole verminder met 31%, 56%, 50% en 28%, respektiewelik. Vegetatiewe groei van Eureka suurlemoen kwekerybome is verminder na die herhaalde (x4) toediening van gibberellienbiosintese inhibeerders. Regalis® teen 4 tot 8 g·L^{-1} en Sunny® teen 10 tot 20 mL·L^{-1} is potensiële kandidate vir verdere veldstudies om die produkte se effek op vrugkleur verbetering van sitrus vrugte te toets.

Introduction

Rind colour is an important cosmetic preference of consumers when purchasing citrus fruit. In general consumers prefer a deep orange rind colour (Krajewski, 1996). As citrus fruit mature, changes in rind colour are due to increased carotenoid and decreased chlorophyll concentrations in the flavedo. This change in rind pigments is mainly due to the senescence of chlorophyllous tissue in the flavedo, and results in the transformation of chloroplasts into chromoplasts. Chloro-chromoplast transformation is a major physiological response affected by environmental, nutritional and hormonal factors (Goldschmidt, 1988).

As part of a larger study to improve rind colour of citrus fruit, an initial study was conducted to determine the concentration of various gibberellin biosynthesis inhibitors required to get a biological response in citrus trees, as measured by vegetative growth. Goldschmidt (1988) showed that factors contributing to invigorating growing conditions are antagonistic to optimal rind colour development.

Vegetative growth in *Citrus* spp. is stimulated by various exogenous factors and nutrients, *viz*. high temperature, high light intensity, nitrogen and water, as well as endogenous hormones, *viz*. gibberellins and cytokinins. Young leaves are a major site of gibberellin biosynthesis (Salisbury and Ross, 1992; Spiegel-Roy and Goldschmidt, 1996). High endogenous gibberellin concentrations enhance stem elongation (Mudzunga, 2000; Salisbury and Ross, 1992), and delay rind colour development of citrus fruit (Garcia-Luis et al., 1985).

Growth retardants, sometimes referred to as gibberellin biosynthesis inhibitors, inhibit vegetative growth in plants by disrupting gibberellin biosynthesis. Aron et al. (1985) demonstrated that when paclobutrazol (Cultar®) was applied at 1 g·L^{-1} on citrus trees just before the onset of the summer flush it reduced shoot length, internode length and the number of shoots developed by 41%, 76% and 44%, respectively. Gilfillan and Lowe (1985) also reported that paclobutrazol increased Satsuma mandarin (*C. unshiu* Marc.) rind colour by 1-2 colour rating units. Uniconazole (Sunny®) reduced shoot length, number of lateral shoots per terminal, number of nodes per terminal and internode length in Wichita pecan [*Carya illinoinensis* (Wangenh.) K. Koch] and Cleopatra mandarin (*C. reticulata* Blanco) trees by blocking the steps before the formation of GA_{12} (Graham and Storey, 2000; Lee et al, 1998; Wheaton, 1989). Prohexadione-calcium (ProCa traded as Regalis®) is used on pome fruit trees (*Malus* and *Pyrus* spp.) to reduce and control vegetative growth (Miller, 2002). Prohexadione-calcium acts primarily as a gibberellin biosynthesis inhibitor, especially 3β-hydroxylation of GA_{20} to GA_1 (Nakayama et al., 1992). ProCa applied on Navelina Navel orange [*C. sinensis* (L.) Osbeck], 2 weeks before anticipated harvest, at 100 mg·L^{-1} improved rind colour. This application of ProCa aided in chlorophyll degradation and carotenoid biosynthesis (Barry and Van Wyk, 2004). Stover et al. (2004) found that two 500 mg·L^{-1} ProCa applications reduced the vegetative growth by ~ 40% across six citrus genotypes tested.

The primary objective of this study was to determine the concentration of various gibberellin biosynthesis inhibitors required to get a vegetative growth response in citrus nursery trees. This information could then be used in a field study to test the effects of gibberellin biosynthesis inhibitors on rind colour of citrus.

Materials and methods

Plant material and site. During the 2005-06 summer growing season, 108 potted nursery trees of Eureka lemon [*C. limon* (L.) Burm. f.] budded on X639 rootstock [Cleopatra mandarin (*C. reticulata* Blanco) × trifoliate orange (*Poncirus trifoliata* Raf.)] of similar size and with at least three strong primary branches were selected at Nucellar Nursery, Simondium, Western Cape province, South Africa (33°50'S, 18°58'E; 160 m alt.). These trees were 21 months old at the start of the experiment.

Treatments applied. Potted nursery trees were randomly allocated to treatments, *viz*. untreated control, 1.6 mL·L^{-1} ProGibb® (4% v/v GA_3), 1, 2, 4 and 8 g·L^{-1} Regalis® (10% v/v prohexadione-calcium), 10 and 20 mL·L^{-1} Sunny® (5% v/v uniconazole) and 10 mL·L^{-1} Cultar® (25% v/v paclobutrazol). Kaolin particle film (Surround®) at 20 g·L^{-1} was applied together with all treatments to easily distinguish new growth flushes throughout the assessment period. Application dates of the treatments (15 Nov. 2005, 27 Dec. 2005, 16 Feb. 2006 and 31 Mar. 2006) were planned to coincide with various growth flushes during the summer growing season.

Data collection. Rootstock and scion diameters were measured 2 cm below and 3 cm above the bud union, at the start of the experiment (15 Nov. 2005), 6 weeks thereafter (27 Dec. 2005) and at the end of the experiment (20 July 2006). Three shoots per tree were selected, marked and measured at the start of the experiment. Thereafter, only the length of the new growth was measured and internodes were counted at each assessment date. Since all shoots did not flush and grow out, data analysis was done on the longest shoot to quantify the treatment effects on growth retardation.

Statistical design and analysis. Experimental layout was a completely randomised block design (CRBD) consisting of twelve single-tree replicates. Blocking was used to reduce the possible effect of experimental error due to lighting and microclimate on within-site variation. Analysis of variance was conducted using the general linear model (GLM) procedure of Statistical Analysis Systems (SAS Inc., Cary, N.C., USA) and least significant difference (LSD) values were used to separate treatment means. Analysis of covariance was conducted with initial stem diameter and shoot growth as covariates.

Results and discussion

Rootstock diameter did not differ among treatments throughout the experiment (Table 5.3.2.1). Significant differences in scion diameter were measured at the onset of the trial and 6 weeks thereafter, but there were no significant differences among treatments at the final measurement (Table 5.3.2.1). When the initial rootstock and scion diameter was fixed by covariance analysis, there was no significant difference on the final rootstock and scion diameters.

Repeated applications of the treatments during the summer growing season did not have an effect on the final rootstock and scion diameters. In this short-term study, i.e. 8 months, there was too little time for a treatment response in rootstock and scion diameters.

Shoot length of the longest shoot was longer for the ProGibb® treatment than for the control, whereas shoot length of the two low concentrations of Regalis® (1 and 2 g·L^{-1}) did not differ from the control (Fig. 5.3.2.1). However, the two high concentrations of Regalis® (4 and 8 g·L^{-1}), both Sunny® treatments (10 and 20 mL·L^{-1}) and the Cultar® treatment (10 mL·L^{-1}) resulted in shorter shoot lengths than the control. Sunny® at 20 mL·L^{-1} significantly retarded growth, resulting in 34% shorter shoot length than the control.

ProGibb® significantly increased shoot length compared to the control by 63%. The present results confirm previous reports that ProGibb® applied at 1.6 mL·L^{-1} stimulates citrus shoot growth (Mudzunga, 2000). This response is not unexpected given the role of gibberellins in enhancing stem elongation (Salisbury and Ross, 1992).

Although the number of nodes on the longest shoot did not differ in any of the treatments from the untreated control (Fig. 5.3.2.2), internode length differed significantly among treatments (Fig. 5.3.2.3). Regalis® at 4 and 8 g·L^{-1}, Sunny® at 20 mL·L^{-1} and Cultar® at 10 mL·L^{-1} reduced internode length relative to the control by 31, 56, 50 and 28%, respectively (Figs. 5.3.2.3 and 5.3.2.4). These findings compare favourably with previous results by Aron et al. (1985) where Cultar® reduced the total growth and internode length of Minneola tangelo (*C. reticulata* Blanco x *C. paradisi* Macf.) trees.

Conclusion

Vegetative growth of Eureka lemon nursery trees was retarded following the repeated (x4) application of gibberellin biosynthesis inhibitors. Since it is unlikely that Cultar® would be registered on citrus due to its persistence in the environment and the plant (Goulston and Shearing, 1985), Regalis® at 4 to 8 g·L^{-1} and Sunny at 10 to 20 mL·L^{-1} are potential candidates for further field studies to test their effects on rind colour enhancement of citrus fruit.

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Table 5.3.2.1. Mean rootstock and scion diameter of Eureka lemon on X639 nursery trees at the start of the experiment (15 Nov. 2005), 6 weeks thereafter (27 Dec. 2005) and at the end of the experiment (20 July 2006)

Treatment (per L)	Rootstock diameter (mm)			Scion diameter (mm)		
	15 Nov. 05	27 Dec. 05	20 Jul. 06	15 Nov. 05	27 Dec. 05	20 Jul. 06
Control	14.3 ns[z]	13.9 ns	14.5 ns	11.1 bc	11.0 bc	11.8 ns
ProGibb 1.6 mL	14.6	14.2	15.3	12.3 a	11.5 abc	12.1
Regalis 1 g	14.1	14.2	14.4	10.9 c	11.2 bc	11.2
Regalis 2 g	14.3	14.2	14.5	11.5 abc	11.0 bc	11.8
Regalis 4 g	15.4	15.3	15.7	11.8 abc	11.3 bc	11.6
Regalis 8 g	13.4	13.5	14.2	11.2 bc	10.7 c	11.3
Sunny 10 mL	15.0	15.3	15.6	12.1 ab	11.9 ab	12.2
Sunny 20 mL	14.8	14.6	15.1	11.4 abc	11.0 bc	11.4
Cultar 10 mL	14.3	14.8	15.3	12.2 a	12.4 a	12.2
P-value	0.2780	0.3159	0.3179	0.0430	0.0482	0.2207
LSD	1.45	1.60	1.41	1.00	1.02	0.91

[z] Means within columns followed by different letters are significantly different (P≤0.05; ns = non significant).

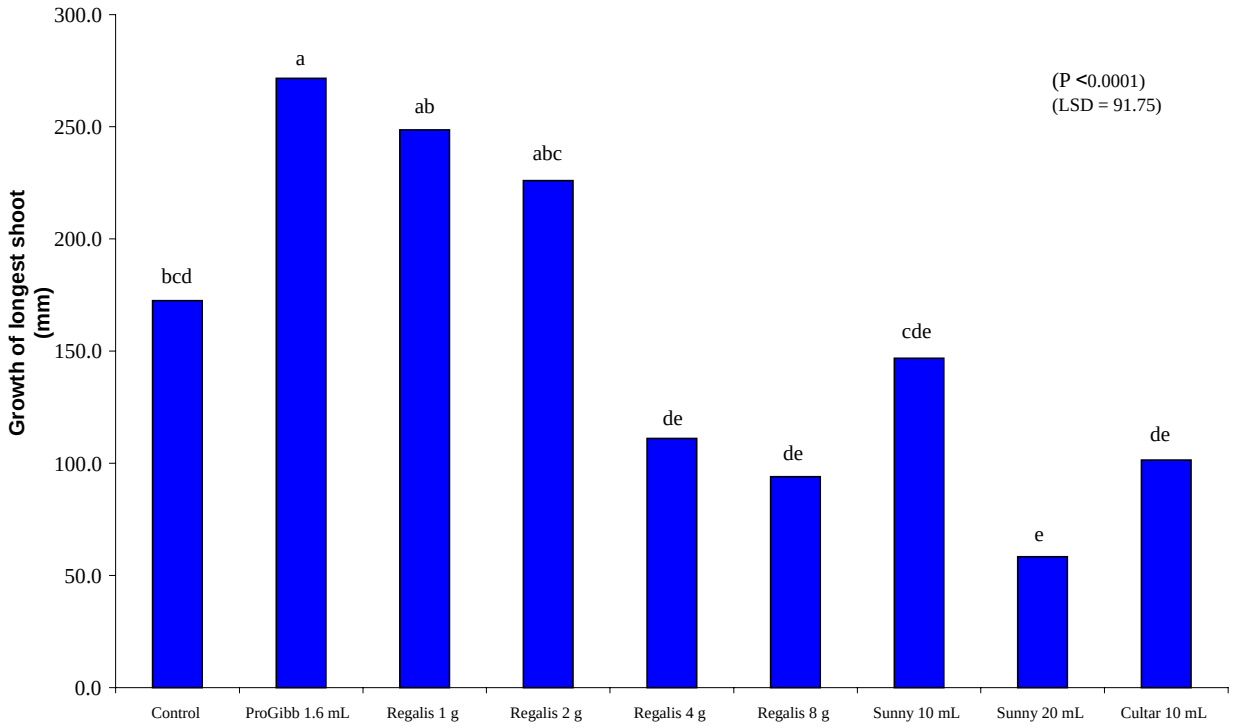

Fig. 5.3.2.1. Shoot length of the longest shoot of Eureka lemon on X639 nursery trees at the end of experiment on 20 July 2006. (Means followed by a different letter are significantly different (P≤0.05).

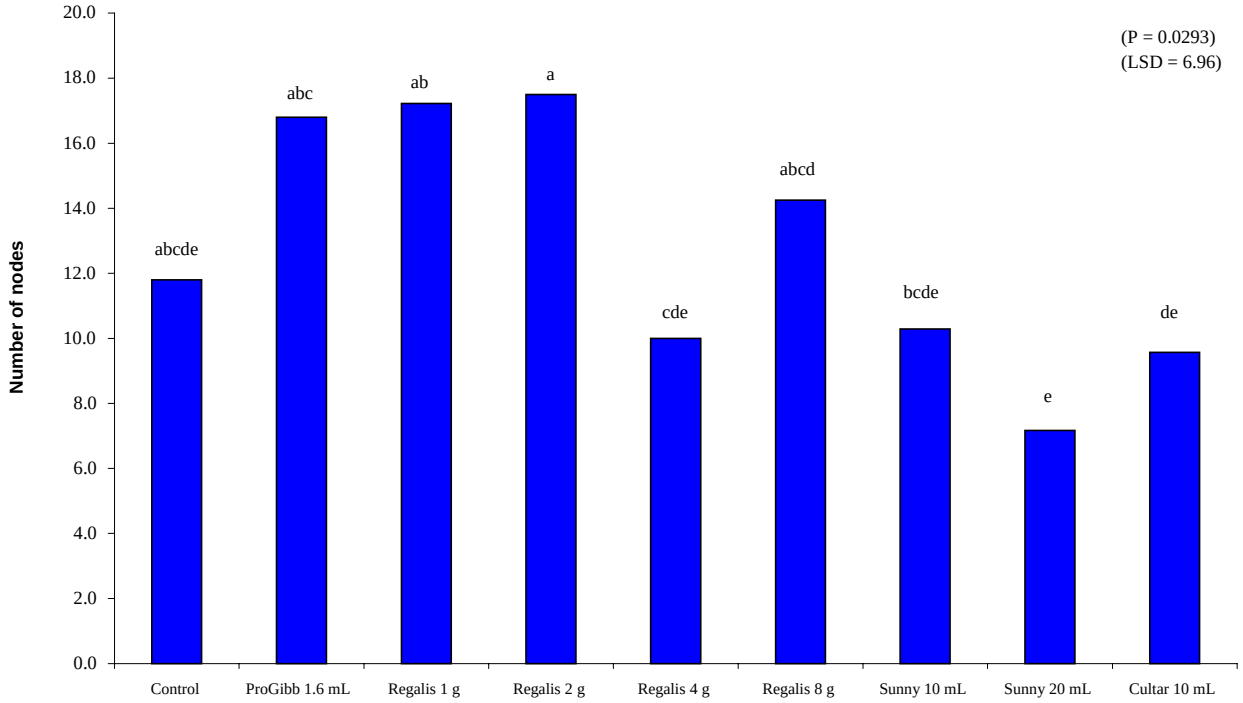

Fig. 5.3.2.2. Number of nodes on the longest shoot of Eureka lemon on X639 nursery trees the end of experiment on 20 July 2006. (Means followed by a different letter are significantly different (P≤0.05).

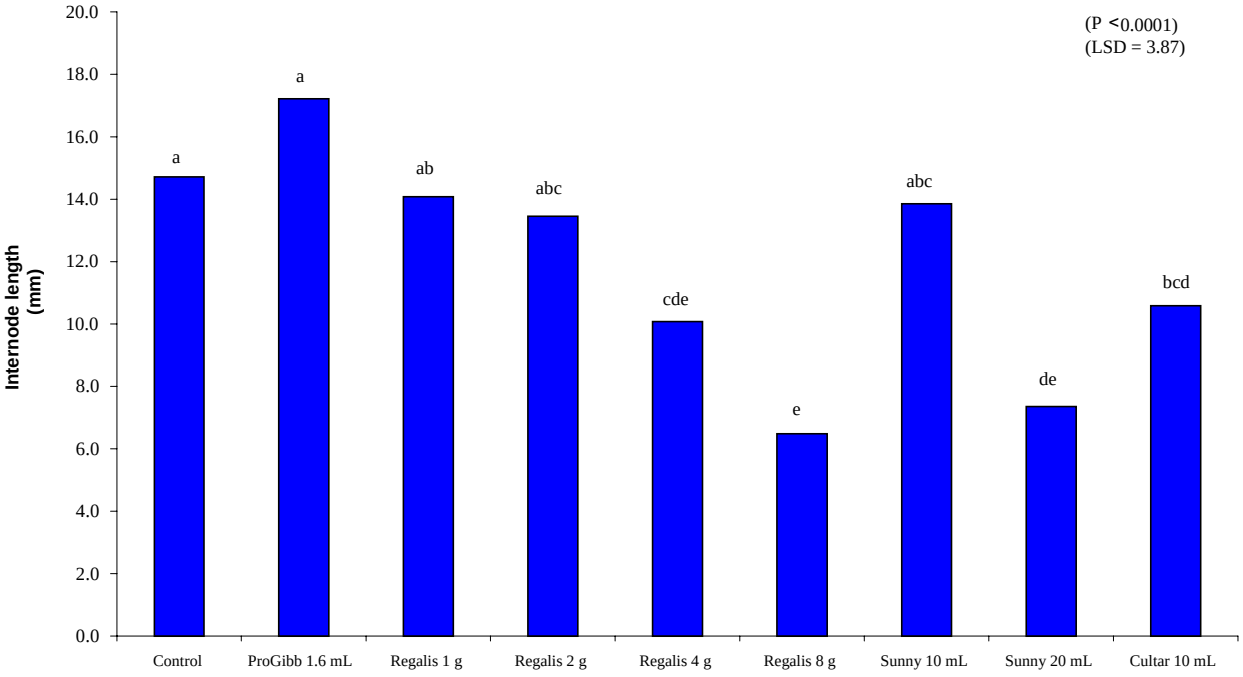

Fig. 5.3.2.3. Internode length of the longest shoot of Eureka lemon on X639 nursery trees the end of experiment on 20 July 2006. (Means followed by a different letter are significantly different (P≤0.05).

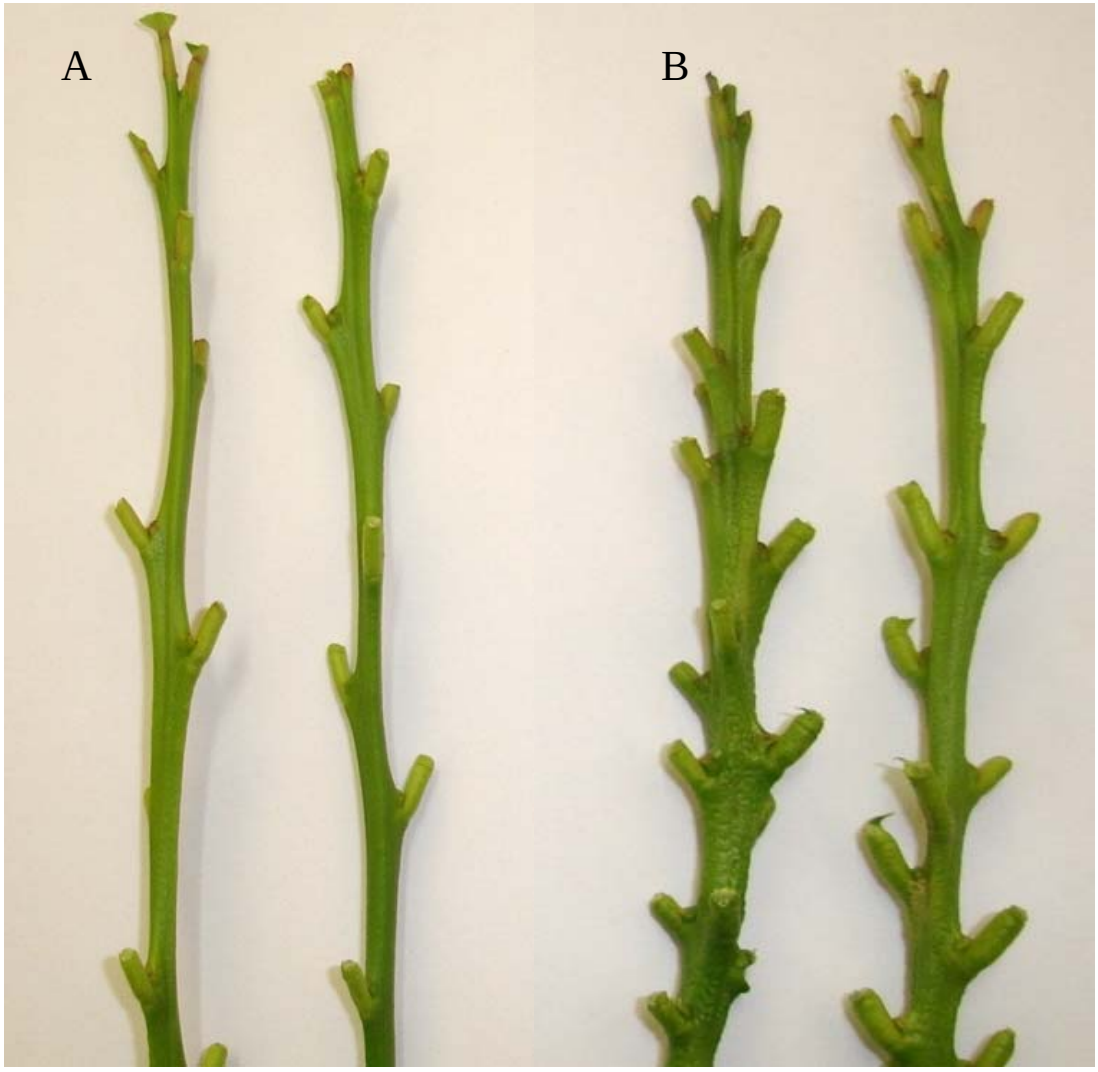

Fig. 5.3.2.4. Eureka lemon shoots illustrating the effect of growth retardants on vegetative growth. A: untreated control; B: 4 g·L^{-1} Regalis®. Note the shortening of internode length by > 30% in the Regalis® treatment compared to the untreated control treatment.

5.3.3 **Preharvest manipulation of chloro-chromoplast transformation by gibberellin biosynthesis inhibitor prohexadione-calcium**

Experiment COL 01/02 by Graham Barry (CRI at SU) and Smit le Roux (SU)

Opsomming

Vrugkleur is een van die hoof kosmetiese voorkeure wat verbruikers in ag neem wanneer sitrusvrugte (*Citrus* spp.) gekoop word. Aanvaarbare vrugkleur word verkry wanneer 'n genoegsame hoeveelheid karotenoïede gesintetiseer word saam met chlorofildegradasie. Daar word aangeneem dat boom vegetatiewe groeikragtigheid en hoë gibberellien en sitokinien vlakke vrugkleur negatief beïnvloed. Die benadering wat in hierdie studie gevolg is om skilkleur te verbeter, was om skilpigmente te manipuleer, deur vegetatiewe groeikrag te verminder. Proheksadioon-kalsium (ProCa; Regalis®) is toegedien op Nules Clementine mandaryn (*Citrus reticulata* Blanco), 'Navelina Navel' en 'Palmer Navel' lemoene [*C. sinensis* (L.) Osbeck], en Eureka suurlemoen [*C. limon* (L.) Burm.f.] gedurende die 2005 en 2006 seisoene teen 200 en 400 mg·L^{-1} aktiewe bestandeel. Kleurkaart evaluasie, colorimeter meetings en pigment analises is gedoen na oes, na etileen ontgroening, en 3 weke na koue opberging. Gedurende die 2005 seisoen, het ProCa vrugkleur betekenisvol verbeter in alle *Citrus* spp. getoets, behalwe vir Eureka suurlemoen, deur karotenoïed konsentrasie te verhoog en chlorofil konsentraise te verlaag in die flavedo van vrugte voor en na etileen ontgroening. Na koue opberging, het vrugkleur nie betekenisvol verskil tussen behandelings nie. Gedurende die 2006 seisoen is vrugkleur na oes betekenisvol verbeter en chlorofildegradasie en karotenoïed sintese gestimuleer op alle *Citrus* spp getoets deur die laat 400 mg·L^{-1} ProCa toediening. Blaar toediening van ProCa teen 'n konsentrasie van 400 mg·L^{-1} toegedien 6 en 3 weke voor verwagte oesdatum het die potensiaal om vooroes vrugkleur van vroeë sitruskultivars te verbeter en die resultate ondersteun die

hipotese dat daar moontlik a verwantskap tussen vegetatiewe groeikrag en vrugkleurontwikkeling van sitrusvrugte mag wees.

Introduction

Rind colour is an important cosmetic preference of consumers when purchasing citrus fruit. In general, consumers prefer a deep-orange rind colour (Krajewski, 1996). As citrus fruit mature, changes in rind colour are due to increased carotenoid and decreased chlorophyll concentrations (Goldschmidt, 1988). "Colour break" of the rind, a colloquial term generally used in the citrus industries of the world, occurs when a decrease in chlorophyll concentration unmasks the presence of carotenoid pigments (El-Zeftawi, 1978; Goldschmidt, 1988). Various factors affect rind colour development, *viz*. genetic, tree age, soil type, temperature, light, irrigation, nutritional and hormonal.

Besides the direct effects of some of these factors on rind colour, various indirect effects may also be important to rind colour development, while the interaction of various seemingly minor factors may delay rind colour development. Factors contributing to invigorating growing conditions are antagonistic to optimal rind colour development (Goldschmidt, 1988). For example, young trees tend to be more vigorous than older, mature trees. This vigour difference may be a major reason why fruit borne on young trees have poorer colour compared to fruit borne on old trees. Colour development is also adversely affected by growth flushes during stage III of fruit development, caused by high autumn temperatures. Such flushes are more common in trees bearing a low crop and in young trees of vigorous rootstock/scion combinations (Krajewski, 1997). Peng and Rabe (1996) found that when deficit irrigation caused the soil water tension to reach -70 kPa, better coloured fruit were obtained, compared to normal irrigation with a soil water tension of -30 kPa. Fruit harvested was not only better coloured, but also had lower chlorophyll levels. Koo (1988) established that excess N (>160 kg/ha/annum) increased the amount of green fruit (from 18% to 32%) when the fruit were physiologically mature and ready for harvest.

Goldschmidt (1988) showed that high gibberellin levels in fruit during maturation delayed chloroplast to chromoplast transformation. Gilfillan et al. (1974) found that when GA_3 was applied at colour break it resulted in unacceptably green fruit at harvest. Gibberellin-treated fruit also resulted in lower carotenoid concentration after full colour development, resulting in paler coloured fruit (Lewis and Coggins, 1964; Rasmussen, 1973). Gilfillan and Lowe (1985), however, reported that paclobutrazol (a gibberellin biosynthesis inhibitor) increased Satsuma mandarin (*C. unshiu* Marc.) rind colour by 1 to 2 colour rating units. These results were achieved when paclobutrazol was applied at 1 $g \cdot L^{-1}$ during November, after fruit drop, January and February, suggesting that paclobutrazol suppressed the November-December growth flush, which may be more important for rind colour development than the January-February growth flush.

Prohexadione-calcium [ProCa; BAS-125W (3-oxido-4propionyl-5-oxo-3-cyclohexene-carboxylate)] traded as Regalis® and Apogee® and developed by BASF (Limburgerhof, Germany) is used on pome fruit trees (*Malus* and *Pyrus* spp.) to reduce and control vegetative growth (Miller, 2002; Stover et al., 2004). Prohexadione-calcium acts primarily as a gibberellin biosynthesis inhibitor, especially 3β-hydroxylation of GA_{20} to GA_1 (Nakayama et al., 1992). Costa et al. (2001) demonstrated that repeated applications of 100 $mg \cdot L^{-1}$ ProCa significantly reduced shoot growth and increased fruit size in pears (*P. communis* L.). Preliminary research by Barry and Van Wyk (2004) showed that when ProCa was applied at 100 $mg \cdot L^{-1}$, 2 weeks before anticipated harvest, rind colour was improved due to chlorophyll degradation and carotenoid synthesis.

In an attempt to reduce vegetative vigour, although this was not measured, and thereby improve rind colour of citrus fruit, various early-maturing citrus cultivars were treated with different concentrations of ProCa at various stages of fruit development. The main objective of this study was to establish the concentration and timing of ProCa applications necessary to improve rind colour by enhancing chlorophyll degradation and carotenoid synthesis.

Materials and methods

Sites and plant material. Four citrus cultivars at different locations in the Western Cape province, South Africa, were used during the 2005 season, *viz*. Nules Clementine mandarin (*C. reticulata* Blanco) at Welgevallen Experimental Farm (Stellenbosch) (33°57'S, 18°53'E; 120 m alt.), Eureka lemon [*C. limon* (L.) Burm.f.] at Jericho (Gt. Drakenstein) (33°52'S, 19°01'E; 160 m alt.), Palmer Navel orange [*C. sinensis* (L.) Osbeck] at Landau (Wellington) (33°35'S, 18°59'E; 120 m alt.) and Navelina Navel orange at Hexrivier (Citrusdal) (32°28'S, 18°58'E; 180 m alt.). The same cultivars were used during the 2006 season, however, Nules Clementine mandarin from Diamant (Paarl) (33°46'S, 18°55; 140 m alt.) and Palmer Navel orange

from Hexrivier (Citrusdal) were used. The main reason for using different sites and plant materials was to test the treatments on different cultivars and to minimise the possibility of experimental loss.

Treatments and experimental design: Nules Clementine mandarin. Prohexadione-calcium (ProCa; Regalis® containing 10% ProCa) was applied as a medium-cover spray with a hand-held spray gun with application rates of 200 and 400 mg·L^{-1} ProCa on 8 and 28 Dec. 2004, 1 Feb. 2005, and 4 (8 Apr. 2005) and 2 (28 Apr. 2005) weeks before anticipated harvest (13 May 2005) during the 2005 season. During the 2006 season, rates of 200 and 400 mg·L^{-1} ProCa were applied on 19 Dec. 2005 and 17 Jan. 2006 constituting an early application treatment, and for the late application treatment rates of 200 and 400 mg·L^{-1} ProCa were applied 6 (28 Mar. 2006) and 3 (12 Apr. 2006) weeks before anticipated harvest (8 May 2006), and compared with an untreated control treatment.

Navelina Navel orange. Prohexadione-calcium was applied as a medium-cover spray with a hand-held spray gun with application rates of 200 and 400 mg·L^{-1} ProCa applied 4 (7 Apr. 2005) and 2 (21 Apr. 2005) weeks before anticipated harvest (5 May 2005) in the 2005 season. During the 2006 season rates of 200 and 400 mg·L^{-1} ProCa were applied on 14 Dec. 2005 and 16 Jan. 2006 constituting an early application treatment, and for the late application treatment rates of 200 and 400 mg·L^{-1} ProCa were applied 6 (8 Mar. 2006) and 3 (23 Mar. 2006) weeks before anticipated harvest (3 May 2006), and compared to an untreated control treatment.

Palmer Navel orange. Prohexadione-calcium was applied as a medium-cover spray with a hand-held spray gun with application rates of 200 and 400 mg·L^{-1} on 8 and 28 Dec. 2004, 1 Feb. 2005, and 4 (22 Apr. 2005) and 2 (6 May 2005) weeks before anticipated harvest (12 May 2005). During the 2006 season of 200 and 400 mg·L^{-1} ProCa were applied on 14 Dec. 2005 and 16 Jan. 2006 constituting an early application treatment, and for the late application treatment rates of 200 and 400 mg·L^{-1} ProCa were applied 6 (4 Apr. 2006) and 3 (25 Apr. 2006) weeks before anticipated harvest on 31 May 2006, and compared to an untreated control treatment.

Eureka lemon. Prohexadione-calcium was applied as a medium-cover spray with a hand-held spray gun with application rates of 200 and 400 mg·L^{-1} on 8 and 28 Dec. 2004, 1 Feb. 2005, 4 (8 Apr. 2005) and 2 (28 Apr. 2005) weeks before anticipated harvest (25 May 2005). In addition, individual fruit and fruit plus leaves were dipped on 4 May 2005 in 200 and 400 mg·L^{-1} ProCa solutions. During the 2006 season rates of 200 and 400 mg·L^{-1} ProCa were applied on 15 Dec. 2005 and 17 Jan. 2006 constituting an early application treatment, and for the late application treatment rates of 200 and 400 mg·L^{-1} ProCa were applied 6 (23 Mar. 2006) and 3 (11 Apr. 2006) weeks before anticipated harvest (12 May 2006), and compared to an untreated control treatment.

Fruit sampling. To limit unwanted, natural variation in rind colour, fruit were sampled from specific canopy positions. Fruit were sampled from the outer, eastern side of trees at a height of 1.5 to 2.0 m. During the 2005 season, 30 fruit were sampled from each tree for Nules Clementine mandarin and Palmer Navel orange of which 10 fruit were used for immediate analysis, and the remaining 20 fruit were degreened. After degreening, 10 fruit were analysed and the remaining 10 fruit were stored at 7.5°C for 2 weeks followed by 1 week at 18°C to simulate early season commercial shipping conditions. For Navelina Navel orange, 20 fruit were sampled at a height of 1.5 to 2.0 m. Ten fruit were used for immediate analysis and the remaining 10 fruit were degreened and then analysed. For Eureka lemon, only the dipped fruit were sampled as the bulk of the crop had been commercially harvested prior to sampling. Ten fruit per replicate were sampled for immediate analysis.

During the 2006 season, 30 fruit from each replicate from both the eastern and western sides of trees were sampled at a 1.5 to 2.0 m height from Nules Clementine mandarin, and Navelina Navel and Palmer Navel orange trees. Ten fruit were used for immediate analysis and the remaining 20 fruit were degreened. After degreening, 10 fruit were analysed and the remaining 10 fruit were stored at 4.5°C for 2 weeks followed by 1 week at 18°C. For Eureka lemon, 20 fruit were sampled on both the eastern and western sides of trees at a height of 1.0 to 1.5 m. Ten fruit were used for immediate analysis and 10 fruit were degreened and then analysed.

Degreening was done at 23°C with a relative humidity of 95%, an ethylene concentration of 2 mg·L^{-1} and a carbon dioxide (CO_2) concentration <0.3% (Krajewski and Pittaway, 2002). Fruit were subjected to a degreening time of 48 hours for Nules Clementine mandarin, and for 72 hours for Navelina Navel and Palmer Navel oranges and Eureka lemon.

Stored fruit were treated with 125 mg·L^{-1} 2,4-D (2,4-dichlorophenoxyacetic acid), 500 mg·L^{-1} Tecto® (thiabendazole) and 120 mg·L^{-1} Sporekill™ (didecyldimethylammonium chloride) drench and waxed with a polyethylene wax.

Data collection: Rind colour. Fruit were colour-rated with the "CRI colour charts, set no. 34, 36 or 37, 2004" for oranges, soft citrus and lemons, respectively (CRI, 2004a, 2004b, 2004c; Appendix 1-3). To limit the variation in rind colour on different sides of fruit, rind colour was also measured objectively on both the "vivid" (orange) and "dull" (green) sides of fruit with a Minolta chromameter (Model CR-400, Minolta Co. Ltd., Tokyo, Japan).

Rind pigments. Rind sampling was done by cutting the flavedo from the fruit. This was done either with a potato peeler (Nules Clementine mandarin) or with a citrus rind zester (Navelina Navel and Palmer Navel oranges and Eureka lemon) during the 2005 season. During the 2006 season, only citrus rind zesters were used for rind sampling on all cultivars. Sampling was done from all 10 fruit in the eight replicates, the pooled flavedo was then immersed into liquid nitrogen and stored at -80°C until completely frozen for a period of at least one day, whereafter the samples were freeze-dried at -56°C until all moisture was removed from the rinds, which lasted 4 days. The samples were then milled (A10 Kika Labortechnic, Kika Werke, GMBH & Co., Staufen, Germany) and sieved through a 500 µm sieve, to a homogenous powder. Samples were then stored in polyethylene vials at -80°C until analysed. All preparation activities were carried out under low light conditions to inhibit the degradation of carotenoids and chlorophyll.

From the freeze-dried rind sample, a 0.1 g sub-sample was added to 10 mL 96% (v/v) aqueous ethanol solvent containing 0.1 g·L^{-1} butylated hydroxytoluene (BHT) and 0.2 g·L^{-1} diethyldithiocarbamate (DDC), both antioxidants to prevent carotenoid degradation. The sample was then vortexed for two 1-minute intervals, whereafter it was stored for 1.5 hours at 4°C to allow the pigment to extract into the solvent. After 1.5 hour storage, the extraction was poured through ashless filter paper (Schleicher & Schuell, Dassel, Germany) to remove rind particles. The filtrated solution was then poured into plastic cuvettes placed into a spectrophotometer, zeroed with a ethanol/antioxidant solvent (Cary 50 conc UV-visible spectrophotometer, Varian Australia (Pty) Ltd, Mulgrave, Victoria, Australia). Absorbance readings were taken at 470, 649 and 664 nm. Absorbance values were used to determine the chlorophyll a (C_a), chlorophyll b (C_b), total chlorophyll (C_{a+b}) and total carotenoids (C_{x+c}) concentrations in µg·g^{-1} dry weight, using the Lightenthaler equations (Lichtenthaler, 1987):

$$C_a = 13.36\ A_{664} - 5.19A_{649}$$
$$C_b = 27.43A_{649} - 8.12A_{664}$$
$$C_{a+b} = A_{664} + 22.24A_{649}$$
$$C_{x+c} = (100A_{470} - 2.13\ C_a - 97.64\ C_b)/209$$

Statistical design and analysis. Experimental layout was a complete randomised block design (CRBD) consisting of eight single-tree replicates. Analysis of variance was conducted using the general linear model (GLM) procedure of Statistical Analysis Systems (SAS Inc., Cary, N.C., USA) and least significant difference (LSD) values were used to indicate any significant differences among treatments.

Results

Nules Clementine mandarin. In the 2005 season, the 400 mg·L^{-1} ProCa treatment significantly improved rind colour rating compared to the untreated control treatment by 0.9 colour units after harvest (Table 5.3.3.1). The 400 mg·L^{-1} ProCa treatment reduced relative greenness (as evidenced by the lower hue angle), and resulted in fruit appearing brighter (higher lightness), and more intensely coloured (higher chroma) after harvest. These differences in rind colour were smaller following ethylene degreening, but rind colour was still significantly better for the 400 mg·L^{-1} ProCa treatment than the control. After cold-storage however, no significant differences were observed among treatments (Table 5.3.3.1). The perceived improvement in rind colour was due to a lower chlorophyll concentration (by ~ 57%), resulting in the improvement of the carotenoid to chlorophyll ratio (P = 0.0860) (Table 5.3.3.2).

In the 2006 season, rind colour rating after harvest was significantly improved by the late 400 mg·L^{-1} ProCa treatment by 0.6 colour units for fruit sampled from the eastern side of trees, and for fruit sampled from the western side of trees by the early 400 mg·L^{-1} treatment by 0.4 colour units, compared to the untreated control treatment (Table 5.3.3.3; Fig. 5.3.3.1). Relative greenness of the rind was reduced by the late 400 mg·L^{-1} ProCa treatment on the eastern side of trees and by the early 400 mg·L^{-1} treatment on the western side of trees (as evidenced by the lower hue angle), brighter fruit (higher lightness) and more intensely coloured fruit (higher chroma) were also the results of the treatments, particularly on the dull side of fruit (Table 5.3.3.3). Ethylene degreening improved rind colour in such a way that no significant differences in hue angle among

treatments could be observed after degreening and after cold-storage compared to the untreated control treatment (Tables 5.3.3.4 to 5.3.3.6). The improvement in rind colour after harvest of fruit sampled from the eastern side of trees from the late 400 mg·L^{-1} ProCa treatment (Table 5.3.3.3) was due to significantly higher carotenoid concentrations (by ~ 25%), resulting in a higher carotenoid to chlorophyll ratio (P = 0.0645) (Table 5.3.3.6).

Navelina Navel orange. In the 2005 season, rind colour rating was significantly improved by the 400 mg·L^{-1} ProCa treatment by 1.3 colour units compared with the untreated control treatment (Table 5.3.3.7). Relative greenness of fruit was reduced (as evidenced by the significantly lower hue angle), and fruit were brighter (higher lightness) and more intensely coloured (higher chroma) at harvest for the 400 mg·L^{-1} ProCa treatment. These differences in rind colour were smaller following ethylene degreening (Table 5.3.3.7). The perceived improvement in rind colour of fruit was due to significantly higher carotenoid concentration (by ~ 15%) and significantly lower chlorophyll concentration (by ~ 41%), resulting in a significantly higher carotenoid to chlorophyll ratio after harvest, and due to a significantly higher carotenoid concentration (by ~ 35%) after ethylene degreening (Table 5.3.3.8).

In the 2006 season, rind colour rating after harvest of fruit sampled from the eastern and western sides of trees was significantly improved by the late 400 mg·L^{-1} ProCa treatment by 0.2 and 0.3 colour units, respectively, compared to the untreated control treatment (Table 5.3.3.9; Fig. 5.3.3.2). As this was the only treatment that improved rind colour, only the late 400 mg·L^{-1} ProCa treatment will be discussed in detail. The late 400 mg·L^{-1} ProCa treatment had a significantly lower hue angle, higher lightness and chroma on both sides of trees, resulting in lower relative greenness of rinds, as well as brighter and more intense coloured fruit (Table 5.3.3.9; Fig. 5.3.3.2). After ethylene degreening (Table 5.3.3.10) and after cold-storage (Table 5.3.3.11), rind colour of the late 400 mg·L^{-1} ProCa treatment was not better than that of the control treatment. This rind colour improvement (Table 5.3.3.9) was due to a lower chlorophyll concentration (by ~ 21%) of fruit sampled from the eastern side of trees, resulting in a significantly higher carotenoid to chlorophyll ratio (Table 5.3.3.12).

Palmer Navel orange. In the 2005 season, rind colour rating of fruit after harvest was significantly improved by both the 200 and 400 mg·L^{-1} ProCa treatments compared to the untreated control treatment by 0.8 colour units (Table 5.3.3.13). After ethylene degreening, the 200 mg·L^{-1} ProCa treatment had a significantly better rind colour rating than the control (by 0.4 colour units), with no differences in rind colour rating after cold-storage (Table 5.3.3.13). Colorimeter measurements indicated that the 200 mg·L^{-1} ProCa treatment improved rind colour of fruit the most, and will therefore be discussed in detail. The 200 mg·L^{-1} ProCa treatment reduced the hue angle, and increased the lightness and chroma of rinds, resulting in a reduction of relative greenness in rinds, as well as brighter and more intensely coloured fruit, after harvest. This perceived rind colour improvement was due to increased carotenoid concentration (by ~ 15%) and a reduction in chlorophyll concentration (by ~ 40%) after harvest, and an increased carotenoid concentration after ethylene degreening (by ~ 25%) and after cold-storage (by ~ 16%), resulting in significantly higher carotenoid to chlorophyll ratios after harvest, after ethylene degreening and after cold-storage (Table 5.3.3.14).

In the 2006 season, rind colour rating after harvest of fruit sampled from the eastern side of trees was significantly improved by the late 200 and 400 mg·L^{-1} ProCa treatments compared to the untreated control treatment by 0.4 colour units (Table 5.3.3.15; Fig. 5.3.3.3). However, these treatments did not affect rind colour rating of fruit sampled from the western side of trees. Colorimeter measurements showed that the late 400 mg·L^{-1} ProCa treatment significantly improved rind colour of fruit, and will therefore be discussed in detail. Relative greenness of fruit sampled from the eastern and western sides of trees was reduced, as evidenced by the lower hue angle. Fruit appeared brighter (higher lightness) and more intensely coloured (higher chroma) on the dull side of fruit sampled from the western side of trees (generally the worst case scenario for rind colour) (personal observation), possibly contributing to a reduction in rind colour variation within trees (Table 5.3.3.15). After ethylene degreening, no significant differences in rind colour occurred between the late 400 mg·L^{-1} ProCa and the untreated control treatment (Tables 5.3.3.16 and 5.3.3.18). After cold-storage, however, all treatments delayed rind colour development, resulting in higher relative greenness (higher hue angle) and less intensely coloured fruit (lower chroma) (Table 5.3.3.17). This perceived rind colour improvement (Table 5.3.3.15) was due to higher carotenoid concentration (by ~ 18%) of fruit sampled from the eastern and western sides of trees, resulting in a higher carotenoid to chlorophyll ratio on the western side of trees (Table 5.3.3.18). The poorer rind colour (Tables 5.3.3.16 and 5.3.3.17) was due to higher chlorophyll concentration of fruit sampled from the western side of trees (Table 5.3.3.18).

Eureka lemon. In the 2005 season, rind colour rating of fruit was significantly improved (by 0.5 colour units) by both the 200 and 400 mg·L^{-1} ProCa treatments when the fruit was dipped and (by 0.4 colour units) by the 400 mg·L^{-1} ProCa treatment when the fruit and leaves were dipped compared to the untreated control

treatment (Table 5.3.3.19). Colorimeter measurements (Table 5.3.3.19) and pigment concentrations (Table 5.3.3.20), however, did not differ among treatments.

In the 2006 season, rind colour rating of fruit sampled from the western side of trees was significantly improved by the early 200 mg·L^{-1} and both the late 200 and 400 mg·L^{-1} ProCa treatment by 0.5 colour units compared to the untreated control treatment (Table 5.3.3.21; Fig. 5.3.3.4). However, after degreening there were no significant differences in rind colour rating among treatments (Table 5.3.3.22) nor in pigment concentration when compared to the untreated control treatment (Table 5.3.3.23). Colorimeter measurements indicated that the late 400 mg·L^{-1} ProCa treatment significantly improved the rind colour on both the eastern and western sides of trees, and will therefore be discussed in detail (Table 5.3.3.21; Fig. 5.3.3.4). The late 400 mg·L^{-1} ProCa treatment significantly reduced the relative greenness of fruit (as evidenced by the lower hue angle) after harvest and after ethylene degreening, fruit also appeared brighter (higher lightness) after harvest, but duller (lower lightness) after ethylene degreening, and were more intensely coloured (higher chroma) after harvest and after ethylene degreening of fruit sampled from both the eastern and western sides of trees, on the vivid and dull sides of fruit (Tables 5.3.3.21 and 5.3.3.22). This perceived improvement in rind colour after harvest was due to a significant reduction in chlorophyll concentration (by ~ 38%), resulting in a significantly lower chlorophyll to carotenoid ratio as well as a significantly higher carotenoid to chlorophyll ratio of fruit sampled from the eastern side of trees (Table 5.3.3.23).

Discussion and conclusion

The late 400 mg·L^{-1} ProCa treatment consistently improved rind colour on all *Citrus* spp. tested. However, these effects were more pronounced after harvest, as ethylene degreening and cold-storage stimulated additional chlorophyll degradation, unmasking the carotenoids, resulting in overall better coloured fruit (El-Zeftawi, 1978; Goldschmidt, 1988; Van Wyk, 2004). Prohexadione calcium in most instances stimulated chlorophyll degradation and carotenoid biosynthesis confirming the preliminary results of Barry and Van Wyk (2004). These changes in pigment concentration resulted in a higher carotenoid to chlorophyll ratio and, therefore, improved rind colour. Gilfillan and Lowe (1985) demonstrated the same response when paclobutrazol, also a gibberellin biosynthesis inhibitor, improved Satsuma mandarin rind colour.

Prohexadione-calcium has been shown to reduce vegetative growth in *Citrus* spp. (Stover et al., 2004; Section 5.3.2), similar to paclobutrazol (Aron et al., 1985; Smeirat and Qrunfleh, 1989) and uniconazole (Wheaton, 1989). Therefore, the improvement of rind colour of citrus fruit in the current study following the application of a gibberellin biosynthesis inhibitor (400 mg·L^{-1} ProCa applied 6 plus 3 weeks before harvest) supports the hypothesis that there may be a relationship between vegetative vigour and rind colour development of citrus fruit, although vegetative vigour was not measured in this study.

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Table 5.3.3.1. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours of harvest, after ethylene degreening and after cold-storage on the vivid (yellow) and dull (green) sides of Nules Clementine mandarin fruit sampled from the eastern side of trees during the 2005 season

Treatment	After harvest		After degreening		After storage	
	Colour rating[z]					
Control	3.5 a[y]		1.3 a		1.1 ns	
ProCa (200 mg·L^{-1})	3.2 a		1.2 b		1.1	
ProCa (400 mg·L^{-1})	2.6 b		1.1 b		1.0	
P-value	0.0006		0.0006		0.2389	
LSD	0.46		0.12		0.07	
	Vivid	Dull	Vivid	Dull	Vivid	Dull
	Hue angle (°)					
Control	74.2 a	83.0 a	64.5 ns	67.7 a	61.0 ns	62.5 ns
ProCa (200 mg·L^{-1})	70.2 b	76.4 b	64.0	66.3 b	61.7	63.2
ProCa (400 mg·L^{-1})	70.3 b	75.8 b	64.7	66.7 ab	61.9	62.7
P-value	<0.0001	<0.0001	0.4553	0.0447	0.3125	0.5606
LSD	1.66	2.23	1.11	1.13	1.23	1.30
	Lightness					
Control	69.9 a	67.1 b	66.2 ns	68.3 a	63.8 ns	64.5 ns
ProCa (200 mg·L^{-1})	68.6 b	67.2 b	65.9	67.0 b	63.7	64.4
ProCa (400 mg·L^{-1})	70.2 a	69.7 a	66.8	67.5 b	64.0	64.4
P-value	<0.0001	0.0006	0.0795	0.0072	0.7285	0.9502
LSD	0.73	1.47	0.77	0.79	0.74	0.73
	Chroma					
Control	69.9 b	64.4 c	71.2 a	71.3 a	68.6 a	69.2 a
ProCa (200 mg·L^{-1})	70.9 ab	67.7 b	69.9 b	69.5 b	67.4 b	67.8 b
ProCa (400 mg·L^{-1})	71.4 a	70.3 a	70.1 b	69.9 b	68.3 a	67.8 b
P-value	0.0456	<0.0001	0.0028	<0.0001	0.0039	<0.0001
LSD	1.23	2.07	0.78	0.80	0.70	0.69

[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).
[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 36, 2004b).

Table 5.3.3.2. Total chlorophyll concentration, total carotenoid concentration, chlorophyll carotenoid ratio and carotenoid chlorophyll ratio following different Prohexadione-calcuim treatments on Nules Clementine mandarin fruit after harvest, after ethylene degreening and after cold-storage of fruit during the 2005 season

Treatment	After harvest	After degreening	After storage
	Carotenoid (µg·g^{-1} DW)		
Control	488.8 b[z]	626.0 ns	961.1 ns
ProCa (200 mg·L^{-1})	595.3 a	665.6	1011.1
ProCa (400 mg·L^{-1})	503.7 b	624.0	937.6
P-value	0.0448	0.5849	0.3754
LSD	89.19	92.92	108.85
	Chlorophyll (µg·g^{-1} DW)		
Control	130.0 ns	25.4 ns	32.0 ns
ProCa (200 mg·L^{-1})	81.0	34.5	35.9
ProCa (400 mg·L^{-1})	55.3	28.9	28.1
P-value	0.0639	0.1210	0.0890
LSD	62.94	8.87	6.89
	Chlorophyll/Carotenoid Ratio		
Control	0.3 a	0.042 ns	0.033 ns
ProCa (200 mg·L^{-1})	0.1 b	0.054	0.036
ProCa (400 mg·L^{-1})	0.1 b	0.047	0.031
P-value	0.0134	0.4600	0.5810
LSD	0.10	0.02	0.01
	Carotenoid/Chlorophyll Ratio		
Control	4.7 ns	28.9 a	30.7 ns
ProCa (200 mg·L^{-1})	12.2	20.1 b	29.6
ProCa (400 mg·L^{-1})	10.7	21.8 ab	34.9
P-value	0.0860	0.0483	0.3581
LSD	6.97	7.32	7.90

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.3. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours of harvest on the vivid (yellow) and dull (green) sides of Nules Clementine mandarin fruit sampled from the eastern and western sides of trees during the 2006 season

Treatment	Eastern		Western	
	Colour rating[z]			
Control	4.6 a[y]		4.3 ab	
ProCa early (200 mg·L^{-1})	4.5 a		4.0 bc	
ProCa early (400 mg·L^{-1})	4.7 a		3.9 c	
ProCa late (200 mg·L^{-1})	4.7 a		4.0 c	
ProCa late (400 mg·L^{-1})	4.0 b		4.4 a	
P-value	<0.0001		0.0065	
LSD	0.28		0.29	
	Eastern, vivid	**Eastern, dull**	**Western, vivid**	**Western, dull**
	Hue angle (°)			
Control	83.2 b	93.0 a	81.4 ab	92.3 a
ProCa early (200 mg·L^{-1})	84.8 ab	94.5 a	79.1 bc	89.7 ab
ProCa early (400 mg·L^{-1})	86.6 a	94.1 a	78.2 c	87.4 b
ProCa late (200 mg·L^{-1})	85.9 ab	95.0 a	80.2 abc	88.1 b
ProCa late (400 mg·L^{-1})	76.3 c	84.5 b	81.8 a	88.3 b
P-value	<0.0001	<0.0001	0.0424	0.0094
LSD	2.97	2.97	2.62	3.01
	Lightness			
Control	67.5 a	59.8 bc	66.6 ab	61.2 c
ProCa early (200 mg·L^{-1})	66.0 ab	59.8 bc	67.3 a	63.6 ab
ProCa early (400 mg·L^{-1})	66.1 ab	61.1 ab	67.5 a	65.5 a
ProCa late (200 mg·L^{-1})	65.0 b	58.4 c	66.7 ab	62.4 bc
ProCa late (400 mg·L^{-1})	66.1 ab	63.0 a	65.4 b	62.2 bc
P-value	0.0329	0.0012	0.0950	0.0002
LSD	1.59	2.12	1.53	1.92
	Chroma			
Control	64.6 a	54.7 bc	64.5 b	56.7 b
ProCa early (200 mg·L^{-1})	62.8 ab	53.9 bc	67.3 a	59.0 ab
ProCa early (400 mg·L^{-1})	63.0 ab	55.9 b	67.0 a	61.5 a
ProCa late (200 mg·L^{-1})	61.2 b	52.3 c	64.4 b	58.2 b
ProCa late (400 mg·L^{-1})	64.9 a	59.2 a	62.1 c	57.6 b
P-value	0.0055	<0.0001	<0.0001	0.0016
LSD	2.14	2.69	2.01	2.45

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 36, 2004b).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.4. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after ethylene degreening on the vivid (yellow) and dull (green) sides of Nules Clementine mandarin orange fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	Eastern		Western	
	Colour rating[z]			
Control	1.3 ns[y]		1.4 ns	
ProCa early (200 mg·L^{-1})	1.4		1.2	
ProCa early (400 mg·L^{-1})	1.3		1.3	
ProCa late (200 mg·L^{-1})	1.3		1.3	
ProCa late (400 mg·L^{-1})	1.1		1.4	
P-value	0.1144		0.0524	
LSD	0.20		0.17	
	Eastern, vivid	**Eastern, dull**	**Western, vivid**	**Western, dull**
	Hue angle (°)			
Control	64.8 ns	67.9 ns	64.2 b	68.2 ns
ProCa early (200 mg·L^{-1})	65.3	68.5	62.9 c	66.9
ProCa early (400 mg·L^{-1})	64.5	68.5	66.0 a	68.7
ProCa late (200 mg·L^{-1})	65.4	68.8	66.0 a	68.4
ProCa late (400 mg·L^{-1})	63.7	67.0	65.1 ab	68.4
P-value	0.2692	0.3025	<0.0001	0.0574
LSD	1.59	1.71	1.30	1.33
	Lightness			
Control	65.2 ns	66.6 a	64.7 b	66.9 ab
ProCa early (200 mg·L^{-1})	65.1	66.8 a	63.4 c	66.2 bc
ProCa early (400 mg·L^{-1})	64.2	66.7 a	65.9 a	67.2 a
ProCa late (200 mg·L^{-1})	65.2	67.1 a	66.1 a	65.6 c
ProCa late (400 mg·L^{-1})	64.1	64.6 b	64.3 b	66.7 ab
P-value	0.1217	0.0013	<0.0001	0.0088
LSD	1.10	1.21	0.95	1.03
	Chroma			
Control	76.6 a	75.4 a	75.8 ab	74.9 a
ProCa early (200 mg·L^{-1})	76.9 a	75.5 a	75.0 b	74.4 ab
ProCa early (400 mg·L^{-1})	74.8 b	74.3 a	76.3 a	74.7 a
ProCa late (200 mg·L^{-1})	74.6 b	74.2 a	75.1 b	73.2 b
ProCa late (400 mg·L^{-1})	74.5 b	71.7 b	73.5 c	73.9 ab
P-value	<0.0001	<0.0001	<0.0001	0.0201
LSD	1.10	1.38	0.84	1.19

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 36, 2004b).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.5. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after cold-storage on the vivid (yellow) and dull (green) sides of Nules Clementine mandarin orange fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	Eastern		Western	
	Colour rating[z]			
Control	1.2 ns[y]		1.1 b	
ProCa early (200 mg·L^{-1})	1.0		1.1 b	
ProCa early (400 mg·L^{-1})	1.1		1.1 b	
ProCa late (200 mg·L^{-1})	1.1		1.1 b	
ProCa late (400 mg·L^{-1})	1.0		1.3 a	
P-value	0.1141		0.0391	
LSD	0.13		0.12	
	Eastern, vivid	**Eastern, dull**	**Western, vivid**	**Western, dull**
	Hue angle (°)			
Control	63.4 ab	65.4 ns	62.5 ns	65.4 ns
ProCa early (200 mg·L^{-1})	62.4 b	65.8	62.2	65.3
ProCa early (400 mg·L^{-1})	63.6 ab	65.8	63.1	66.4
ProCa late (200 mg·L^{-1})	64.6 a	65.7	63.9	66.2
ProCa late (400 mg·L^{-1})	64.1 a	66.2	63.0	65.7
P-value	0.0167	0.8606	0.0730	0.3793
LSD	1.40	1.38	1.35	1.38
	Lightness			
Control	64.2 ab	64.1 bc	62.9 ns	64.9 ab
ProCa early (200 mg·L^{-1})	63.1 c	65.2 a	62.9	65.0 ab
ProCa early (400 mg·L^{-1})	64.0 abc	64.8 ab	63.5	65.7 a
ProCa late (200 mg·L^{-1})	64.6 a	63.2 c	63.4	64.1 b
ProCa late (400 mg·L^{-1})	63.3 bc	64.2 abc	63.5	64.0 b
P-value	0.0101	0.0018	0.5763	0.0040
LSD	0.98	1.09	1.00	0.99
	Chroma			
Control	69.5 a	68.3 ab	68.7 ns	68.1 a
ProCa early (200 mg·L^{-1})	68.6 ab	69.1 a	68.3	68.4 a
ProCa early (400 mg·L^{-1})	68.7 a	68.2 ab	68.8	68.7 a
ProCa late (200 mg·L^{-1})	68.8 a	66.7 c	68.2	67.6 ab
ProCa late (400 mg·L^{-1})	67.6 b	67.3 bc	68.6	66.9 b
P-value	0.0242	0.0038	0.5831	0.0453
LSD	0.99	1.31	0.90	1.14

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 36, 2004b).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.6. Carotenoid, chlorophyll, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest, after ethylene degreening and after cold-storage of Nules Clementine mandarin fruit sampled from the eastern and western sides of trees during the 2006 season

Treatment	After harvest	After degreening	After storage	After harvest	After degreening	After storage
	Eastern			**Western**		
	Carotenoid (µg·g^{-1} DW)					
Control	461.5 bc[x]	890.2 ns	928.5 ns	498.3 ns	930.6 ns	865.5 ns
ProCa early (200 mg·L^{-1})	456.0 bc	831.1	884.9	507.4	898.5	793.9
ProCa early (400 mg·L^{-1})	431.5 c	811.6	845.9	503.8	851.8	892.3
ProCa late (200 mg·L^{-1})	538.0 ab	862.1	838.8	502.4	880.4	814.7
ProCa late (400 mg·L^{-1})	617.1 a	968.5	833.0	575.2	985.4	815.6
P-value	0.0093	0.5211	0.7107	0.7511	0.5177	0.5133
LSD	105.11	178.47	173.69	121.81	146.42	137.64
	Chlorophyll (µg·g^{-1} DW)					
Control	243.0 b	45.6 ns	nd[z]	237.1 ns	34.4 b	nd
ProCa early (200 mg·L^{-1})	294.0 b	39.8	nd	193.8	28.0 b	nd
ProCa early (400 mg·L^{-1})	291.6 b	53.8	nd	189.6	26.8 b	nd
ProCa late (200 mg·L^{-1})	403.6 a	36.8	nd	290.6	38.7 b	nd
ProCa late (400 mg·L^{-1})	236.0 b	33.9	nd	349.6	59.1 a	nd
P-value	0.0277	0.4062	nd	0.2332	0.0082	nd
LSD	109.01	22.35	nd	154.74	15.74	nd
	Chlorophyll/Carotenoid Ratio					
Control	0.55 ns	0.05 ns	nc[y]	0.51 ns	0.04 ns	nc
ProCa early (200 mg·L^{-1})	0.68	0.05	nc	0.41	0.03	nc
ProCa early (400 mg·L^{-1})	0.69	0.07	nc	0.40	0.03	nc
ProCa late (200 mg·L^{-1})	0.78	0.04	nc	0.60	0.05	nc
ProCa late (400 mg·L^{-1})	0.38	0.04	nc	0.58	0.06	nc
P-value	0.0710	0.3147	nc	0.5302	0.0888	nc
LSD	0.28	0.03	nc	0.31	0.02	nc
	Carotenoid/Chlorophyll Ratio					
Control	2.19 ns	20.04 ns	nc	2.31 ns	28.48 ns	nc
ProCa early (200 mg·L^{-1})	1.95	22.40	nc	3.13	40.61	nc
ProCa early (400 mg·L^{-1})	1.61	17.80	nc	4.65	32.98	nc
ProCa late (200 mg·L^{-1})	1.41	27.58	nc	2.01	23.98	nc
ProCa late (400 mg·L^{-1})	2.95	29.26	nc	2.75	20.45	nc
P-value	0.0645	0.2092	nc	0.2408	0.0543	nc
LSD	1.05	11.00	nc	2.61	14.02	nc

[z] Chlorophylls were not detectable (nd) by spectrophotometry.
[y] Ratios could not be calculated (nc) due to the non detectable chlorophylls.
[x] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.7. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours of harvest and after ethylene degreening on the vivid (yellow) and dull (green) sides of Navelina Navel orange fruit sampled from the eastern side of trees during the 2005 season

Treatment	After harvest		After degreening	
	Colour rating[z]			
Control	5.2 a[y]		2.2 a	
ProCa (200 mg·L^{-1})	4.4 b		2.1 a	
ProCa (400 mg·L^{-1})	3.9 c		1.8 b	
P-value	<0.0001		0.0001	
LSD	0.29		0.19	
	Vivid	**Dull**	**Vivid**	**Dull**
	Hue angle (°)			
Control	91.4 a	104.0 a	76.0 ns	78.4 b
ProCa (200 mg·L^{-1})	87.9 b	99.9 b	76.0	80.0 a
ProCa (400 mg·L^{-1})	84.2 c	96.9 c	76.2	79.2 ab
P-value	<0.0001	<0.0001	0.9277	0.0398
LSD	1.89	1.95	1.02	1.19
	Lightness			
Control	68.4 b	56.9 b	70.0 ns	67.4 b
ProCa (200 mg·L^{-1})	69.6 ab	58.6 b	69.7	66.6 b
ProCa (400 mg·L^{-1})	70.0 a	61.2 a	70.7	68.6 a
P-value	0.0013	<0.0001	0.0545	0.0019
LSD	1.37	1.76	0.82	1.12
	Chroma			
Control	64.8 c	52.5 c	71.8 ns	69.7 ab
ProCa (200 mg·L^{-1})	66.9 b	54.9 b	71.7	68.8 b
ProCa (400 mg·L^{-1})	69.0 a	58.2 a	72.4	70.8 a
P-value	<0.0001	<0.0001	0.2839	0.0352
LSD	1.74	2.18	0.95	1.49

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.8. Total chlorophyll concentration, total carotenoid concentration, chlorophyll carotenoid ratio and carotenoid chlorophyll ratio following different Prohexadione-calcium treatments on Navelina Navel orange fruit after harvest and after ethylene degreening of fruit during the 2005 season

Treatment	After harvest	After degreening
	Carotenoid (µg·g^{-1} DW)	
Control	193.9 b[z]	187.5 b
ProCa (200 mg·L^{-1})	210.0 ab	283.5 a
ProCa (400 mg·L^{-1})	229.3 a	289.5 a
P-value	0.0462	0.0069
LSD	27.54	66.73
	Chlorophyll (µg·g^{-1} DW)	
Control	211.1 a	22.0 ns
ProCa (200 mg·L^{-1})	158.7 ab	25.1
ProCa (400 mg·L^{-1})	124.7 b	22.9
P-value	0.0343	0.7470
LSD	64.15	8.60
	Chlorophyll/Carotenoid Ratio	
Control	1.1 a	0.5 ns
ProCa (200 mg·L^{-1})	0.8 b	0.1
ProCa (400 mg·L^{-1})	0.6 b	0.1
P-value	0.0105	0.3062
LSD	0.30	0.68
	Carotenoid/Chlorophyll Ratio	
Control	1.1 b	10.0 ns
ProCa (200 mg·L^{-1})	1.5 ab	12.4
ProCa (400 mg·L^{-1})	2.1 a	13.6
P-value	0.0211	0.3256
LSD	0.74	4.98

[z] Means within columns followed by different letters are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.9. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours of harvest on the vivid (yellow) and dull (green) sides of Navelina Navel orange fruit sampled from the eastern and western sides of trees during the 2006 season

Treatment	Eastern		Western	
	Colour rating[z]			
Control	5.0 b[y]		5.4 a	
ProCa early (200 mg·L^{-1})	5.3 a		5.4 a	
ProCa early (400 mg·L^{-1})	5.1 ab		5.3 a	
ProCa late (200 mg·L^{-1})	5.0 bc		5.3 a	
ProCa late (400 mg·L^{-1})	4.8 c		5.1 b	
P-value	<0.0001		0.0030	
LSD	0.17		0.21	
	Eastern, vivid	**Eastern, dull**	**Western, vivid**	**Western, dull**
	Hue angle (°)			
Control	99.2 a	110.5 a	101.5 a	111.2 a
ProCa early (200 mg·L^{-1})	100.2 a	111.6 a	98.8 b	110.5 ab
ProCa early (400 mg·L^{-1})	99.3 a	110.1 a	97.4 b	109.7 b
ProCa late (200 mg·L^{-1})	96.6 b	108.3 b	99.3 ab	108.1 c
ProCa late (400 mg·L^{-1})	92.9 c	102.4 c	94.9 c	104.3 d
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	2.26	1.69	2.35	1.48
	Lightness			
Control	64.5 b	53.5 c	62.4 b	54.8 c
ProCa early (200 mg·L^{-1})	65.0 b	53.5 c	64.9 a	54.9 c
ProCa early (400 mg·L^{-1})	65.4 b	54.7 bc	65.3 a	55.5 c
ProCa late (200 mg·L^{-1})	68.0 a	56.0 b	65.0 a	57.2 b
ProCa late (400 mg·L^{-1})	69.1 a	59.8 a	66.5 a	59.3 a
P-value	<0.0001	<0.0001	0.0004	<0.0001
LSD	1.82	1.53	1.81	1.44
	Chroma			
Control	59.2 c	47.4 cd	57.6 c	48.7 c
ProCa early (200 mg·L^{-1})	58.3 c	46.0 d	59.7 b	48.3 c
ProCa early (400 mg·L^{-1})	59.6 c	48.1 bc	60.8 b	49.4 c
ProCa late (200 mg·L^{-1})	62.2 b	49.7 b	59.4 bc	51.30 b
ProCa late (400 mg·L^{-1})	64.1 a	54.8 a	63.1 a	53.90 a
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	1.90	1.86	2.14	1.72

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.10. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after ethylene degreening on the vivid (yellow) and dull (green) sides of Navelina Navel orange fruit sampled from the eastern and western sides of trees during the 2006 season

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).

Treatment	Eastern		Western	
	Colour rating[z]			
Control	2.0 c[y]		2.3 c	
ProCa early (200 mg·L^{-1})	2.2 b		2.6 ab	
ProCa early (400 mg·L^{-1})	2.3 b		2.5 b	
ProCa late (200 mg·L^{-1})	2.5 a		2.7 a	
ProCa late (400 mg·L^{-1})	2.2 b		2.3 c	
P-value	<0.0001		<0.0001	
LSD	0.18		0.20	
	Eastern, vivid	**Eastern, dull**	**Western, vivid**	**Western, dull**
	Hue angle (°)			
Control	76.8 bc	78.2 c	78.9 a	80.3 a
ProCa early (200 mg·L^{-1})	76.6 c	79.2 bc	77.6 dc	79.8 ab
ProCa early (400 mg·L^{-1})	76.9 bc	78.9 bc	77.8 bc	80.4 a
ProCa late (200 mg·L^{-1})	78.5 a	81.4 a	78.8 ab	80.7 a
ProCa late (400 mg·L^{-1})	77.7 ab	79. b	76.8 d	78.6 b
P-value	0.0011	<0.0001	0.0002	0.0332
LSD	1.01	1.26	1.03	1.37
	Lightness			
Control	70.8 a	70.1 ns	70.7 ns	69.8 ns
ProCa early (200 mg·L^{-1})	69.5 b	69.1	69.7	68.8
ProCa early (400 mg·L^{-1})	70.7 a	69.6	70.3	69.3
ProCa late (200 mg·L^{-1})	71.3 a	69.6	70.5	68.9
ProCa late (400 mg·L^{-1})	70.9 a	69.8	70.4	69.2
P-value	0.0001	0.5096	0.1043	0.4403
LSD	0.78	1.10	0.75	1.16
	Chroma			
Control	71.9 a	71.5 a	71.4 ns	70.1 a
ProCa early (200 mg·L^{-1})	70.4 b	69.5 b	70.2	68.1 b
ProCa early (400 mg·L^{-1})	71.1 ab	69.8 b	70.6	68.5 ab
ProCa late (200 mg·L^{-1})	70.3 b	68.9 b	69.9	67.5 b
ProCa late (400 mg·L^{-1})	70.8 b	69.9 b	71.0	69.8 a
P-value	0.0032	0.0069	0.0992	0.0092
LSD	0.93	1.40	1.19	1.68

[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.11. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after cold-storage on the vivid (yellow) and dull (green) sides of Navelina Navel orange fruit sampled from the eastern and western sides of trees during the 2006 season

Treatment	Eastern		Western	
	Colour rating[z]			
Control	1.0 c[y]		1.2 ns	
ProCa early (200 mg·L^{-1})	1.1 ab		1.3	
ProCa early (400 mg·L^{-1})	1.1 bc		1.2	
ProCa late (200 mg·L^{-1})	1.2 a		1.2	
ProCa late (400 mg·L^{-1})	1.0 bc		1.2	
P-value	0.0155		0.4630	
LSD	0.09		0.15	
	Eastern, vivid	**Eastern, dull**	**Western, vivid**	**Western, dull**
	Hue angle (°)			
Control	71.8 c	73.0 b	73.8 ns	74.1 b
ProCa early (200 mg·L^{-1})	72.6 bc	73.1 b	73.4	74.2 b
ProCa early (400 mg·L^{-1})	72.3 bc	73.6 b	72.7	73.3 b
ProCa late (200 mg·L^{-1})	73.9 a	74.7 a	74.1	75.6 a
ProCa late (400 mg·L^{-1})	72.9 ab	73.7 b	73.2	73.4 b
P-value	0.0027	0.0134	0.0706	0.0002
LSD	1.11	1.05	1.01	1.07
	Lightness			
Control	66.4 ns	66.8 ns	66.9 ns	66.8 ns
ProCa early (200 mg·L^{-1})	66.5	66.5	66.5	66.5
ProCa early (400 mg·L^{-1})	66.7	66.9	66.8	66.6
ProCa late (200 mg·L^{-1})	67.4	67.2	67.3	67.2
ProCa late (400 mg·L^{-1})	67.0	66.6	66.5	66.4
P-value	0.1399	0.4157	0.1992	0.2163
LSD	0.81	0.81	0.72	0.77
	Chroma			
Control	66.5 ns	67.0 ns	67.9 ns	67.5 ns
ProCa early (200 mg·L^{-1})	67.5	67.7	67.2	67.3
ProCa early (400 mg·L^{-1})	67.2	67.4	67.5	67.6
ProCa late (200 mg·L^{-1})	67.2	66.7	66.8	66.6
ProCa late (400 mg·L^{-1})	66.6	66.0	66.9	66.7
P-value	0.2921	0.0601	0.1603	0.2721
LSD	1.06	1.17	1.03	1.12

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.12. Carotenoid concentration, chlorophyll concentration, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest, after degreening and after storage of Navelina Navel orange fruit sampled from the eastern and western sides of trees during the 2006 season

Treatment	After harvest	After degreening	After storage	After harvest	After degreening	After storage
	Eastern			Western		
	Carotenoid (µg·g^{-1} DW)					
Control	263.3 ns[z]	298.5 ns	454.5 ns	238.3 ns	326.5 ns	390.5 ns
ProCa early (200 mg·L^{-1})	261.6	268.5	427.8	248.7	304.7	390.5
ProCa early (400 mg·L^{-1})	258.8	327.1	447.1	250.2	313.0	395.0
ProCa late (200 mg·L^{-1})	262.2	316.2	437.2	240.2	336.8	370.1
ProCa late (400 mg·L^{-1})	255.0	297.5	407.9	262.4	354.6	393.7
P-value	0.9425	0.1070	0.4180	0.4662	0.2097	0.8130
LSD	22.16	34.85	54.34	28.82	45.23	50.37
	Chlorophyll (µg·g^{-1} DW)					
Control	384.7 ab	21.8 ns	18.1 ab	418.1 ns	18.7 ns	21.1 ns
ProCa early (200 mg·L^{-1})	443.8 a	21.1	15.2 b	436.4	21.4	19.5
ProCa early (400 mg·L^{-1})	409.8 a	24.9	13.9 b	416.2	21.5	16.5
ProCa late (200 mg·L^{-1})	402.3 a	20.7	18.3 ab	402.6	14.7	21.8
ProCa late (400 mg·L^{-1})	302.5 b	24.9	23.7 a	359.1	16.1	18.7
P-value	0.0252	0.8703	0.0295	0.5829	0.1832	0.8546
LSD	84.93	10.69	6.12	98.32	7.02	11.11
	Chlorophyll/Carotenoid Ratio					
Control	1.47 a	0.07 ns	0.04 b	1.75 ns	0.06 ns	0.05 ns
ProCa early (200 mg·L^{-1})	1.69 a	0.08	0.04 b	1.74	0.07	0.05
ProCa early (400 mg·L^{-1})	1.57 a	0.08	0.03 b	1.67	0.07	0.04
ProCa late (200 mg·L^{-1})	1.53 a	0.07	0.04 b	1.67	0.04	0.06
ProCa late (400 mg·L^{-1})	1.18 b	0.09	0.06 a	1.37	0.05	0.05
P-value	0.0102	0.8742	0.0208	0.1096	0.1147	0.8269
LSD	0.28	0.04	0.02	0.32	0.03	0.03
	Carotenoid/Chlorophyll Ratio					
Control	0.71 b	15.69 ns	27.48 ab	0.57 ns	20.46 ns	23.67 ns
ProCa early (200 mg·L^{-1})	0.59 b	13.66	38.99 a	0.61	16.02	27.08
ProCa early (400 mg·L^{-1})	0.65 b	13.70	38.13 a	0.61	16.00	26.00
ProCa late (200 mg·L^{-1})	0.67 b	16.56	24.48 ab	0.63	24.34	24.48
ProCa late (400 mg·L^{-1})	0.93 a	16.03	17.70 b	0.77	24.44	23.95
P-value	0.0017	0.7960	0.0323	0.0845	0.0659	0.9913
LSD	0.16	5.99	14.95	0.14	7.80	15.68

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.13. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours of harvest, after ethylene degreening and after cold-storage on the vivid (yellow) and dull (green) sides of Palmer Navel orange fruit sampled from the eastern side of trees during the 2005 season

Treatment	After harvest		After degreening		After storage	
	Colour rating[z]					
Control	5.3 a[y]		2.9 a		1.9 ns	
ProCa (200 mg·L^{-1})	4.5 b		2.5 b		1.8	
ProCa (400 mg·L^{-1})	4.5 b		2.8 ab		1.9	
P-value	<0.0001		0.0129		0.3968	
LSD	0.24		0.30		0.19	
	Vivid	**Dull**	**Vivid**	**Dull**	**Vivid**	**Dull**
	Hue angle (°)					
Control	90.7 a	105.4 a	77.1 a	82.2 a	73.8 a	78.0 a
ProCa (200 mg·L^{-1})	83.8 b	95.7 c	75.0 b	78.4 c	72.8 ab	75.0 c
ProCa (400 mg·L^{-1})	84.2 b	98.2 b	74.5 b	79.9 b	72.3 b	76.6 b
P-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0258	<0.0001
LSD	2.28	2.09	1.23	1.37	1.11	1.10
	Lightness					
Control	67.6 b	61.6 c	70.9 ab	74.2 a	69.2 ab	72.7 a
ProCa (200 mg·L^{-1})	70.6 a	66.7 a	71.2 a	73.4 a	69.5 a	71.5 b
ProCa (400 mg·L^{-1})	70.1 a	63.6 b	70.2 b	71.3 b	68.6 b	71.1 b
P-value	<0.0001	<0.0001	0.0263	<0.0001	0.0331	<0.0001
LSD	1.49	1.75	0.75	1.09	0.66	0.70
	Chroma					
Control	67.7 b	56.7 c	75.8 b	73.0 b	73.5 a	75.1 a
ProCa (200 mg·L^{-1})	73.3 a	63.3 a	78.3 a	75.6 a	73.8 a	74.9 a
ProCa (400 mg·L^{-1})	72.6 a	60.5 b	76.8 b	73.2 b	72.7 b	73.6 b
P-value	<0.0001	<0.0001	0.0016	0.0003	0.0175	0.0002
LSD	2.36	2.15	1.36	1.42	0.80	0.76

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.14. Total chlorophyll concentration, total carotenoid concentration, chlorophyll carotenoid ratio and carotenoid chlorophyll ratio following different Prohexadione-calcium treatments on Palmer Navel orange fruit after harvest, after ethylene degreening and after cold-storage of fruit during the 2005 season

Treatment	After harvest	After degreening	After storage
	Carotenoid (µg·g^{-1} DW)		
Control	298.1 ns[z]	256.8 b	510.5 b
ProCa (200 mg·L^{-1})	349.3	340.7 a	605.5 a
ProCa (400 mg·L^{-1})	298.3	331.9 a	566.1 ab
P-value	0.0543	<0.0001	0.0161
LSD	47.33	32.77	62.48
	Chlorophyll (µg·g^{-1} DW)		
Control	334.7 a	67.9 ns	39.6 ns
ProCa (200 mg·L^{-1})	201.3 b	52.2	37.5
ProCa (400 mg·L^{-1})	230.1 b	68.2	52.7
P-value	0.0247	0.3242	0.1397
LSD	97.99	24.66	16.42
	Chlorophyll/Caroteniod		
Control	1.1 a	0.3	0.08 ns
ProCa (200 mg·L^{-1})	0.6 b	0.2	0.07
ProCa (400 mg·L^{-1})	0.8 b	0.2	0.09
P-value	0.0036	0.0647	0.3211
LSD	0.29	0.09	0.04
	Carotenoid/Chlorophyll		
Control	0.9 b	4.0 b	13.2 b
ProCa (200 mg·L^{-1})	2.3 a	8.7 a	20.5 a
ProCa (400 mg·L^{-1})	1.4 b	5.5 b	11.7 b
P-value	0.0049	0.0177	0.0303
LSD	0.76	3.16	6.81

[z]Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.15. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours of harvest on the vivid (yellow) and dull (green) sides of Palmer Navel orange fruit sampled from the eastern and western sides of trees during the 2006 season

Treatment	Eastern		Western	
	Colour rating[z]			
Control	4.0 b[y]		4.6 bc	
ProCa early (200 mg·L^{-1})	4.4 a		4.9 a	
ProCa early (400 mg·L^{-1})	4.5 a		4.9 ab	
ProCa late (200 mg·L^{-1})	3.6 c		4.6 c	
ProCa late (400 mg·L^{-1})	3.6 c		4.5 c	
P-value	<0.0001		0.0027	
LSD	0.33		0.25	
	Eastern, vivid	**Eastern, dull**	**Western, vivid**	**Western, dull**
	Hue angle (°)			
Control	77.8 bc	87.9 b	84.7 b	94.5 b
ProCa early (200 mg·L^{-1})	81.3 a	91.3 a	87.1 a	98.5 a
ProCa early (400 mg·L^{-1})	79.6 ab	90.1 ab	85.2 ab	95.4 b
ProCa late (200 mg·L^{-1})	77.8 bc	85.2 c	83.3 bc	91.8 c
ProCa late (400 mg·L^{-1})	76.2 c	82.0 d	82.3 c	88.9 d
P-value	<0.0001	<0.0001	0.0005	<0.0001
LSD	1.85	2.49	2.26	2.27
	Lightness			
Control	69.9 ns	66.3 ns	69.2 ns	62.4 b
ProCa early (200 mg·L^{-1})	68.8	65.0	67.9	61.0 b
ProCa early (400 mg·L^{-1})	69.5	65.3	67.8	62.3 b
ProCa late (200 mg·L^{-1})	69.2	66.6	68.9	64.6 a
ProCa late (400 mg·L^{-1})	69.0	66.1	68.7	64.6 a
P-value	0.0897	0.1616	0.0656	<0.0001
LSD	0.88	1.46	1.12	1.54
	Chroma			
Control	73.3 a	65.2 ab	68.8 a	59.1 b
ProCa early (200 mg·L^{-1})	69.6 c	62.0 c	66.1 c	56.3 c
ProCa early (400 mg·L^{-1})	70.4 bc	63.2 bc	66.5 bc	58.6 b
ProCa late (200 mg·L^{-1})	71.3 b	65.9 a	68.5 a	61.4 a
ProCa late (400 mg·L^{-1})	71.6 b	66.6 a	67.9 ab	61.7 a
P-value	<0.0001	0.0001	0.0044	<0.0001
LSD	1.43	2.17	1.68	2.12

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.16. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after ethylene degreening on the vivid (yellow) and dull (green) sides of Palmer Navel orange fruit sampled from the eastern and western sides of trees during the 2006 season

Treatment	Eastern		Western	
	Colour rating[z]			
Control	3.0 cd[y]		3.5 c	
ProCa early (200 mg·L^{-1})	3.4 b		4.4 a	
ProCa early (400 mg·L^{-1})	3.8 a		4.0 b	
ProCa late (200 mg·L^{-1})	3.3 bc		3.9 b	
ProCa late (400 mg·L^{-1})	2.8 d		3.8 bc	
P-value	<0.0001		<0.0001	
LSD	0.39		0.34	
	Eastern, vivid	**Eastern, dull**	**Western, vivid**	**Western, dull**
	Hue angle (°)			
Control	72.1 cd	80.3 b	76.6 ns	85.6 bc
ProCa early (200 mg·L^{-1})	73.4 bc	80.4 b	78.6	88.7 a
ProCa early (400 mg·L^{-1})	77.3 a	84.6 a	77.2	86.0 b
ProCa late (200 mg·L^{-1})	74.7 b	80.6 b	76.8	84.0 bc
ProCa late (400 mg·L^{-1})	71.5 d	76.7 c	77.3	83.4 c
P-value	<0.0001	<0.0001	0.1214	<0.0001
LSD	1.50	2.09	1.60	2.28
	Lightness			
Control	69.2 bc	68.2 bc	71.2 ns	66.7 a
ProCa early (200 mg·L^{-1})	69.9 ab	69.6 a	70.5	64.7 b
ProCa early (400 mg·L^{-1})	68.9 a	67.0 c	70.8	66.1 ab
ProCa late (200 mg·L^{-1})	70.3 a	68.1 bc	70.5	66.8 a
ProCa late (400 mg·L^{-1})	70.6 c	68.7 ab	70.6	67.0 a
P-value	<0.0001	0.0072	0.2867	0.0448
LSD	0.76	1.36	0.74	1.64
	Chroma			
Control	75.1 a	69.5 a	74.0 a	65.8 a
ProCa early (200 mg·L^{-1})	74.1 b	69.8 a	72.5 bc	62.2 b
ProCa early (400 mg·L^{-1})	73.1 c	66.2 b	73.3 ab	65.0 a
ProCa late (200 mg·L^{-1})	72.7 c	68.8 a	71.9 c	65.7 a
ProCa late (400 mg·L^{-1})	73.5 bc	70.5 a	71.5 d	65.9 a
P-value	<0.0001	0.0017	<0.0001	0.0151
LSD	0.78	2.08	0.92	2.45

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.17. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after cold-storage on the vivid (yellow) and dull (green) sides of Palmer Navel orange fruit sampled from the eastern and western sides of trees during the 2006 season

Treatment	Eastern		Western	
	Colour rating[z]			
Control	1.5 c[y]		1.7 c	
ProCa early (200 mg·L^{-1})	1.9 b		2.4 b	
ProCa early (400 mg·L^{-1})	2.2 a		2.6 ab	
ProCa late (200 mg·L^{-1})	2.2 a		2.8 a	
ProCa late (400 mg·L^{-1})	2.3 a		2.6 ab	
P-value	<0.0001		<0.0001	
LSD	0.29		0.30	
	Eastern, vivid	**Eastern, dull**	**Western, vivid**	**Western, dull**
	Hue angle (°)			
Control	70.6 b	74.2 b	72.7 b	77.1 b
ProCa early (200 mg·L^{-1})	72.4 a	76.3 a	74.2 a	79.8 a
ProCa early (400 mg·L^{-1})	72.7 a	76.7 a	73.7 ab	78.2 b
ProCa late (200 mg·L^{-1})	71.9 a	76.0 a	74.8 a	79.9 a
ProCa late (400 mg·L^{-1})	70.6 b	74.5 b	73.7 ab	77.6 b
P-value	<0.0001	0.0002	0.0032	<0.0001
LSD	1.05	1.30	1.09	1.52
	Lightness			
Control	67.4 bc	69.4 a	68.3 c	69.2 a
ProCa early (200 mg·L^{-1})	68.5 a	68.8 ab	69.0 ab	66.8 c
ProCa early (400 mg·L^{-1})	68.6 a	69.0 a	68.6 bc	68.0 b
ProCa late (200 mg·L^{-1})	68.0 ab	68.1 bc	69.3 a	67.2 bc
ProCa late (400 mg·L^{-1})	67.1 c	67.6 c	68.8 abc	68.2 ab
P-value	<0.0001	0.0001	0.0364	<0.0001
LSD	0.67	0.85	0.68	1.09
	Chroma			
Control	73.6 a	74.6 a	74.2 a	73.5 a
ProCa early (200 mg·L^{-1})	73.7 a	72.1 bc	73.3 b	69.0 bc
ProCa early (400 mg·L^{-1})	73.4 ab	72.7 b	73.1 bc	70.8 b
ProCa late (200 mg·L^{-1})	72.6 bc	71.0 c	72.9 bc	68.4 c
ProCa late (400 mg·L^{-1})	72.0 c	70.9 c	72.4 c	70.0 bc
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	0.79	1.45	0.75	1.86

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.18. Carotenoid concentration, chlorophyll concentration, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest, after degreening and after storage of Palmer Navel orange fruit sampled from the eastern and western sides of trees during the 2006 season

Treatment	After harvest	After degreening	After storage	After harvest	After degreening	After storage
	Eastern			Western		
	Carotenoid (µg·g^{-1} DW)					
Control	368.9 b[z]	431.6 ns	703.8 ns	379.5 b	414.3 ns	688.1 ns
ProCa early (200 mg·L^{-1})	385.7 b	449.6	702.1	366.4 b	394.2	639.2
ProCa early (400 mg·L^{-1})	409.9 ab	424.7	693.8	354.1 b	410.4	625.3
ProCa late (200 mg·L^{-1})	440.2 a	483.0	726.6	410.5 ab	433.7	687.0
ProCa late (400 mg·L^{-1})	448.7 a	499.8	766.9	460.8 a	440.3	660.0
P-value	0.0041	0.4073	0.5258	0.0089	0.3495	0.6192
LSD	46.82	94.93	96.40	62.09	51.11	95.53
	Chlorophyll (µg·g^{-1} DW)					
Control	115.4 ns	25.4 ns	25.6 ns	192.6 ns	55.4 ns	19.1 b
ProCa early (200 mg·L^{-1})	123.1	45.3	23.2	193.7	89.4	38.6 a
ProCa early (400 mg·L^{-1})	128.4	72.1	30.5	223.1	74.7	44.7 a
ProCa late (200 mg·L^{-1})	122.7	50.2	37.9	160.5	65.3	29.7 ab
ProCa late (400 mg·L^{-1})	118.2	41.4	32.6	151.3	68.7	36.7 a
P-value	0.9893	0.1213	0.4151	0.4782	0.5828	0.0315
LSD	52.79	34.01	16.70	83.43	43.86	15.88
	Chlorophyll/Carotenoid Ratio					
Control	0.32 ns	0.06 ns	0.04 ns	0.52 ns	0.15 ns	0.03 c
ProCa early (200 mg·L^{-1})	0.32	0.10	0.03	0.55	0.23	0.06 ab
ProCa early (400 mg·L^{-1})	0.32	0.18	0.05	0.62	0.18	0.07 a
ProCa late (200 mg·L^{-1})	0.28	0.11	0.05	0.39	0.15	0.04 bc
ProCa late (400 mg·L^{-1})	0.27	0.09	0.04	0.34	0.17	0.06 ab
P-value	0.9234	0.0743	0.5566	0.0926	0.5584	0.0221
LSD	0.14	0.08	0.03	0.22	0.12	0.03
	Carotenoid/Chlorophyll Ratio					
Control	3.33 ns	18.32 ab	28.32 ns	2.30 b	13.02 ns	37.13 a
ProCa early (200 mg·L^{-1})	5.07	10.26 b	34.50	2.20 b	5.97	18.43 b
ProCa early (400 mg·L^{-1})	3.25	10.72 b	25.25	1.70 b	6.06	15.97 c
ProCa late (200 mg·L^{-1})	3.88	10.81 b	25.32	3.02 ab	8.76	30.20 a
ProCa late (400 mg·L^{-1})	4.41	19.95 a	24.36	4.41 a	13.21	18.79 b
P-value	0.3960	0.0374	0.2387	0.0196	0.3106	<0.0001
LSD	2.02	8.15	9.84	1.63	9.22	8.87

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.19. Hue angle, lightness and chroma responses following different Prohexadione-calcium treatments on Eureka lemons after harvest for yellow and green sides of fruit during the 2005 season

Treatment	Fruit dipped		Fruit and leaves dipped	
	Colour rating[z]			
Control	5.2 a[y]		4.8 a	
ProCa (200 mg·L^{-1})	4.7 b		4.9 a	
ProCa (400 mg·L^{-1})	4.7 b		4.4 b	
P-value	0.0047		0.0086	
LSD	0.38		0.36	
	Fruit dipped Vivid	Fruit and leaves dipped Vivid	Fruit dipped Dull	Fruit and leaves dipped Dull
	Hue angle (°)			
Control	105.8 ns[z]	103.7 ns	110.6 ns	106.8 ns
ProCa (200 mg·L^{-1})	103.1	102.3	108.0	108.5
ProCa (400 mg·L^{-1})	104.1	102.7	108.6	106.4
P-value	0.1611	0.7164	0.1478	0.3358
LSD	2.81	3.63	2.70	2.89
	Lightness			
Control	67.8 a	70.4 ns	60.5 ns	62.3 ns
ProCa (200 mg·L^{-1})	69.9 a	69.2	61.8	63.2
ProCa (400 mg·L^{-1})	70.0 b	69.8	61.8	62.1
P-value	0.0413	0.5929	0.6897	0.8150
LSD	1.93	2.36	3.62	3.69
	Chroma			
Control	51.8 ns	52.1 ns	50.0 ns	52.1 ns
ProCa (200 mg·L^{-1})	51.8	53.7	50.2	50.3
ProCa (400 mg·L^{-1})	51.0	53.2	50.7	52.0
P-value	0.5781	0.2579	0.7832	0.1616
LSD	1.76	2.04	1.81	2.09

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 37, 2004c).
[y] Means within columns followed by different letters are significantly different (P<0.05; ns = non significant).

Table 5.3.3.20. Total chlorophyll concentration, total carotenoid concentration, chlorophyll carotenoid ratio and carotenoid chlorophyll ratio following different Prohexadione-calcium treatments on Eureka lemons after harvest of fruit during the 2005 season

Treatment	Fruit dipped	Fruit and leaves dipped
	Carotenoid (µg·g^{-1} DW)	
Control	82.8 ns[z]	81.0 b
ProCa (200 mg·L^{-1})	86.7	100.5 a
ProCa (400 mg·L^{-1})	82.5	84.5 b
P-value	0.8997	0.0136
LSD	24.62	11.70
	Chlorophyll (µg·g^{-1} DW)	
Control	301.8 ns	224.8 ns
ProCa (200 mg·L^{-1})	276.2	286.9
ProCa (400 mg·L^{-1})	252.3	236.2
P-value	0.6549	0.3781
LSD	126.99	106.68
	Chlorophyll/Carotenoid Ratio	
Control	3.6 ns	2.8 ns
ProCa (200 mg·L^{-1})	3.1	2.8
ProCa (400 mg·L^{-1})	3.0	2.8
P-value	0.1755	0.9745
LSD	0.72	1.24
	Carotenoid/Chlorophyll Ratio	
Control	0.3 ns	0.3 ns
ProCa (200 mg·L^{-1})	0.3	0.4
ProCa (400 mg·L^{-1})	0.3	0.4
P-value	0.2626	0.9573
LSD	0.08	0.16

[z]Means within columns followed by different letters are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.21. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours of harvest on the vivid (yellow) and dull (green) sides of Eureka lemon fruit sampled from the eastern and western sides of trees during the 2006 season

Treatment	Eastern		Western	
	Colour rating[z]			
Control	4.9 ns[y]		4.8 a	
ProCa early (200 mg·L^{-1})	4.4		4.3 b	
ProCa early (400 mg·L^{-1})	4.7		4.5 ab	
ProCa late (200 mg·L^{-1})	4.7		4.3 b	
ProCa late (400 mg·L^{-1})	4.6		4.3 b	
P-value	0.1039		0.0318	
LSD	0.36		0.40	
	Eastern, vivid	**Eastern, dull**	**Western, vivid**	**Western, dull**
	Hue angle (°)			
Control	104.3 a	108.1 a	104.4 a	108.3 a
ProCa early (200 mg·L^{-1})	103.8 ab	106.0 abc	102.2 b	105.5 b
ProCa early (400 mg·L^{-1})	102.5 ab	106.5 ab	101.6 b	106.5 ab
ProCa late (200 mg·L^{-1})	101.6 bc	104.7 bc	101.8 b	104.7 b
ProCa late (400 mg·L^{-1})	100.1 c	104.3 c	99.6 c	104.6 b
P-value	0.0003	0.0034	<0.0001	0.0011
LSD	1.95	2.17	1.96	2.04
	Lightness			
Control	71.3 ns	62.7 b	69.7 c	63.5 c
ProCa early (200 mg·L^{-1})	71.6	65.9 a	72.2 ab	67.7 a
ProCa early (400 mg·L^{-1})	70.0	65.6 a	71.3 b	65.1 bc
ProCa late (200 mg·L^{-1})	71.4	67.2 a	71.6 b	68.1 a
ProCa late (400 mg·L^{-1})	71.9	67.8 a	73.3 a	67.1 ab
P-value	0.1792	0.0004	<0.0001	0.0004
LSD	1.55	2.45	1.50	2.35
	Chroma			
Control	52.9 b	51.2 c	53.6 ns	51.6 bc
ProCa early (200 mg·L^{-1})	51.8 b	51.1 c	53.1	52.3 abc
ProCa early (400 mg·L^{-1})	52.0 b	51.7 bc	52.4	51.4 c
ProCa late (200 mg·L^{-1})	53.0 b	52.7 ab	52.6	52.7 ab
ProCa late (400 mg·L^{-1})	54.8 a	53.4 a	53.9	53.2 a
P-value	0.0004	0.0037	0.2173	0.0287
LSD	1.49	1.43	1.51	1.24

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 37, 2004c).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.22. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after ethylene degreening on the vivid (yellow) and dull (green) sides of Eureka lemon fruit sampled from the eastern and western sides of trees during the 2006 season

Treatment	Eastern		Western	
	Colour rating[z]			
Control	3.4 ns[y]		3.2 ns	
ProCa early (200 mg·L^{-1})	3.4		3.0	
ProCa early (400 mg·L^{-1})	3.0		3.0	
ProCa late (200 mg·L^{-1})	3.5		3.3	
ProCa late (400 mg·L^{-1})	3.4		3.0	
P-value	0.2239		0.3489	
LSD	0.40		0.40	
	Eastern, vivid	**Eastern, dull**	**Western, vivid**	**Western, dull**
	Hue angle (°)			
Control	94.8 a	96.6 a	95.0 a	96.2 ab
ProCa early (200 mg·L^{-1})	94.6 a	96.6 a	94.3 a	95.3 bc
ProCa early (400 mg·L^{-1})	94.2 ab	94.8 b	94.6 a	95.7 ab
ProCa late (200 mg·L^{-1})	94.9 a	96.3 a	94.5 a	96.7 a
ProCa late (400 mg·L^{-1})	93.2 b	95.3 ab	92.3 b	94.6 c
P-value	0.0075	0.0292	<0.0001	0.0019
LSD	1.10	1.33	1.10	1.15
	Lightness			
Control	76.3 a	72.2 ns	75.3 a	74.1 ns
ProCa early (200 mg·L^{-1})	75.6 a	72.5	75.3 a	73.4
ProCa early (400 mg·L^{-1})	75.6 a	73.6	75.1 ab	74.3
ProCa late (200 mg·L^{-1})	75.7 a	73.3	75.6 a	73.5
ProCa late (400 mg·L^{-1})	74.5 b	73.4	74.3 b	74.0
P-value	0.0005	0.3452	0.0276	0.6968
LSD	0.89	1.63	0.89	1.40
	Chroma			
Control	54.8 b	54.8 ns	54.2 b	54.2 b
ProCa early (200 mg·L^{-1})	52.8 b	53.9	53.4 b	54.6 b
ProCa early (400 mg·L^{-1})	53.0 b	54.2	53.1 b	53.8 b
ProCa late (200 mg·L^{-1})	54.4 b	54.9	54.7 b	54.9 b
ProCa late (400 mg·L^{-1})	57.4 a	55.8	58.6 a	57.3 a
P-value	<0.0001	0.1114	<0.0001	<0.0001
LSD	2.03	1.60	2.07	1.50

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 37, 2004c).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.3.3.23. Carotenoid concentration, chlorophyll concentration, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest and after degreening of Eureka lemon fruit sampled from the eastern and western sides of trees during the 2006 season

Treatment	After harvest	After degreening	After harvest	After degreening
	Eastern		Western	
	Carotenoid (µg·g^{-1} DW)			
Control	140.0 a[z]	75.6 ab	125.5 ns	73.0 b
ProCa early (200 mg·L^{-1})	114.9 b	69.4 b	112.5	74.1 b
ProCa early (400 mg·L^{-1})	121.6 b	69.5 b	127.6	79.2 ab
ProCa late (200 mg·L^{-1})	126.5 ab	76.6 ab	116.3	76.0 b
ProCa late (400 mg·L^{-1})	116.4 b	84.0 a	124.8	91.0 a
P-value	0.0286	0.0276	0.2696	0.0352
LSD	16.89	10.20	16.83	12.87
	Chlorophyll (µg·g^{-1} DW)			
Control	333.4 a	49.3 ns	274.6 ns	44.2 ns
ProCa early (200 mg·L^{-1})	275.1 ab	53.8	230.8	39.7
ProCa early (400 mg·L^{-1})	272.4 ab	43.0	282.9	46.1
ProCa late (200 mg·L^{-1})	242.0 bc	52.1	251.9	53.0
ProCa late (400 mg·L^{-1})	206.3 c	48.5	208.9	42.2
P-value	0.0028	0.6267	0.2320	0.5942
LSD	63.33	13.89	75.32	17.50
	Chlorophyll/Carotenoid Ratio			
Control	2.36 a	0.65 ns	2.18 ns	0.61 ns
ProCa early (200 mg·L^{-1})	2.36 a	0.79	2.06	0.55
ProCa early (400 mg·L^{-1})	2.28 ab	0.62	2.24	0.60
ProCa late (200 mg·L^{-1})	1.94 bc	0.68	2.16	0.71
ProCa late (400 mg·L^{-1})	1.77 c	0.58	1.71	0.49
P-value	0.0077	0.2294	0.2673	0.5239
LSD	0.40	0.20	0.58	0.26
	Carotenoid/Chlorophyll Ratio			
Control	0.43 b	1.55 ns	0.47 ns	1.90 ns
ProCa early (200 mg·L^{-1})	0.44 b	1.35	0.51	1.92
ProCa early (400 mg·L^{-1})	0.45 b	1.79	0.48	1.97
ProCa late (200 mg·L^{-1})	0.53 ab	1.62	0.47	1.48
ProCa late (400 mg·L^{-1})	0.59 a	1.75	0.67	2.62
P-value	0.0145	0.3158	0.1511	0.1316
LSD	0.11	0.46	0.20	0.89

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

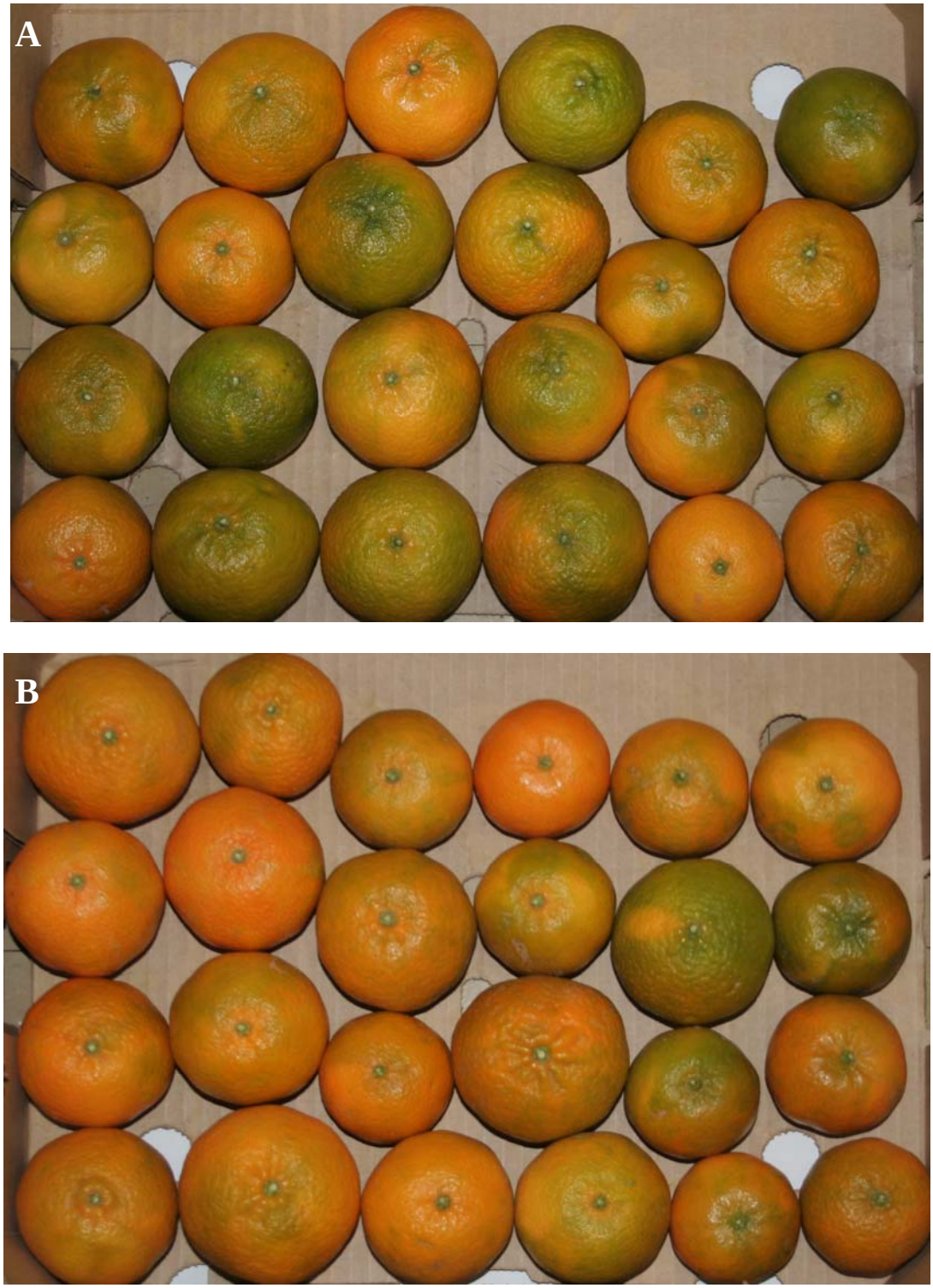

Fig. 5.3.3.1. Photographs of Nules Clementine mandarin fruit taken after harvest of fruit sampled during the 2006 season from the eastern side of trees to illustrate the effect of ProCa on rind colour enhancement. A: untreated control; B: late 400 $mg \cdot L^{-1}$ ProCa treatment. Note the more intensely coloured fruit of the ProCa treatment compared to the untreated control treatment.

Fig. 5.3.3.2. Photographs of Navelina Navel orange fruit taken after harvest of fruit sampled during the 2006 season from the eastern side of trees to illustrate the effect of ProCa on rind colour enhancement. A: untreated control; B: late 400 $mg \cdot L^{-1}$ ProCa treatment. Note the more intensely coloured fruit of the ProCa treatment compared to the untreated control treatment.

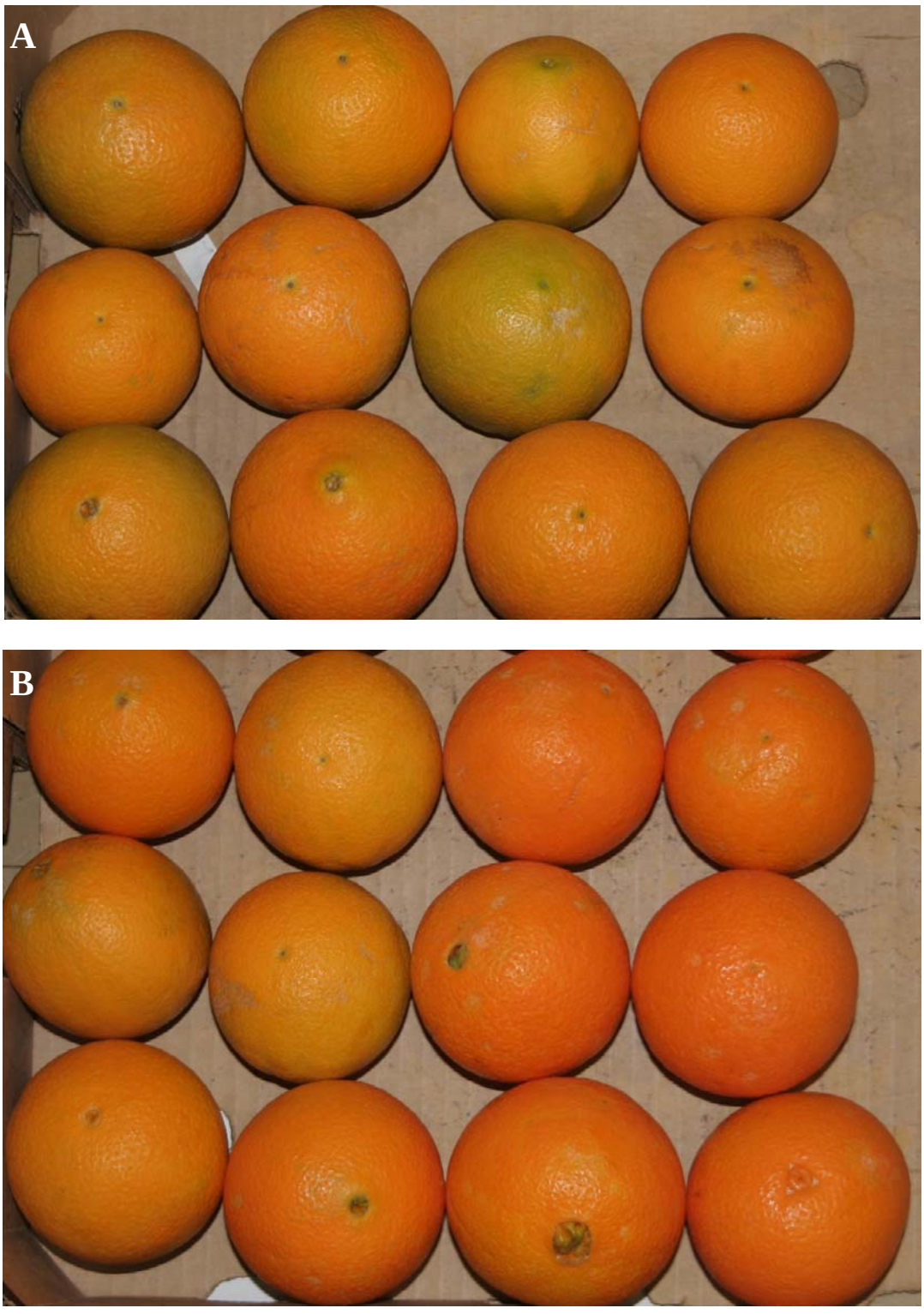

Fig. 5.3.3.3. Photographs of Palmer Navel orange fruit taken after harvest of fruit sampled during the 2006 season from the eastern side of trees to illustrate the effect of ProCa on rind colour enhancement. A: untreated control; B: late 400 mg·L^{-1} ProCa treatment. Note the more intensely coloured fruit of the ProCa treatment compared to the untreated control treatment.

Fig. 5.3.3.4. Photographs of Eureka lemon fruit taken after harvest of fruit sampled during the 2006 season from the eastern side of trees to illustrate the effect of ProCa on rind colour enhancement. A: untreated control; B: late 400 $mg \cdot L^{-1}$ ProCa treatment. Note the more intensely coloured fruit of the ProCa treatment compared to the untreated control treatment.

5.3.4 **Improving colour of physiologically mature citrus fruit**

By M. Mosoeunyane, I. Bertling and J. Bower (UKZNP)

Opsomming

Om vrugkleur van fisiologiese ryp sitrusvrugte te verbeter, is 'n vooroes en 'n na-oes benadering gevolg in 2006. As vooroes benadering is die effek van blaartoediening van molibdeen (Mo) en tungsten (W) op vrugkleur van vroeë Valencia, Turkey en Navel vrugte ondersoek. As deel van die na-oes benadering is vrugte blootgestel aan koue en warm temperature om die effek van koue en hitte skok op vrugkleurontwikkeling te ondersoek. Om te bepaal tot watter temperature die perikarp en pulp van vrugte afgekoel word wanneer in 'n 4ºC koelkamer geplaas word, is perikarp en pulp temperature oor 'n 24 h periode bepaal. Die perikarp en pulp temperature van Navel, Turkey en Valencia vrugte gepluk by die geel-groen stadium (T5) het geval tot 3.95ºC (perikarp) en 5.78ºC (pulp) binne 9 uur, wat aandui dat perikarp temperature lugtemperatuur van die koelkamer kan bereik, terwyl pulpweefsel betekenisvol hoër sal bly. Die effek van dompeling van T5 vrugte in 1 en 10 µM oplossings van Mo en W om vrugkleur te verbeter is ook ondersoek. Vrugte is na dompeling verskuif na 'n 4ºC koelkamer en gevolglik hitte skok ontvang in 'n waterbad. Die blootstelling van die 3 sitrustipes aan koue gevolg deur hitte skok het ontgroening betekenisvol verbeter in vergelyking met die kontrole of met vrugte wat net koue skok ontvang het. Verder, koue gevolg deur hitte skok was verantwoordelik vir die ontwikkeling van 'n skil abnormaliteit in na-oes Mo en W behandelde Valencia en W behandelde Navel vrugte. Die abnormaliteit is ook waargeneem na koue/hitte na-oes bahandeling op Navel vrugte van bome wat blaarbespuitings van W ontvang het. Navel vrugte wat voor- en na-oes behandelings van Mo ontvang het, het nie die abnormaliteit getoon nie. Voor- en na-oes behandeling van Navel vrugte met Mo blyk om weerstand teen die abnormaliteit te ontwikkel. Die abnormaliteit was nie teenwoordig op Turkey vrugte wat dieselfde behandelings ontvang het nie. Dehalwe, fisiologiese respons van sitrusvrugte, behandel (voor- of na-oes) met W, koue en gevolg deur hitte skok is kultivar afhanklik. Blootstelling van ryp sitrusvrugte aan koue en hitte skok kan gebruik word as 'n na-oes metode om kleurverandering te bewerkstellig. Blootstelling van Navel en Valencia vrugte aan koue water by 4ºC vir een uur (HC) of 'n warm waterbad vir 30 sekondes (HWD) of 'n koue waterbad gevolg deur 'n warm waterbad (HC & HWD) het chlorofil afbraak beïnvloed. Die HC & HWD behandeling het die laagste chlorofil konsentrasies van al die behandelings tot gevolg gehad. Hidroverkoeling van vrugte vir een uur of 'n dompeling in warm water vir 30 sekondes (HWD) het karotenoïed konsentasie verhoog in Navel en Valencia T5 skil. Dit was duidelik in vrugte versamel 14 dae na koue/hitte behandeling (DAT). HC & HWD het 'n soortgelyke effek op T5 Navel vrugte gehad, maar nie op Valencia vrugte nie. Om die fisiologiese prosesse van die koue en hitte behandelings te verstaan is daar gepoog om peroksidase aktiwiteit (guaiacol peroksides (GPX), as 'n maatstaf van stres en weerstand teen stress in warm en/of koue water behandelde vrugte te bepaal. 'n Betekenisvolle afname in GPX aktiwiteit in Valencia vrugte a.g.v. van die HC, HWD en HC & HWD behandelings het voorgekom binne sewe dae na behandeling. Hierdie afname is gevolg deur 'n toename in GPX aktiwiteit. Die toename, behalwe in die HC behandelde vrugte, het egter nie die versamelde kontrole vrugte oorskry nie. 'n Soortgelyke respons (verlaging in GPX aktiwiteit) is waargeneem by Navel vrugte, behalwe by die HWD 30s vrugte. Alhoewel daar 'n verhoging was 7 dae na behandeling in T4 vrugte, was die aktiwiteit steeds laer of gelyk aan die kontrole. Die resultate demonstreer dat warm en koue water behandeling kleurverandering versnel van Valencia, Turkey en Navel vrugte moontlik a.g.v. "stres" van die behandelings. Hierdie stres is heel moontlik die oorsaak vir die toename in karotenoïed konsentrasie in die skil. Die meganisme hierby betrokke moet verder ondersoek word.

Introduction

Fruit colour is the most important external quality parameter in citrus, enticing the consumer to purchase the fruit on offer. The colouring of fruit occurs due to alteration in temperature regimes once the fruit is physiologically mature. Cold treatments have been found to induce the colour change and artificial cold exposure results in colour change. Our previous experiments (CRI annual research report Mosoeunyane, Bertling and Bower, 2005) have indicated that, besides cold (Ramos and Rodriquez-Amaya, 1987), certain micronutrients affect the carotenoid pathway (Lee and Milborrow, 1997, Milborrow, 2001) and could therefore be involved in colour change in citrus.

Furthermore it has been speculated that the seasonal changes in weather patterns, as citrus fruit mature, result in a cold stress experienced by the fruit. Carotenoid synthesis in particular is increased due to stress (Young and Britton, 1990). Measuring the effect of a treatment to enhance colour on "stress indicators" could provide an important tool to determine the ability of a treatment to enhance the carotenoid concentration in the stressed tissue. Most importantly, pigment profiles, alterations in chlorophyll as well as carotenoid concentration following such treatment have not been reported. This is particularly important if such postharvest treatments were to be included in a packing line. It is vital to determine how severe a colour

change can be affected by such treatment as well as after which time-frame such an alteration in colour would be visible.

Furthermore, it is important to investigate if such treatments which positively affect flavedo colour also alter other fruit quality parameters as TSS and total acid and sugar concentration in order to be able to determine the potential for commercial usage.

In order to affect external colour of early maturing citrus fruit, four different approaches were taken and their effect on other fruit quality parameters evaluated.

Materials and methods

Preharvest treatments to affect colour change

Foliar application of micronutrients. The concentration of Mo used for foliar application to citrus trees was adopted from Longbottom (*pers. com.*) (0 g, 0.101 g and 0.202 g per tree). Trees to which previously 0, 0.202 and 0.404 g Mo or W had been applied were given a boost of 0.05 g Mo and W nine weeks after the first application of Mo and W (ca. 27 weeks after full bloom). A drift retardant (Control Mist®) and a wetting and sticker agent (Nu-film P®) were added to each treatment solution according to the manufacturers' instructions.

Determination of fruit quality parameters

Leaf and fruit sampling and tissue preparation. Prior to the nutrient application, three fruit and forty leaves per tree were sampled and transported in a cooler box to the Horticultural Science UKZN-PMB laboratory. Subsequently samples were stored at -20°C until processed. Half of the sampled leaves were freeze-dried while the other half was shock frozen and stored at -20°C. The fruit rind was peeled off, 50% was freeze-dried while the other half was shock frozen with liquid nitrogen and subsequently stored at -20°C. The pulp was treated similarly. One half was dried while the other half was kept at -20°C for TSS analysis.

Extraction of sugars from citrus tissue. The ground, freeze-dried plant material (100 mg) was suspended in 10 mL chilled 80% (v/v) ethanol and homogenized for two minutes on ice. The supernatant was separated from the pellet and an additional 10 ml of solvent added to the pellet for re-extraction. The pH of the extract was measured, and if necessary, adjusted with 0.1N NaOH to pH 7± 0.02. The pooled extracts were heated in a hot water bath at 80ºC for 15 minutes. Subsequently, the homogenate was centrifuged (10 000 rpm, 15', 4ºC), dried *in vacuo* and stored at -20ºC until analysis.

Determination of soluble solutes in the pulp of Navel and Turkey fruit. The sampled pulp was thawed at room temperature and the juice extracted using a juice extractor. The juice was centrifuged and the soluble solute concentration was subsequently determined in 100 µl of supernatant using a refractometer. Analysis of variance showed that treatments had a significant effect (P=0.009) on soluble solutes (Brix %) in Navel and Turkey juice. Fruit from trees foliar-fed with 0.404 g Mo or W had a higher Brix% even though that was not statistically different from the control (Fig. 5.3.4.1). On the other hand, foliar application of 0.202 g W resulted in statistically higher Brix%.

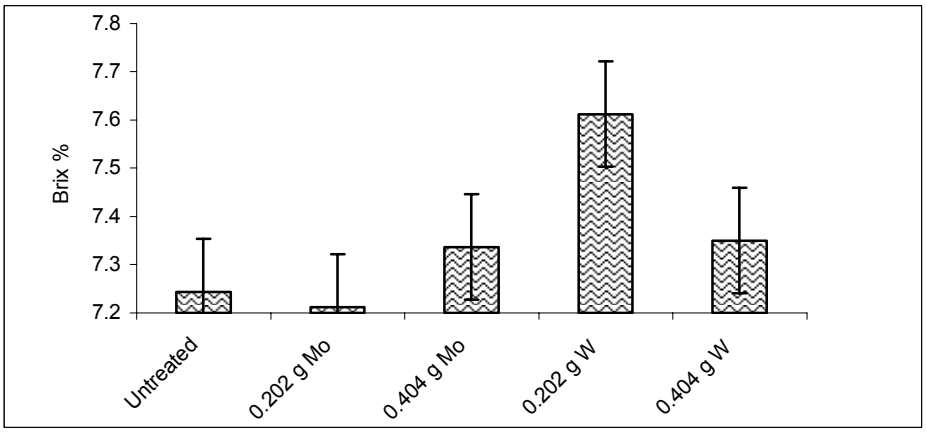

Fig. 5.3.4.1. Soluble solutes (Brix%) in Turkey and Navel juice.

This experiment is not completed yet; further evaluations are needed as the cooler season is approached (May-June 2007) in order to determine the effect of Mo and W applications on early colour development.

Postharvest treatments to affect colour change

Determining temperature changes of citrus pericarp and pulp during storage in a 4°C cold room. In order to determine to which temperature fruit rind and juice vesicles cool in which time frame, Turkey and Navel T5 colour grade (yellowish-green) fruit were picked and stored in a 4°C cold room for 24 hours. The pulp temperature was measured after 0, 3, 6, 9, 12 and 24 hours of exposure using a digital thermometer. Eight fruit were sampled and a probe was inserted through to the centre of the pulp. Eight mini-sensors were used to measure temperature changes of the pericarp (1 mini-sensor per fruit). A small flap was cut on Turkey pericarp with a sharp thin knife in order to insert a thermocouple beneath the pericarp. The thermocouple was inserted and the injured area covered with tape in order to impose as little alteration as possible to the metabolism of the fruit. A further thermocouple was adhered to the surface of the peel of Navel fruit with a tape.

Temperature changes of citrus pericarp and pulp during storage in a 4°C cold room. On average the pericarp temperature of Navel, Turkey and Valencia fruit took 8 to 9 hours to drop to 3.95°C, while that of the pulp reached 5.97°C within the same period (Figs. 5.3.4.2 A and 5.3.4.2 B, respectively). Fruit were, therefore, cold-shocked in a 4ºC cold room for 9 hours.

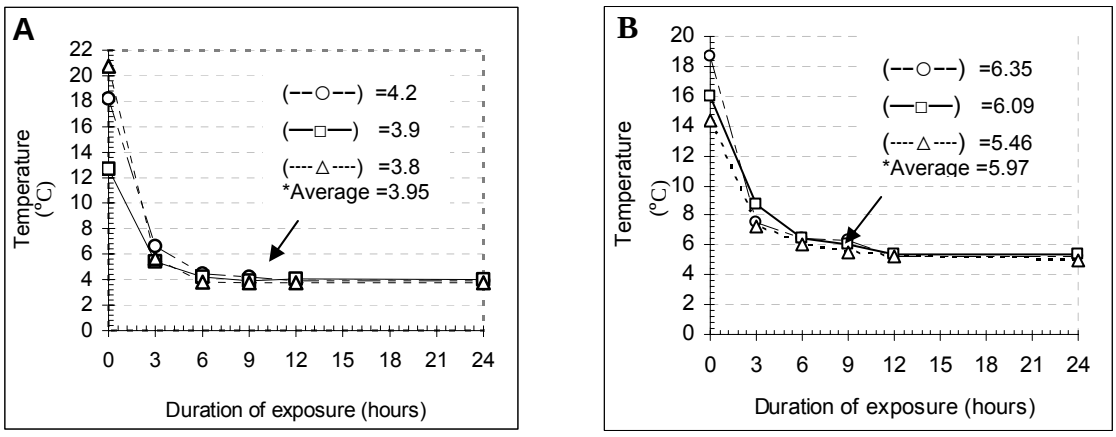

Fig. 5.3.4.2. Temperature changes of citrus pericarp (**A**) and pulp (**B**) during incubation in a 4ºC cold room. Pulp temperature was measured at the centre of the fruit with a digital thermometer. The pericarp temperature of Turkey (--○--) and Valencia (----△----) was measured with a mini-sensor just beneath the pericarp. A small flap was cut to accommodate the thermocouple. The pericarp temperature of Navel (—□—) was measured with the same instrument on the surface of the pericarp. The thermocouple of the mini-sensor was adhered to the surface of the pericarp with a tape.
*Average is the mean of pericarp or pulp temperature (ºC) at the 9th hour of incubation in a 4ºC cold room.

In vitro effect of Mo and W coupled with air-cooling and hot water dipping treatments

Method. Fruit were picked at the yellowish-green (T5) stage from OrangeWood Farm in Cramond, KZN. They were immersed in 1 and 10 µM solutions of Mo and W for 2 hours. Subsequently, fruit were incubated in a 4ºC cold room for 9 hours and thereafter heat-shocked in a hot water bath. The duration of the incubation period was based on the previous experiment (Fig. 5.3.4.2), indicating that pericarp temperature reaches the 4ºC plateau after 9 hours. This experiment was a follow-up of the results obtained by Oberholster (2001), which showed that incubating Valencia flavedo discs for 96 hours in certain Mo and W solutions resulted in a significant increase in carotenoid concentration.

Results. The exposure of Valencia fruit, treated with micronutrients or untreated, to cold air followed by a dip in hot water resulted in the development of a peel disorder (Fig. 5.3.4.3). Similar results were observed on Navel fruit treated with 1 and 10 µM W and untreated fruit. Navel fruit treated with Mo (Fig. 5.3.4.5 A and C) and Turkey fruit (Fig. 5.3.4.4) treated with both, Mo or W, did not develop this peel disorder.

Effect of cold and heat-shock on skin colour change

Method. Citrus fruit (Navel, Turkey and Valencia) were picked at two physiological maturity stages, T5 and T4, from OrangeWood Farm in Cramond in April, May and June 2006. Fruit were cold-shocked in a 4ºC water bath (1 hour) followed by a heat-shock in a hot water bath (2 minutes) within 24 hours of picking.

Subsequently, the fruit were waxed and kept at room temperature for a maximum of 14 days. Skin colour change was monitored and pictures were taken 7 and 14 days after treatment (DAT).

Results. Cold-shock followed by heat-shock enhanced degreening of fruit significantly compared to untreated fruit and those that were subjected to cold-shock alone (Figs. 5.3.4.6, 5.3.4.7 and 5.3.4.8). The current results accord with the preliminary results obtained in 2005.

Cold and heat-shock in field-Mo- or W-treated fruit

Method. Citrus fruit (Navel and Turkey) that had been foliar-sprayed in December 2005 with 500 mL, 0.20 g or 0.404 gL^{-1} of Mo or W were picked at the T5 stage in April, May and June 2006, from OrangeWood Farm in Cramond, KZN. Fruit were cold-shocked in a 4ºC water bath (1 hour) followed by a heat-shock in a hot water bath (2 minutes) within 24 hours of picking. Subsequent to that, fruit were waxed and kept at room temperature for a maximum of 14 days. Control fruit (treated and untreated) were kept at room temperature. Skin colour change was monitored and pictures were taken 7 and 14 DAT.

Results. Figs. 5.3.4.9 and 5.3.4.10 reveal that cold followed by heat-shock resulted in skin colour change from yellowish-green to yellow/orange in field-treated and untreated fruit. On the other hand, fruit from foliar-sprayed trees that were kept at room temperature without postharvest shock treatment did not de-green significantly. Nonetheless, Navel fruit that were foliar-fed with two levels of W developed a skin disorder similar to those observed in postharvest W-treated fruit after being subjected to cold- and heat-shock (Figs. 5.3.4.9 G and I).

Postharvest temperature treatments may influence colour change in Navel and Valencia peel

Material and methods

Plant material. T4 and T5 Navel and Valencia fruit were picked from OrangeWood Farm, Cramond, KZN.

Experimental treatments. Fruit were hydro-cooled at 4°C for an hour followed by a brief dip into a hot water bath. Other fruit were simply hydro-cooled for one hour (no dip into a hot water bath); another batch was dipped in a hot water bath for 30 seconds. Control fruit were not treated. Treated and untreated fruit were waxed and kept in boxes in the dark at room temperature (≤ 20ºC) and sampled at various intervals.

Extraction of pigments. Flavedo tissue of citrus fruit was scraped from the equatorial region of fruit with a peeler, immediately frozen in liquid nitrogen, stored at -20ºC and lyophilized. Dry flavedo tissue was ground in an ice-cold mortar with a pestle. The ground flavedo was homogenized in 10 mL chilled 95% ethanol (v/v) with an UltraTurrax® on ice. The supernatant was centrifuged at 5000 RPM (table top centrifuge) for 5 minutes.

Spectrophotometric determination of total carotenoid concentration. For chlorophyll and carotenoid determination, absorbance readings of the extracts were recorded at different wavelengths (470, 648.6, 664.2 nm; Lichtenthaler, 1987). The calculations for the determination of the total carotenoid concentration (C_{x+c}) were performed using equations provided by the same author. Carotenoid concentrations were expressed as $\mu g\ g^{-1}$ exocarp.

Results. Exposing Navel and Valencia fruit to a cold water bath for one hour (HC) or a hot water bath for 30 seconds (HWD) or a cold water bath followed by a hot water dip (HC&HWD) positively influenced chlorophyll degradation. However, HC&HWD treatment resulted in a significantly lower chlorophyll concentration than other treatments including the control, except for T4 fruit 7 DAT (Table 5.3.4.1).

Hydro-cooling (HC) fruit for one hour or a brief dip in hot water for 30 seconds (HWD) resulted in an increase in carotenoid concentration in Navel and Valencia fruit picked at the T5 stage 14 days after treatment (DAT) (Table 5.3.4.2). In addition to that, the dip of fruit into a cold water bath for one hour followed by a dip into a hot water bath for 2 minutes (HC&HWD) increase the carotenoid concentration. In Navel fruit the carotenoid concentration increased beyond the level of the control while in Valencia carotenoids increased compared to the benchmark fruit but not the control.

Table 5.3.4.1. Total chlorophyll concentration (µg/g dry flavedo weight) ± standard deviation (SD) in Navel and Valencia (n = 6) fruit picked at two colour grades

Citrus type	Treatments	Colour grade of fruit at picking			
		T4		T5	
		7 DAT	14 DAT	7 DAT	14 DAT
Navel	Benchmark*	$158.9571^{\pm 4.347}$		$161.5469^{\pm 3.5300}$	
	Control	$72.83^{\pm 3.0464}$	$57.46^{\pm 1.9033}$	$103.15^{\pm 1.5098}$	$50.45^{\pm 2.8368}$
	HC only	$51.87^{\pm 1.6317}$	$39.47^{\pm 1.2131}$	$87.83^{\pm 1.9851}$	$89.97^{\pm 2.4932}$
	HWD	$37.06^{\pm 0.5008}$	$44.21^{\pm 2.7787}$	$89.81^{\pm 1.7885}$	$54.19^{\pm 1.7661}$
	HC & HWD	$37.78^{\pm 1.3462}$	$21.94^{\pm 1.5846}$	$43.15^{\pm 1.4523}$	$34.72^{\pm 2.0656}$
Valencia	Benchmark*	$81.6667^{\pm 1.2552}$		$102.082^{\pm 1.7519}$	
	Control	$56.42^{\pm 2.4999}$	$67.41^{\pm 0.6246}$	$87.56^{\pm 2.1232}$	$80.10^{\pm 2.4315}$
	HC only	$58.62^{\pm 1.9579}$	$59.23^{\pm 5.1268}$	$78.61^{\pm 1.9753}$	$76.22^{\pm 0.8651}$
	HWD	$54.88^{\pm 2.0324}$	$54.21^{\pm 2.1265}$	$45.64^{\pm 2.1163}$	$69.41^{\pm 3.5933}$
	HC & HWD	$47.48^{\pm 1.9398}$	$45.55^{\pm 2.3362}$	$36.10^{\pm 2.7694}$	$38.87^{\pm 0.08275}$
	$F_{prob.}$	<.001			
	$L.S.D_{0.05}$	2.494			

Key: Control = fruit stored at room temperature from day zero. HC & HWD = hydro-cooled at 4ºC for one hour and dipped in 60ºC hot water for 2 minutes. HWD = hot water dip (60ºC) for 30 seconds. DAT = days after treatment (post-harvest temperature treatment) on which treated fruit were sampled.
*Benchmark fruit were sampled on day zero.

Table 5.3.4.2. Total carotenoid concentration (µg/g dry flavedo weight) ± standard deviation (SD) in Navel and Valencia (n = 6) fruit picked at two colour grades

Citrus type	Treatments	Colour grade of fruit at picking			
		T4		T5	
		7 DAT	14 DAT	7 DAT	14 DAT
Navel	Benchmark*	$194.2067^{\pm 3.7494}$		$185.9104^{\pm 3.7796}$	
	Control	$206.12^{\pm 1.9635}$	$221.29^{\pm 1.3584}$	$173.29^{\pm 2.2555}$	$192.31^{\pm 1.9537}$
	HC only	$208.17^{\pm 2.8576}$	$205.89^{\pm 1.6486}$	$178.69^{\pm 4.7163}$	$199.41^{\pm 1.2078}$
	HWD	$117.18^{\pm 2.7524}$	$238.81^{\pm 2.3057}$	$191.43^{\pm 3.5841}$	$209.01^{\pm 1.3337}$
	HC & HWD	$203.60^{\pm 2.9521}$	$210.41^{\pm 2.8148}$	$160.97^{\pm 1.8968}$	$198.03^{\pm 2.6212}$
Valencia	Benchmark*	$169.8072^{\pm 4.7305}$		$147.7829^{\pm 2.5411}$	
	Control	$205.50^{\pm 5.4531}$	$257.09^{\pm 1.0457}$	$216.21^{\pm 0.3186}$	$247.93^{\pm 1.5718}$
	HC only	$214.54^{\pm 1.5803}$	$252.73^{\pm 3.0998}$	$220.04^{\pm 2.3049}$	$261.05^{\pm 2.5640}$
	HWD	$217.39^{\pm 1.2166}$	$227.56^{\pm 1.3048}$	$206.55^{\pm 1.4343}$	$255.59^{\pm 2.3952}$
	HC & HWD	$178.07^{\pm 2.0121}$	$216.27^{\pm 2.1361}$	$207.15^{\pm 1.4257}$	$216.87^{\pm 1.3073}$
	$F_{prob.}$	<.001			
	$L.S.D_{0.05}$	2.650			

Key: Control = Stored at room temperature. HC & HWD = hydro-cooled at 4ºC for one hour and dipped in a 60ºC hot water bath for 2 minutes. HWD = hot water dip (60ºC) for 30 seconds. DAT = days after treatment (post-harvest temperature treatment) on which treated fruit were sampled.
*Benchmark fruit were sampled on day zero.

<u>Cold and hot water bath induced changes in peroxidase activity in the peel of Navel and Valencia fruit</u>

Many fruit and vegetables of tropical and subtropical origin exhibit physiological dysfunctions when exposed to non-freezing temperatures below 12ºC. As a result, several physiological and biochemical alterations occur in response to chilling stress. Plant resistance to different stresses is often a result of an increase in protective enzymes such as peroxidase and catalase. Peroxidases have free radical scavenging properties under chilling conditions (Burris, 1960) and play a role in protecting plant cells from reactive oxygen species (ROS). Therefore, it was hypothesised that postharvest dipping of fruit into a 4ºC and/or a 60ºC water bath would stress citrus fruit resulting in high production of ROS and therefore in increased enzymatic antioxidant

activity. Most peroxidases are involved in hydrogen peroxide elimination, making them very useful indicators of oxidative stress. Therefore, a spectrophotometric determination of guaiacol peroxidase, as an indicator of peroxidase activity, was carried out on lyophilised flavedo tissue.

Material and methods

Plant material and treatment conditions. Navel and Valencia fruit of the T4 and T5 colour grade were picked at OrangeWood Farm in Cramond, KZN. Within 24 hours of picking fruit were separated and divided into treatments. Control fruit were stored at room temperature (20ºC). Hydro-cooled fruit (HC) were subjected to a 4ºC water bath for one hour. HC&HWD fruit were treated like the HC ones and thereafter dipped in a 60ºC water bath for 2 minutes. HWD fruit were dipped in a 60ºC hot water bath for 30 seconds only. The different holding times in the hot water bath of these two treatments emanated from previous experiments, indicating that a similar flavedo temperature is reached by 20ºC stored fruit if subjected to a 30 s 60ºC hot water bath dip as by 4ºC fruit after 2 min in a hot water bath of the same temperature. Benchmark fruit were sampled, peeled and stored at -20ºC. Treated (HC, HC&HWD and HWD) and untreated (Control) fruit were waxed prior to storage at room temperature. Samples from these treatments were collected 7 and 14 days after treatment (DAT).

Guaiacol peroxidase extraction procedure. The activity of guaiacol peroxidase (GPX) was determined as follows. Lyophilised citrus flavedo, chilled with liquid nitrogen, was ground to a powder using a chilled pestle and mortar. The powder (100 mg), to which PVP was added, was homogenised in 5 mL of ice-cold extraction buffer using an Ultra-Turrax T_{25} homogeniser at maximum speed on ice for one minute. The extraction buffer consisted of 0.1 M TRIS-HCl buffer, pH 8, to which 1 mM EDTA was added. The homogenate was filtered through two layers of muslin cloth. The filtrate was centrifuged at 16 000 rpm for 15 minutes at 4ºC. The reaction mixture contained 5 mM guaiacol and 5 mM H_2O_2 in 0.2 M phosphate buffer at pH 5.8.

Spectrophotometric determination of peroxidase activity. GPX activities were determined immediately after extraction. The reaction of total guaiacol peroxidase was initiated by addition of 200 μL of enzyme extract to 800 μL of reaction mixture. The increase in absorbance due to formation of a brown coloured tetraguaiacol was monitored on a DU® 800 UV/Vis spectrophotometer at 470 nm 0 and 60 seconds after addition of the enzyme extract at ambient temperature. The difference in absorbance (ΔA_{470}) was divided by the tetraguaiacol molar extinction coefficient (26.6 mM^{-1} cm^{-1}) and the enzyme activity expressed as μmol of H_2O_2 min^{-1} mg^{-1} protein, taking into consideration that 4.0 mol of H_2O_2 are reduced to produce 1.0 mol of tetraguaiacol (Plewa *et al*., 1991). A blank was the reaction mixture without the additional 200μL enzyme.

Results. A significant decrease in GPX activity in Valencia fruit of the HC, HWD, and HC & HWD treatments was found within seven days after treatment (Table 5.3.4.3). This decrease was followed by an increase in GPX activity. However, the increase - except in the HC treated fruit - did not surpass that of the benchmark fruit. A similar response (reduction in GPX activity) was observed in Navel fruit except for the HWD fruit. Although there was an increase 7 DAT in T4 fruit this activity was still lower than or equal to that of control fruit. The decrease in GPX activity may be related to a change from stressful temperature to relatively optimal or less stressful environment (20ºC) where fruit were stored after treatment. The variation in GPX activity 14 DAT may be attributed to the interaction between cultivar, sampling date, colour grade and treatments. The HC treatment induced significantly higher activity in Valencia fruit than control Valencia 14 DAT in T4 and T5 fruit, indicating that such treatments could have increased the carotenoid concentration due to the stressfullness of this particular treatment.

Table 5.3.4.3. Guaiacol peroxidase (GPX) activity (μmol H_2O_2 min^{-1} mg^{-1} protein) in the peel of Navel and Valencia fruit dipped in cold and/or hot water. Control fruit were stored at room temperature (ca. ≤ 20ºC). Treated fruit were stored at room temperature for maximum of 14 DAT. Each point represents the mean value of six measurements

Citrus type	Treatments	Colour grade			
		T4		T5	
		7 DAT	14 DAT	7 DAT	14 DAT
		Guaiacol Peroxidase activity		(μmol H_2O_2 min^{-1} mg^{-1} protein)	
Valencia	Benchmark*	0.05084^{b}		0.04695^{b}	
	Control	0.05014^{b}	0.05001^{b}	0.04830^{b}	0.04831^{b}
	HC Only	0.04812^{c}	0.05523^{a}	0.04688^{b}	0.05816^{a}
	HWD 30s	0.04476^{d}	0.04449^{d}	0.04695^{b}	0.04433^{d}
	HC & HWD**	0.04381^{e}	0.04825^{c}	0.04270^{c}	0.03978^{f}

Citrus type	Treatments	Colour grade			
		T4		T5	
		7 DAT	14 DAT	7 DAT	14 DAT
Navel	Benchmark*	0.03669[a]		0.03264[b]	
	Control	0.03850[a]	0.0364[a]	0.03107[bc]	0.03007[c]
	HC Only	0.03363[c]	0.03631[b]	0.02857[d]	0.03036[c]
	HWD 30s	0.02887[d]	0.03037[d]	0.03448[a]	0.03365[a]
	HC & HWD**	0.03226[c]	0.03339[c]	0.02444[e]	0.02774[d]
	$F_{prob.}$	<.001			
	$L.S.D_{0.05}$	0.0019			

Key: Control = Stored at room temperature, HC = hydro-cooled at 4ºC for one hour, **HWD = hot water dip (60ºC) for 2 minutes, HWD = hot water dip (60ºC) for 30 seconds. DAT = days after treatment (post-harvest temperature treatment) on which treated fruit were sampled.

Conclusions

GPX activity in both T4 and T5 Valencia fruit, was increased by HC treatment while HWD increased the activity in T5 Navel, possibly indicating a stress response to the treatment. Further investigation into other stress parameters needs to be conducted as GPX is only one of the enzymatic anti-oxidants. Determination of total antioxidants as well as activity of other antioxidant enzymes need to be carried out in order to reveal the physiological processes behind the hydrocooling and the hot water bath treatments.

Re-application of molybdenum and tungsten to Navel and Valencia trees

Molybdenum nutrition is an essential component to healthy plant growth (Kaiser *et al*., 2005). Molybdate is highly mobile in plants where foliar absorption and translocation occur quickly (Williams *et al.,* 2004). Molybdenum fertilization through foliar sprays can effectively supplement internal Mo deficiency and rescue the activity of molybdo-enzymes (Kaiser *et al*., 2005). Mo and W are important in two-electron redox reactions (Johnson *et al*., 1996). It has been reported that tungsten assumes a role similar to that of Mo in bacteria (Adams, 1994). Further, Mo and W play a role in high temperature conditions (Johnson *et al*., 1996).

Materials and methods. Molybdenum (Mo), supplied as sodium molybdate, and tungsten (W), supplied in the form of sodium tungstate were dissolved in ultra-pure water. An amount of 0.2 g or 0.4 gL^{-1} of each nutrient mineral (Mo or W) was dissolved according to Longbottom (*pers. com*) in a total of 15 L water. No Mo or W was supplied to control trees. A drift retardant (ControlMist®) and a wetting and sticker agent (Nu-filmP®) had been added to each solution according to the manufacturers' instructions. The solution was sprayed at a rate of 1000 mL per tree.

Procedure. One thousand millilitres of molybdate or tungstate was sprayed onto each tree on a fine day 19 weeks after full bloom.

Experimental design. The experimental design was a randomized complete block design. Each row was considered a block. Every treatment level was replicated six times.

Results. Results of this experiment have not yet been analysed. Analysis will continue in 2007.

Overall conclusions

The approach to improve citrus colour through Mo and W application has resulted in a positive response as a postharvest approach. Whether the preharvest application of Mo and W will have a similar positive effect needs further investigation, data pertaining this experiment will be available probably in July or August 2007.

We have clearly demonstrated that - through hydrocooling and a dip into a hot water - colour of citrus fruit at the T4 and T5 stage can be improved. This change in colour appears through chlorophyll degradation and/ or carotenoid synthesis. The question whether this change in colour is due to the stressfulness of the treatment cannot be answered yes, as the enzymatic antioxidant system investigated did not give a clear indication whether the treatments were stressful or not. Therefore other experiments need to be conducted to understand the action of the hydrocooling/ hot water dipping on the production of antioxidants.

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Valencia 2006

Figure A. Immersed in **10 µM Mo** (2 hrs). Air-cooled (9 Hrs), dipped in hot water (2 min) and stored at room temperature. Picture was taken **7 DAT**.

Figure B. Immersed in **10 µM W** (2 hrs). Air-cooled (9 Hrs), dipped in hot water (2 min) and stored at room temperature. Picture was taken **7 DAT**.

Figure C. Immersed in **1 µM W** (2 hrs). Air-cooled (9 Hrs), dipped in hot water (2 min) and stored at room temperature. Picture was taken **7 DAT**.

Figure D. Immersed in **1 µM Mo** (2 hrs). Air-cooled (9 Hrs), dipped in hot water (2 min) and stored at room temperature. Picture was taken **7 DAT**.

Figure E. 0 elements. Stored at room temperature. Picture was taken **7 DAT**.

Figure F. 0 elements. Air-cooled (9 hrs) & dipped in hot water (2 min) and stored at room temperature. Picture was taken **7 DAT**.

Fig. 5.3.4.3. Colour changes of T5 Valencia fruit immersed in 1 and 10 µM solutions of Mo and W. Control (o elements) fruit were not treated.

Turkey 2006

Figure A. 10 μM Mo. Air cooled (9 hrs) and dipped in hot water (2 min). Stored at room temperature. Picture was taken 7 DAT.

Figure B. 10 μM W. Air cooled (9 hrs) and dipped in hot water (2 min). Stored at room temperature. Picture was taken 7 DAT.

Figure C. 1 μM Mo. Air cooled (9 hrs) and dipped in hot water (2 min). Stored at room temperature. Picture was taken 7 DAT.

Figure D. 1 μM W. Air cooled (9 hrs) and dipped in hot water (2 min). Stored at room temperature. Picture was taken 7 DAT.

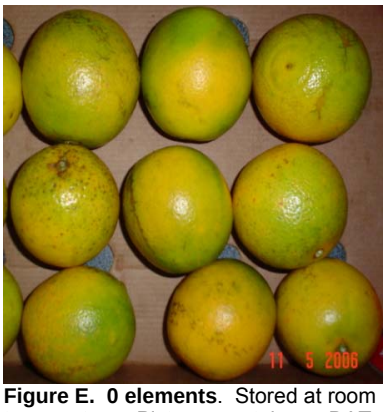

Figure E. 0 elements. Stored at room temperature. Picture was taken 7 DAT.

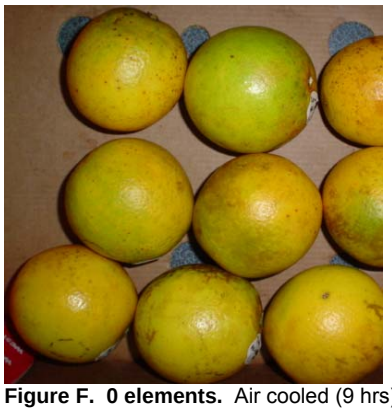

Figure F. 0 elements. Air cooled (9 hrs) and dipped in hot water (2 min). Stored at room temperature. Picture was taken 7 DAT.

Fig. 5.3.4.4. Colour changes of T5 Turkey fruit immersed in 1 and 10 μM solutions of Mo and W. Control (o elements) fruit were not treated.

Navel 2006

Figure A. Immersed in **10 µM Mo**. Air cooled at 4°C (9 hrs) and dipped in hot water bath (2 min). Picture was taken 7 DAT.

Figure B. Immersed in **10 µM W**. Air cooled at 4°C (9 hrs) and dipped in hot water bath (2 min). Picture was taken 7 DAT.

Figure C. Immersed in **1 µM Mo**. Air cooled at 4°C (9 hrs) and dipped in hot water bath (2 min). Picture was taken 7 DAT.

Figure D. Immersed in **1 µM W**. Air cooled at 4°C (9 hrs) and dipped in hot water bath (2 min). Picture was taken 7 DAT.

Figure E. Navel fruit stored at room temperature. They were neither air-cooled nor dipped in hot water. Picture was taken 7 DAT.

Figure F. 0 elements. Air cooled at 4°C (9 hrs) and dipped in hot water bath (2 min). Picture was taken 7 DAT.

Fig. 5.3.4.5. Colour changes of T5 Navel fruit immersed in 1 and 10 µM solutions of Mo and W. Control (o elements) fruit were not treated.

Figure A. Control. T5 at room temperature. Picture was taken 7 DAT.

Figure B. Control. T4 at room temperature.Picture was taken 7 DAT..

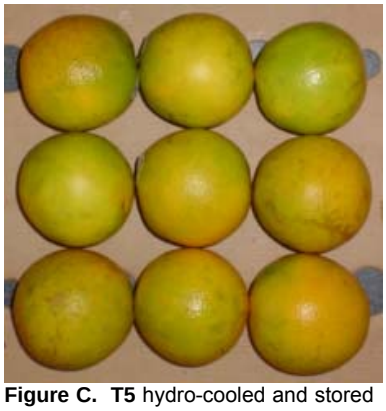

Figure C. T5 hydro-cooled and stored at room temperature. Picture was taken 7 DAT.

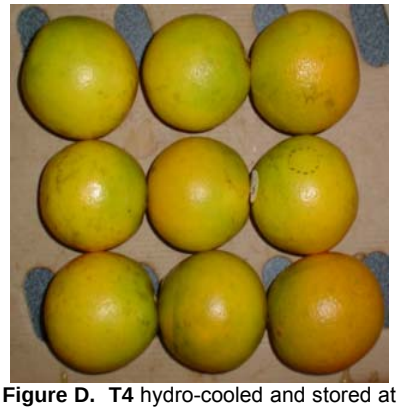

Figure D. T4 hydro-cooled and stored at room temperature. Picture was taken 7 DAT.

Figure E. T5 hydro-cooled and dipped in hot water. Stored at room temperature. Picture was taken 7 DAT.

Figure F. T4 hydro-cooled and dipped in hot water. Stored at room temperature. Picture was taken 7 DAT

Fig. 5.3.4.6. Colour changes of T5 and T4 Turkey fruit subjected to cold (1 hour) and heat (2 minutes) shock. Control fruit were stored at room temperature.

Navel 2006

Figure A. **T5** at room temperature. Picture was taken 7 DAT.

Figure B. **T4** at room temperature. Picture was taken 7 DAT.

Figure C. **T5** Hydro-cooled only. Picture was taken 7 DAT.

Figure D. **T4** Hydro-cooled only. Picture was taken 7 DAT.

Figure E. T5 Hydro-cooled and dipped in hot water. Picture was taken 7 DAT.

Figure F. **T4** Hydro-cooled and dipped in hot water. Picture was taken 7 DAT.

Fig. 5.3.4.7. Colour changes of T5 and T4 Navel fruit subjected to cold (1 hour) and heat (2 minutes) shock. Control fruit were stored at room temperature.

Valencia 2006

Figure A. T5 at room temperature. Picture was taken 7 DAT.

Figure B. T4 at room temperature. Picture was taken 7 DAT.

Figure C. T5 hydro-cooled (1 hr) only. Picture was taken 7 DAT.

Figure D. T4 hydro-cooled (1 hr) only. Picture was taken 7 DAT.

Figure E. T5 hydro-cooled and dipped in hot water. Picture was taken 7 DAT.

Figure F. T4 hydro-cooled and dipped in hot water. Picture was taken 7 DAT.

Fig. 5.3.4.8. Colour changes of T5 and T4 Valencia fruit subjected to cold (1 hour) and heat (2 minutes) shock. Control fruit were stored at room temperature.

Navel 2006

Figure A. Control. Navel not treated in field. Hydro cooled for an hour and dipped in hot water for two minutes. Stored at room temperature. Picture was taken 7 DAT.

Figure B. Control. Navel not treated in field. Stored at room temperature. Picture was taken 7 DAT.

Figure C. 0.202 g Mo. Navel treated in the field. Hydro cooled for an hour and dipped in hot water for two minutes. Stored at room temperature. Picture taken 7 DAT.

Figure D. 0.202 g Mo. Navel treated in the field. Stored at room temperature. Picture taken 7 DAT.

Figure E. 0.404 g Mo. Navel treated in the field. Hydro cooled for an hour and dipped in hot water for two minutes. Stored at room temperature. Picture taken 7 DAT.

Figure F. 0.404 g Mo. Navel treated in the field. Stored at room temperature. Picture taken 7 DAT.

Fig. 5.3.4.9 (A to F). Colour changes of T5 Navel fruit subjected to cold (1 hour) and heat (2 minutes) shock. Fertilizer treated fruit were picked from trees foliarly sprayed with two levels (0.202 and 0.404 g) of Mo and W in December 2005. Control fruit were stored at room temperature.

Figure G. 0.404 g W. Navel treated in the field. Hydro cooled an hour and dipped in hot water for two minutes. Stored at room temperature. Picture taken 7 DAT.

Figure H. 0.404 g W. Navel treated in the field. Stored at room temperature. Picture taken 7 DAT.

Figure I. 0.202 g W. Navel treated in the field. Hydro cooled an hour and dipped in hot water for two minutes. Stored at room temperature. Picture taken 7 DAT.

Figure J. 0.202 g W. Navel treated in the field. Stored at room temperature. Picture taken 7 DAT.

Fig. 5.3.4.9 (G to J). Colour changes of T5 Navel fruit subjected to cold (1 hour) and heat (2 minutes) shock. Fertilizer treated fruit were picked from trees foliarly sprayed with two levels (0.202 and 0.404 g) of Mo and W in December 2005. Control fruit were stored at room temperature.

Turkey 2006

Figure A. 0.202 g W foliarly sprayed. Fruit hydro-cooled and dipped in hot water. Picture was taken 7 DAT.

Figure B. 0.202 g W foliarly sprayed. Fruit stored at room temperature. Picture was taken 7 DAT.

Figure C. 0.404 g W foliarly sprayed. Fruit hydro-cooled and dipped in hot water. Picture was taken 7 DAT.

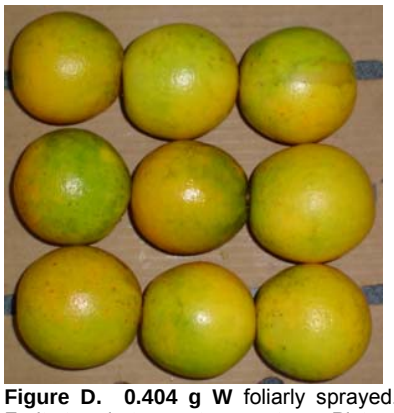

Figure D. 0.404 g W foliarly sprayed. Fruit stored at room temperature. Picture was taken 7 DAT.

Figure E. 0.202 g Mo foliarly sprayed. Fruit hydro-cooled and dipped in hot water. Picture was taken 7 DAT.

Figure F. 0.202 g Mo foliarly sprayed. Fruit stored at room temperature. Picture was taken 7 DAT.

Fig. 5.3.4.10 (A to F). Colour changes of T5 Turkey fruit subjected to cold (1 hour) and heat (2 minutes) shock. Fertilizer treated fruit were picked from trees foliarly sprayed with two levels (0.202 and 0.404 g) of Mo and W in December 2005. Control fruit were stored at room temperature.

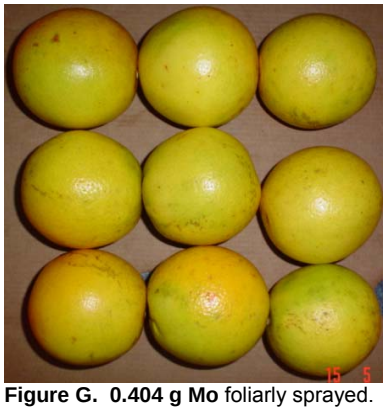

Figure G. 0.404 g Mo foliarly sprayed. Fruit hydro-cooled and dipped in hot water. Picture was taken 7 DAT.

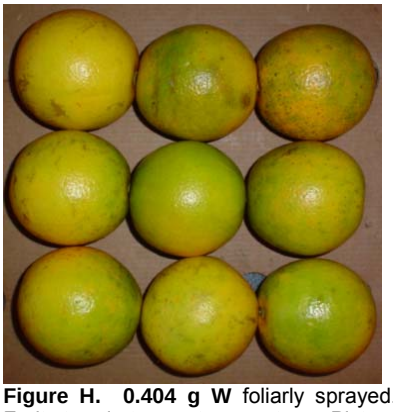

Figure H. 0.404 g W foliarly sprayed. Fruit stored at room temperature. Picture was taken 7 DAT.

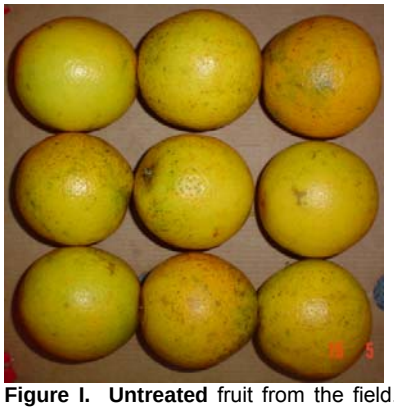

Figure I. Untreated fruit from the field. Fruit hydro-cooled and dipped in hot water. Picture was taken 7 DAT.

Figure J. Untreated fruit from the field. Fruit stored at room temperature. Picture was taken 7 DAT.

Fig. 5.3.4.10 (G to J). Colour changes of T5 Turkey fruit subjected to cold (1 hour) and heat (2 minutes) shock. Fertilizer treated fruit were picked from trees foliarly sprayed with two levels (0.202 and 0.404 g) of Mo and W in December 2005. Control fruit were stored at room temperature.

5.3.5 Reduction of acidity of high acid citrus cultivars using alternatives to calcium arsenate

Experiment ACID 01/02 by Graham Barry (CRI at SU)

Opsomming

Die doel van hierdie navorsing was om suur te verlaag in hoë suur sitrus kultivars deur alternatiewe tot kalsiumarsenaat te gebruik. Behandelings is toegedien 6 weke na volblom en vrugte is versamel elke vier weke vanaf vroeg Jul. 2006 tot oestyd om veranderings in suur oor tyd aan te teken. Gedurende die 2005-06 seisoen, het 'n enkele toediening van 1 of 2% MAP toegedien 6 weke na volblom op Delta en Midknight Valencia lemoene suur betekenisvol verlaag teenoor die kontrole met ≈0.3%, d.w.s. 'n intermediêre effek tussen die effek van Ca-arsenaat en die kontrole. Sapinhoud en Brix was nie deur die behandelings geaffekteer nie. Die verlaging in suur blyk om verband te hou met hoër blaar P-vlakke waar P-bevattende produkte toegedien is. Alhoewel Phytex® nie die suurvlakke betekenisvol verlaag het nie by die konsentrasies toegedien, was daar 'n tendens tot laer suur by die Phytex® behandeling.

Introduction

Calcium and lead arsenates were developed in Florida and used commercially to reduce juice acidity and advance maturity of oranges and grapefruit since the 1920s (Davies, 1986). However, there are no suitable alternative options to reduce acidity in high acid citrus cultivars or cultivars grown under conditions of slow acid degradation. In addition, the apparent early marketing window for grapefruit cannot be met due to high

acidity of the fruit at that time. Therefore, an obvious need exists to find suitable alternative products that could accelerate acid degradation in citrus fruit.

Monoammonium phosphate (MKP) and monopotassium phosphate (MAP) have been shown to reduce acidity in Shamouti orange and Star Ruby grapefruit in Israel (Lavon et al., 1996) and in Satsuma mandarin in Japan (Kuretani and Terao, 1986; Kuretani et al., 1986), and may provide an alternative option to calcium arsenate. Additional benefits of MKP and MAP include a potential reduction in rind coarseness in Shamouti orange and Temple tangor (Rabe and co-workers, 2001 CRI Group Annual Report; Mudau, 2001). Encouraging results with Temple tangor from this work in 2001 prompted me to analyse the fruit acidity of the Temple tangors in 2002 from MKP and MAP treated trees. From these data, 1% MKP or MAP applied 6 weeks after full bloom (WAFB) resulted in significantly lower acidity (by 0.2-0.3% acidity) and consequently higher ratio.

Promising results with MAP were achieved in the 2002 and 2003 seasons (2002 and 2003 CRI Group Annual Research Reports) when acidity was reduced to an intermediate level between that of calcium arsenate and the untreated control, but there were indifferent results in the 2004 and 2005 seasons (2004 and 2005 CRI Group Annual Research Reports).

The aim of this research was to reduce fruit acidity using alternatives to calcium arsenate with emphasis on mainline cultivars, e.g. Valencia orange and grapefruit, by testing various concentrations of MAP to determine the optimal treatment concentration to reduce acidity. Furthermore, multiple applications of MAP and alternative P sources were tested, together with other chemical compounds containing molybdenum and vanadium. In California, preliminary results indicated that tryptophan may reduce acidity of citrus fruit (Lovatt, pers. comm.).

Materials and methods

Plant material and treatments. A commercial Delta Valencia orange orchard at Paardekop, Citrusdal, and a commercial Midknight Valencia orange orchard at Sandrivier, Wellington were used in this study. The trees used were selected for uniformity in tree size and health.

The MAP and MKP treatments were applied ≈6 WAFB on 21 Nov. 2005 when fruit diameter was 15-20 mm and the air temperature was 21°C on a cool, overcast day. The second 1% MAP treatment was applied 1 week later, and the tryptophan treatment was applied on 16 Jan. and 5 Apr. 2006 when fruit size was 25-30 mm (to coincide with similar timings used in California). A randomized complete block design with eight single-tree replicates was used. Treatments included 1 and 2% MAP, 1 and 2% MKP, 1% MAP applied twice, calcium arsenate, Phytex®, Molybdate, tryptophan, and an untreated control. A medium-cover spray was used to apply spray material until just before run-off. On average, 7.5 L of spray material was applied per tree.

Data collection and statistical analysis. Fruit acidity, from six replicates, was determined every 4 weeks from 3 Jul. 2006 until maturity to map seasonal changes in acidity. Ten fruit of similar size were sampled from the east side of trees. At maturity on 14 Aug. (Midknight) and 8 Sept. (Delta) 2006, eight replicates were sampled, where 10 similar-sized fruit per replicate from each of the east and west sides of trees were taken for juice quality analysis. Juice content, Brix (by refractometer), titratable acidity (TA) and the ratio were determined using standard procedures. Leaf samples of five replicates were taken ≈12 weeks after spray application in mid-Feb 2006. These samples were used for N, P, K and Mo analysis. Data were analysed using SAS.

Results and discussion

Delta Valencia orange, Citrusdal

Internal fruit quality

Juice and Brix contents were consistently unaffected by the treatments, and the ratio of Brix-to-acid largely mirrored that of acidity. Therefore, only acidity data will be presented and discussed. From the first sampling date (4 July 2006) until maturity and commercial harvest (8 Sep. 2006), acidity of fruit from the Ca-arsenate treatment was significantly lower than that of all other treatments (Fig. 5.3.5.1; Table 5.3.5.1). This response is consistent with previous results.

Of the other treatments, a single application 6 WAFB of 1 or 2% MAP resulted in significantly lower acidity than the untreated control by ≈0.3% acidity, i.e. an intermediate effect between that of Ca-arsenate and the untreated control (Fig. 5.3.5.1; Table 5.3.5.1).

At the rates applied, tryptophan, molybdate, Phytex® and 2% MKP did not significantly reduce acidity relative to the untreated control (Fig. 5.3.5.1; Table 5.3.5.1), although there is a trend towards lower acidity for the Phytex® treatment.

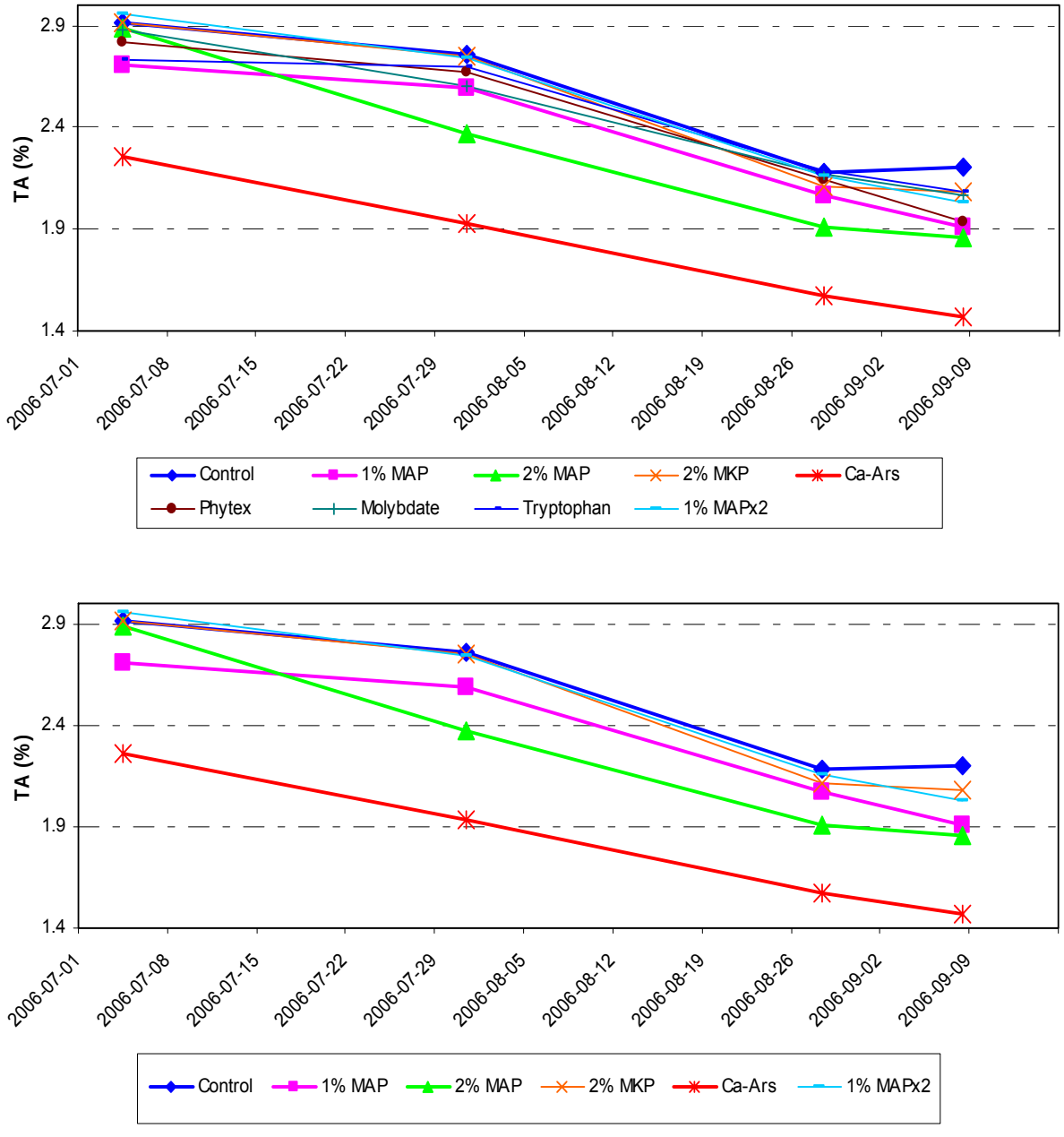

Fig. 5.3.5.1. Effect of various products on fruit acidity of Delta Valencia orange (Paardekop, Citrusdal). Treatments were applied on 21 November 2005 and fruit were sampled four-weekly from early July until maturity to demonstrate changes in acidity over time and to overcome issues of sampling error. The second graph does not include the three treatments that did not affect acidity to allow the reader to more clearly see the data points of the other treatments.

Table 5.3.5.1. Effect of various products on fruit acidity of Delta Valencia orange (Paardekop, Citrusdal). Treatments were applied on 21 November 2005 and fruit were sampled four-weekly from early July until maturity to demonstrate changes in acidity over time and to overcome issues of sampling error. Means within columns not followed by the same letter are significantly different from one another at *P*=0.05

Treatment	Rate (L^{-1})	04 Jul.		31 Jul.		28 Aug.		08 Sept.	
Control	-	2.91	a	2.76	a	2.18	a	2.20	a
1% MAP	1 kg	2.71	a	2.59	ab	2.07	ab	1.91	b
2% MAP	2 kg	2.89	a	2.37	b	1.91	b	1.86	b
1% MAPx2	1 kg	2.96	a	2.74	a	2.16	ab	2.03	ab
2% MKP	2 kg	2.91	a	2.75	a	2.11	ab	2.08	ab
Ca-Ars	100 g	2.26	b	1.93	c	1.57	c	1.47	c
Phytex	1 L	2.82	a	2.67	ab	2.14	ab	1.94	ab
Molybdate	50 g	2.88	a	2.60	ab	2.17	ab	2.07	ab
Tryptophan	20.4 g	2.73	a	2.70	a	2.19	a	2.08	ab
p-value		0.0097		<0.0001		0.0003		0.0002	
LSD(5%)		0.36		0.31		0.26		0.28	
CV(%)		11.1		10.2		10.8		13.9	
n		6		6		6		8	

Leaf nutrient levels

Leaf analysis for N, P, K and Mo showed that the treatments did not affect leaf N or K levels (Table 5.3.5.2). However, P levels were elevated relative to the untreated control following the application of P-containing products (MAP and MKP), with a trend towards high leaf P levels with increasing quantity of P applied. Leaf Mo levels were signicantly higher for the molybdate and Phytex® treatments. Overall, macronutrient levels were close to or within the optimal range for all treatments at the time of sampling. The relatively small difference in leaf P levels between the control and MAP or MKP treatments (0.2-0.3%) coupled with the small difference in acidity between these treatments (0.3% acidity) suggests that a small increase in leaf P concentration is insufficient to translate into a response in acidity, and a larger increase in leaf P level may be required to achieve a larger reduction in acidity.

Table 5.3.5.2. Effect of various products on leaf N, P, K and Mo levels of Delta Valencia orange (Paardekop, Citrusdal). Treatments were applied on 21 November 2005 and leaves were sampled from five replicates in mid-February 2006. Means within columns not followed by the same letter are significantly different from one another at *P*=0.05.

Treatment	N (%)	P (%)	K (%)	Mo ($mg \cdot kg^{-1}$)
Control	2.37 NS	0.11 d	1.68 NS	0.88 c
1% MAP	2.30	0.13 abc	1.80	0.56 c
2% MAP	2.30	0.14 ab	2.00	0.63 c
1% MAPx2	2.32	0.14 a	1.42	0.52 c
2% MKP	2.16	0.13 abc	1.59	0.63 c
Ca-Ars	2.27	0.11 d	1.64	3.20 bc
Phytex	2.32	0.11 cd	1.92	7.94 b
Molybdate	2.38	0.12 bc	1.96	28.71 a
Tryptophan	2.18	0.10 d	1.49	1.45 c
P-value	0.3471	0.0001	0.1381	<0.0001
LSD (5%)	0.199	0.0162	0.463	5.62
CV %	6.8	10.4	21.0	88.5
Norms	2.2-2.6	0.11-0.15	0.9-1.8	

Midknight Valencia orange, Wellington

Internal fruit quality

Juice and Brix contents were consistently unaffected by the treatments, and the ratio of Brix-to-acid largely mirrored that of acidity. Therefore, only acidity data will be presented and discussed. From the first sampling date (3 Jul. 2006) until maturity and commercial harvest (14 Aug. 2006), acidity of fruit from the Ca-arsenate treatment was significantly lower than that of the untreated control (Fig. 5.3.5.2; Table 5.3.5.3). This response is consistent with previous results. However, the acidity of fruit from the Ca-arsenate treatment was not different from that of the MAP and MKP treatments. Therefore, the single applications of 1 or 2% MAP and MKP applied 6 WAFB resulted in significantly lower acidity than the untreated control by ≈0.2% acidity. However, at the earlier sampling dates, indications were that the MAP and MKP treatments had an intermediate effect between that of Ca-arsenate and the untreated control (Fig. 5.3.5.2; Table 5.3.5.3).

At the rates applied, tryptophan, molybdate and Phytex® did not significantly reduce acidity relative to the untreated control (Fig. 5.3.5.2; Table 5.3.5.3), although there is a trend towards lower acidity for the Phytex® treatment.

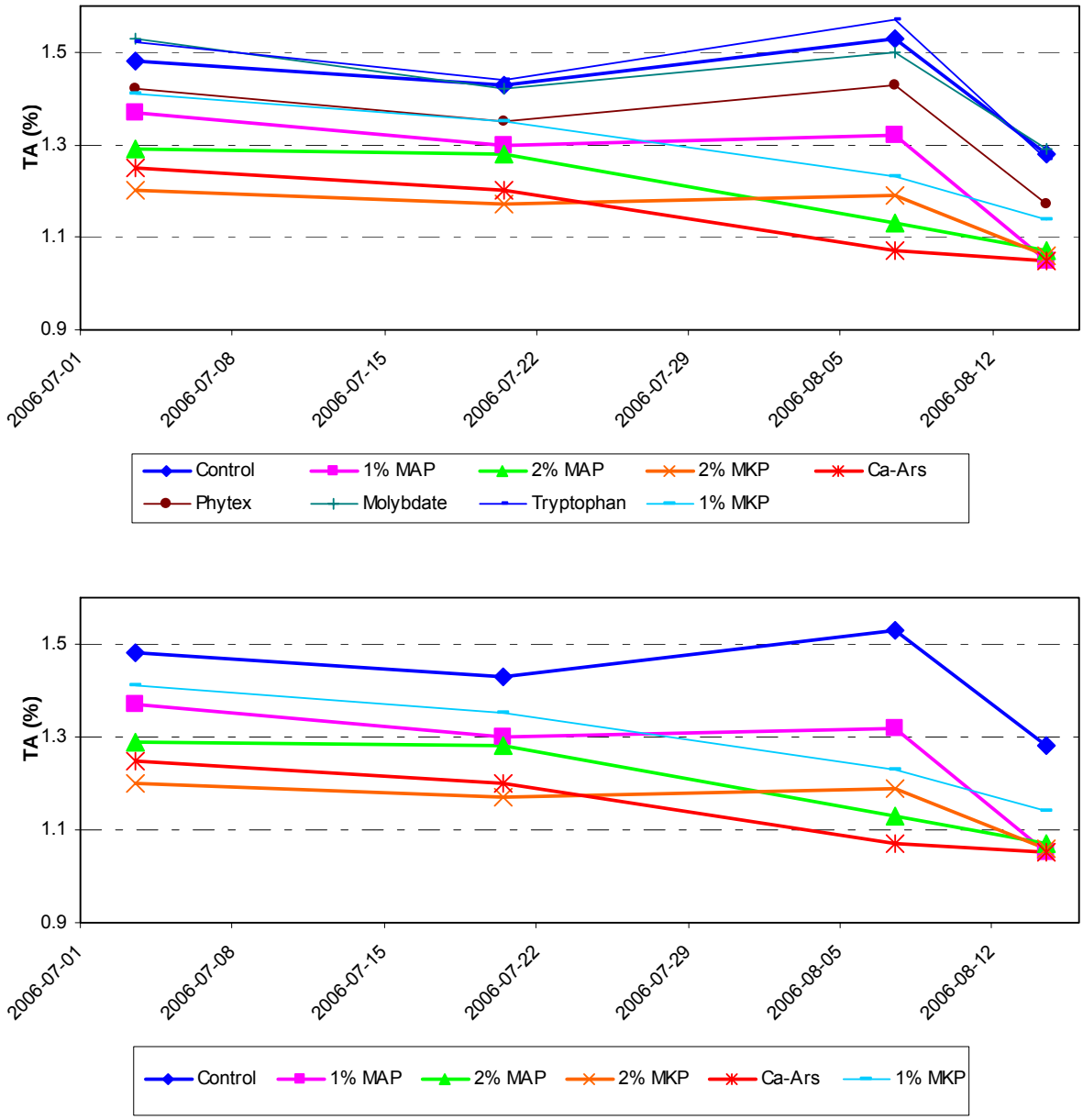

Fig. 5.3.5.2. Effect of various products on fruit acidity of Midknight Valencia orange (Sandrivier, Wellington). Treatments were applied on 22 November 2005 and fruit were sampled four-weekly from early July until maturity to demonstrate changes in acidity over time and to overcome issues of sampling error. The second

graph does not include the three treatments that did not affect acidity to allow the reader to more clearly see the data points of the other treatments.

Table 5.3.5.3. Effect of various products on fruit acidity of Midknight Valencia orange (Sandrivier, Wellington). Treatments were applied on 22 November 2005 and fruit were sampled four-weekly from early July until maturity to demonstrate changes in acidity over time and to overcome issues of sampling error. Means within columns not followed by the same letter are significantly different from one another (*P*=0.05)

Treatment	Rate (L^{-1})	03 Jul.		20 Jul.		07 Aug.		14 Aug.	
Control	-	1.48	ab	1.43	ab	1.53	a	1.28	ab
1% MAP	1 kg	1.37	bcd	1.30	bcd	1.32	bc	1.05	d
2% MAP	2 kg	1.29	cde	1.28	bcd	1.13	de	1.07	cd
1% MKP	1 kg	1.41	abc	1.35	abc	1.23	cd	1.14	cd
2% MKP	2 kg	1.20	e	1.17	d	1.19	cde	1.06	d
Ca-Ars	100 g	1.25	de	1.20	cd	1.07	e	1.05	d
Phytex	1 L	1.42	abc	1.35	abc	1.43	ab	1.17	bc
Molybdate	50 g	1.53	a	1.42	ab	1.50	a	1.29	a
Tryptophan	20.4 g	1.52	ab	1.44	a	1.57	a	1.27	ab
p-value		<0.0001		0.0033		<0.0001		<0.0001	
LSD(5%)		0.15		0.15		0.15		0.11	
CV(%)		8.6		9.2		9.8		8.9	
n		6		6		6		8	

Leaf nutrient levels

Leaf analysis for N, P, K and Mo showed that the treatments did not affect leaf N levels (Table 5.3.5.4). Leaf P levels were elevated relative to the untreated control following the application of P-containing products (MAP, MKP and Phytex®), with a trend towards higher leaf P levels with increasing quantity of P applied. Leaf K levels varied among treatments, but were not excessive. Excess K levels are known to result in high acidity levels. Leaf Mo levels were significantly higher for the molybdate treatment. Overall, macronutrient levels were close to or within the optimal range for all treatments at the time of sampling. The relatively small difference in leaf P levels between the control and 2% MAP or MKP treatments (0.3%) coupled with the small difference in acidity between these treatments (0.2% acidity) suggests that a small increase in leaf P concentration is insufficient to translate into a response in acidity, and a larger increase in leaf P level may be required to achieve a larger reduction in acidity.

Table 5.3.5.4. Effect of various products on leaf N, P, K and Mo levels of Midknight Valencia orange (Sandrivier, Wellington). Treatments were applied on 22 November 2005 and leaves were sampled from five replicates in mid-February 2006. Means within columns not followed by the same letter are significantly different from one another at *P*=0.05.

Treatment	N (%)	P (%)	K (%)	Mo ($mg \cdot kg^{-1}$)
Control	2.34 NS	0.13 de	1.25 cd	2.93 b
1% MAP	2.49	0.14 cd	1.49 abc	2.62 b
2% MAP	2.41	0.16 a	1.63 ab	2.48 b
1% MKP	2.40	0.14 bcd	1.36 bcd	3.20 b
2% MKP	2.53	0.16 ab	1.55 ab	4.36 b
Ca-Ars	2.30	0.13 de	1.18 d	3.86 b
Phytex	2.52	0.16 abc	1.71 a	4.12 b
Molybdate	2.47	0.13 de	1.49 abc	217.00 a
Tryptophan	2.37	0.12 e	1.22 cd	3.07 b
P-value	0.0678	<0.0001	0.0070	<0.0001
LSD (5%)	0.161	0.0184	0.302	17.95
CV %	5.2	10.2	16.5	51.7
Norms	2.2-2.6	0.11-0.15	0.9-1.8	

Conclusions

During the 2005-06 season, a single application of 1 or 2% MAP applied 6 WAFB to Delta and Midknight Valencia orange resulted in significantly lower acidity than the untreated control by ≈0.3% acidity, i.e. an intermediate effect between that of Ca-arsenate and the untreated control. Juice and Brix contents were not affected by the treatments. This reduction in acidity appeared to be related to slightly high leaf P levels where P-containing products were applied. From these data it is not possible to establish whether there is a direct relationship between leaf P levels 12 weeks after treatment and fuit acidity. Although Phytex® did not significantly reduce acidity at the rate applied, there was a trend towards lower acidity for the Phytex® treatment.

Future treatments should include earlier (4 WAFB) and double applications of MAP and MKP, higher rate of application or double application of Phytex®, and preharvest application of these P-containing products in an attempt to increase aconitase enzyme activity during the acid degradation stage of fruit development.

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6 PROGRAMME: CULTIVAR AND ROOTSTOCK EVALUATION

6.1 PROGRAMME SUMMARY

By Graham H. Barry (Manager: Cultivar Development, CRI)

Maximising the long-term global competitiveness of the Southern African citrus producer requires on-going innovation at various levels in the fruit value-chain. Product differentiation, through the commercialisation of new citrus cultivars, provides a principal means by which to achieve this goal.

CRI's Cultivar Development division aims to facilitate the rapid access to growers of new citrus cultivars to meet the changing requirements of the markets and to provide independent and objective information on all citrus cultivars to enhance grower decision-making. To this end, the cultivar evaluation component of CRI's Cultivar Development division will be actively involved in the establishment and field evaluation of all citrus cultivars.

Programopsomming

CRI se Cultivarontwikkelingsdivisie mik daarna om die beskikbaarheid van nuwe sitruscultivars te versnel en sodoende aan die markbehoeftes te voorsien. Verder wil ons onafhanklike inligting beskikbaar stel om besluitneeming van produsente te verbeter. Om dié doelwit to bereik sal CRI se Cultivarontwikkelingsdivisie betrokke wees by die evaluasie van alle sitruscultivars.

6.2 PROGRAMME INTRODUCTION

The need for independent and objective cultivar information

Citrus production is a long-term, capital-intensive endeavour with a relatively long interval between the start of orchard preparation and when the economic break-even point is achieved. As a primary producer, the citrus grower is the ultimate risk-taker in the production and marketing value-chain. Therefore, the correct choice of cultivar is one of the most important pre-planting decisions that a grower must make. To make such decisions requires credible information.

The introduction and commercialisation of new citrus cultivars in South Africa during the 1980s and 1990s was dynamic and aggressive and was driven by the citrus industry. Numerous cultivars were introduced and commercialised during this era, and contributed to South Africa's position as the southern hemisphere leader in citrus supply. Yet to remain competitive, there is still an acute need to seek out, evaluate and commercialise new citrus cultivars.

Following de-regulation of the citrus industry in the late-1990's and re-structuring of research and technical support services to the industry between 2002 and 2004, led to uncertainty in the role of industry-driven cultivar evaluation. In addition, this era also witnessed the advent of cultivar management companies and an increase in the number of privately-managed citrus cultivars. In 2004, the citrus industry decided to become actively involved in cultivar development, and established a Cultivar Development division within CRI in 2005.

Recognising the need of citrus growers to have access to impartial, credible, decision-making information related to cultivar planting options, the Cultivar Development division will, among other things, address the need to provide independent and objective information on all citrus cultivars through coordinated field evaluation of privately-managed and so-called "open" citrus cultivars. The ultimate purpose of this endeavour will be to maximise competitiveness of South African citrus growers through enhancing their access to new citrus cultivars and related information.

Overall objectives of cultivar evaluation

The evaluation of new citrus cultivars serves three principle purposes, namely, to

1. describe a cultivar's characteristics,
2. determine broad climatic suitability of a cultivar, and ultimately to
3. determine commercial potential in the market.

Planting decisions remain the responsibility of the grower and should be made in consultation with marketers, private cultivar agents (where appropriate), and other cultivar specialists. Commercial decisions and processes of privately-managed cultivars remain the right of private cultivar agents.

Cultivars cannot be fully evaluated under all possible conditions within a relatively short timespan. Therefore, latent defects cannot be accounted for and a degree of risk in cultivar choice will probably always exist. Therefore, the role of cultivar evaluation is to describe a cultivar's characteristics and to determine the broad climatic suitability of a cultivar, and thereby minimise the potential commercial risks involved in their commercial use. Longer-term production and postharvest information will be generated from semi-commercial or commercial plantings.

Cultivar evaluation guidelines

Guiding principles for cultivar evaluations

1. *Evaluation objectives*
   1.1. The ultimate objective of cultivar evaluation is to provide citrus growers with impartial, credible information upon which they can make planting decisions.
      - To achieve this objective, statistically-sound experiments and publishable scientific findings are not required. Also, exhaustive evaluations of all aspects of a cultivar's production in all regions and postharvest responses cannot be conducted. In the short-term, therefore, general cultivar characteristics will be described and broad climatic suitability of a cultivar will be determined. Longer-term, semi-commercial or commercial plantings will be used to "fill in the gaps" with respect to detailed observations.
      - Furthermore, it is not the intention of the cultivar evaluation component of Cultivar Development to slow down the process of commercialisation of new citrus cultivars, but rather to facilitate the flow of cultivars through the system, including direct involvement in cultivar evaluation.
   1.2. "Fast-track" or initial evaluations will be conducted to determine the general characteristics of a cultivar as opposed to the long-term performance potential. Therefore, there will be an element of risk to growers in an endeavour to avoid unnecessary delays in planting new cultivars.

2. *Site selection*
   2.1. Since cultivar characteristics are climate-dependent, evaluations will be conducted in climatically suitable growing regions for each cultivar group.
      - New cultivars will be established at sites according to a "cultivar group x climatic region matrix" in the major citrus-producing regions.
      - Where appropriate, information generated from these evaluations will, by necessity, be used to determine cultivar suitability in other similar sites.
   2.2. Suitable nursery and grower co-operators are essential.
      - Relevant non-propagation agreements must be signed by nursery and grower co-operators.
      - The evaluation site should preferably be within a commercial orchard and must consist of uniform and suitable soil type, and not be near a windbreak.

3. *Trial design*
   3.1. The main commercial cultivar in the cultivar group will serve as the control for comparison with new cultivars in the same cultivar group.
   3.2. Budded trees of the same (or similar) age on a standard rootstock should preferably be used. However, in some cases topworked trees will be evaluated. In such cases, tree condition must be taken into account when evaluation results are interpreted.
   3.3. A minimum of five trees per cultivar to be planted or topworked.
   3.4. Randomisation of cultivars within the test plot is not required.
   3.5. Factorial type experiment designs to be avoided.

4. *Evaluation criteria*
   4.1. Cultivar development strategy by cultivar group
      - Navel oranges: To select cultivars with improved fruit set (and yield), packout (wind blemish, creasing, oleo, mealybug, Alternaria core rot), rind colour and internal fruit quality (juice content, granulation, acidity), and extended harvest period (especially earlier maturity).
        - Early maturity, deep orange rind colour.
      - Midseason oranges: To select suitable midseason orange cultivars which compliment Navel and Valencia supply or provides a niche product. Specific requirements include acceptable fruit size, rind colour and texture, peelability, flavour and pigmentation (in the case of blood oranges).
        - Lower priority; acceptable size, consistent pigmentation and flavour.
      - Valencia oranges: To select cultivars with improved and consistent productivity, fruit size, rind colour, peelability and internal fruit quality (seedlessness, ratio), and extended harvest period (both earlier and later maturity).

    - Large, seedless, early and late maturity.
- Grapefruit: To select cultivars with improved fruit size and eating quality (reduced bitterness, higher ratio) and extended harvest period (both earlier and later).
    - Lower priority; reduced bitterness, improved flavour, large fruit size, red.
- Shaddocks: Low priority; acceptable size (smaller), thinner rinds, uniform segmentation, higher juice content and acceptable flavour.
- Lemons: To select cultivars with elongated fruit shape, high juice content and seedless cultivars with improved productivity. Trees should be thornless and compatible with a wide range of rootstocks.
- Satsuma mandarins: To select cultivars with acceptable fruit size, improved rind colour and internal fruit quality (Brix and acidity, ratio), and extended harvest period (both earlier and later maturity).
    - Early and late maturity with acceptable size, colour and flavour.
- Clementine mandarins: To select larger fruited cultivars with acceptable rind colour and flavour, and extended harvest period (especially earlier, but also later maturity).
    - Large-fruited, early maturity.
- Mandarins: To select late maturing, seedless mandarins with acceptable fruit size, good peelability, rind colour and flavour.
    - Late maturing, seedless, easy-peeling mandarin with acceptable size, colour and flavour.

PROGRAM INLEIDING

Die behoefte vir onafhanklike en objektiewe kultivar inligting

Sitrus produksie is ’n langtermyn, kapitaal-intensiewe belegging met ’n relatiewe lang periode tussen boord voorbereiding en wanneer die ekonomiese gelykbreek punt bereik word. Die sitrus produsent dra die finale risiko in die produksie en bemarking waardetoevoegings ketting. Die mees belangrikste besluit wat voor aanplanting gedoen moet word is die kultivar keuse. Akkurate inligting moet beskikbaar wees om hierdie finale besluit te kan neem.

Die invoer en kommersialisering van nuwe sitrus kultivars in Suid Afrika gedurende die 1980s en 1990s was dinamies en aggresief gewees, en deur die sitrus bedryf geinspireer. Gedurende hierdie era is daar verskeie kultivars ingevoer en gekommersialiseer. Dit het bygedra tot Suid Afrika se posisie as die suidelike halfrond leiers in die verskaffing van sitrus. Om dus kompeterend te bly, is daar ’n noodsaaklikheid om nuwe kultivars te vind, te evalueer en te kommersialiseer.

Die de-regulering van die sitrusbedryf in die laat 1990s en herstrukturering van die navorsings- en tegniese ondersteunings dienste tydens 2002 en 2004, het gelei tot onsekerheid in die rol van industrie-gedrewe kultivar evaluasie. Bykomend tot hierdie era, het kultivar bestuurs maatskappye ontstaan, asook ’n toename in die aantal privaat-bestuurde kultivars. In 2004, het die sitrus industrie besluit om akteif betrokke te raak by kultivar ontwikkeling, met die stigting van ’n Kultivar Ontwikkelings afdeling binne CRI gedurende 2005.

Met die gewaarwording van die sitrus produsent se behoefte om toegang tot onpartydige, kredietwaardige inligting vir besluitneming, met betrekking tot kultivar aanplantings opsies te verkry. Die Kultivar Ontwikkelings afdeling, sal onder andere, die behoefte aanspreek om onafhanklike en objekteiwe informasie van alle sitrus kultivars deur gekoordineerde veld evalusasies van privaat-bestuurde en so-genaamde “oop” sitrus kultivars, beskikbaar te stel. Die finale doel van hierdie projekte sal wees om die Suid Afrikaanse sitrus produsend se mededingings vermoë te verbeter deur beskikbaarstelling van nuwe kultivars en toepaslike inligting.

Algemene doelwitte van kultivar evaluasies

Die evaluasie van nuwe sitrus kultivars behels hoofsaaklik drie doelstellings, naamlik, om

1. ’n kultivar se eienskappe te beskryf,
2. die breë klimaats aanpasbaarheid van die kultivar te bepaal, en laastens
3. die kommersiele potensiaal in die mark te bepaal.

Aanplantings besluite bly die verantwoordelikheid van die produsent en moet gedoen word in konsultasie met die bemarkers, privaat kultivar agente (waar van toepassing), en ander kultivar spesialiste. Kommersiële besluite en prosesse van privaat-bestuurde kultivars bly die reg van privaat kultivar agente.

Kultivars kan nie ten volle ge-evalueer word onder alle moontlike kondisies in 'n relatiewe kort tydbestek nie. Daarom kan verantwoordelik nie aanvaar word vir onvoorsiene foute nie, en daar sal altyd 'n mate van risiko wees by die keuse van 'n kultivar. Daarom sal die doel van kultivar evaluasies wees om die kultivar eienskappe te beskryf en die breë klimaats aanpasbaarheid van die kultivar vas te stel. Hierdeur word die potensiele kommersiële risiko verminder met betrekking tot die kommersiële gebruik. Langtermyn produksie- en vooroes inligting sal gegenereer word van semi-kommersiële of kommersiële aanplantings.

Kultivar evaluasie riglyne

Leidende beginsels vir kultivar evaluasies

1. *Evaluasie doelwitte*
   1.1. Die belangrikste doelwit van kultivar evaluasies is om die sitrus produsent met onpartydige, kredietwaardige inligting vir aanplantings besluite te voorsien.
      - Om hierdie doelwit te bereik, behels nie statisties-korrekte eksperimente en publiseerbare wetenskaplike bevindinge nie. Daar kan ook nie onbeperkte evaluasies van alle aspekte van 'n kultivar se produksie in alle areas en na-oes gedrag gedoen word nie. Daarom, oor die korttermyn, sal algemene kultivar eienskappe beskryf word en breë klimaats aanpasbaarheid vasgestel word. Oor die langtermyn sal semi-komersiele of kommersiele aanplantings gebruik word om "gapings te vul" ten opsigte van volledige observasies.
      - Buitendien is dit nie die kultivar evaluasie komponent van die Kultivar Onwikkelings afdeling se bedoeling om die kommersialeserings proses van die nuwe sitrus kultivar te vertraag nie, maar eerder om die vloei van kultivars deur die sisteem te akkommodeer, asook direkte betrokkenheid by die kultivar se evaluasie te verseker.
   1.2. "Spoedige opvolg" of aanvangs evaluasies sal uitgevoer word om die algemene eienskappe van die kultivar, asook die langtermyn potensiaal te bepaal. Daarom sal daar 'n mate van risiko bestaan vir produsente met die aanplant van nuwe kultivars, deur spoedige aanplantings sonder om te veel tyd te verspeel.

2. *Keuse van perseel*
   2.1. Kultivar eienskappe is klimaats-gebonde, daarom sal evaluasies uitgevoer word in klimaats aangepaste produksie areas vir elke kultivar groep.
      - Nuwe kultivars sal aangeplant word by persele volgens 'n "kultivar groep x klimaat streek matriks" in die hoof sitrus produserende areas.
      - Waar van toepassing, sal inligting wat uit hierdie evaluasies verkry word, indien nodig, gebruik word om kultivar aanpasbaarheid in ander soortgelyke areas te bepaal.

   2.2. Geskikte kwekery en produsent samewerking is essensieel.
      - Toepaslike nie-vermeerderings ooreenkomste moet onderteken word deur beide kwekery en produsente.
      - Die evaluasie perseel moet verkieslik binne 'n kommersiele boord wees, met univorme en geskikte grondtipe en nie naby 'n windbreek wees nie.

3. *Proef ontwerp*
   3.1. Die hoof kommersiële kultivar in die kultivar groep sal as kontrole dien vir die vergelyking van die nuwe kultivar in dieselfde kultivar groep.
   3.2. Gekweekte bome van dieselfde (of soortgelyke) ouderdom op 'n standaard onderstam moet verkieslik gebruik word. In sekere gevalle sal oorgewerkte bome ge-evalueer word. In sekere gevalle, moet boom kondisies in ag geneem word wanneer die evaluasie resultate ge-interpreteer word.
   3.3. 'n Minimum van vyf bome per kultivar moet aangeplant en oorgewerk word, en randomisering van kultivars binne die proef blok word nie vereis nie.
   3.4. Fakulteitsfunksie-tipe eksperiment ontwerpe moet vermy word.

4. *Evaluasie kriteria*
   4.1. Kultivar ontwikkelings strategie by kultivar groep
      - Nawel lemoene: Selekteer kultivars met beter vrugset (en oes), uitpak (windskade, kraakskil, oleo, wolluis, Alternaria kern vrot), skilkleur en interne vrugkwaliteit (sapinhoud, granulering, suur), en verlengde oes tydperk (veral vroeë rypheid).
        - Vroeë rypheid, diep oranje skilkleur.

- Midseisoen lemoene: Selekteer aanvaarbare midseisoen lemoene, kultivars wat nawels en valencia's komplementeer en wat 'n gekikte nis produk verskaf. Spesifieke vereistes sluit in aanvaarbare vruggrootte, skilkleur, tekstuur, skilbaarheid, smaak en pigmentasie (in die geval van bloedlemoene).
  - Laer prioriteit, aanvaarbare grootte, konsekwente pigmentasie en smaak.
- Valencia lemoene: Selekteer kultivars met verbeterde en konstante produksie, vruggrootte, skilkleur, skilbaarheid en interne vrugkwaliteit (saadloosheid, verhouding), verlengde oes periode (beide vroeg en laat rypheid).
  - Groot, saadlose, vroeg en laat rypheid.
- Pomelo's: Selekteer kultivars met verbeterde vruggrootte en eetkwaliteit (verlaagde bitter smaak, hoër verhouding) verlengde oes periode (beide vroeg en laat).
  - Laer prioriteit, laer bitter smaak, verbeterde smaak, groot vruggrootte, rooi.
- Shaddocks: Laer prioriteit, aanvaarbare grootte (kleiner), dunner skil, eenvormige segmente, hoër sapinhoud en aanvaarbare smaak.
- Suurlemoene: Selekteer kultivars met 'n langwerpige vrug vorm, hoë sapinhoud en saadlose vrugte met verbeterde produktiwiteit. Bome moet doringloos wees en verenigbaar met 'n groot reeks onderstamme.
- Satsuma manderyne: Selekteer kultivars met aanvaarbare vruggrootte, verbeterde skilkleur en interne vrugkwaliteit (Brix en suur, verhouding), en verlengde oesperiode (beide vroeg en laat rypheid).
  - Vroeë en laat rypwording met aanvaarbare grootte, kleur en smaak.
- Clementine manderyne: Selekteer kultivars wat grootter vrugte produseer met aanvaarbare skilkleur en smaak, en verlengde oesperiode (veral vroeër, maar ook later ryp wording).
  - Groot vruggrootte, vroeg rypwording.
- Manderyne: Selekteer laat rypwordende, saadlose manderyne met aanvaarbare vruggrootte, goeie skilbaarheid, skilkleur en smaak.
  - Laat rypwording, saadloos, skil maklik met aanvaarbare vruggrootte, kleur en smaak.

6.3 PROJECT: CULTIVAR EVALUATION

6.3.1 Project summary: Cape areas

Satsumas: The objective of this project is to find suitable, high quality, early maturing and early colouring selections for the early marketing season and to overcome production peaks by extending the harvest season both early and later. Satsuma x Nova looks promising as an early maturing selection. Primosole is early maturing but shows variable potential at this early stage. The commercial Dobashi Beni trees are not yet in production. A single trial site with all the late maturing selections has been established.

Clementines: The aim of this work is to flatten out existing midseason Clementine production peaks by extending the harvest period both earlier and particularly later and to provide selections of superior external colour, internal quality and larger fruit size. Clemenpons is early maturing with good quality but only medium fruit size. Trunks have galls and tree size is variable. The long term effect of the galls needs to be established. Tardif de Janvier I bore well but with only medium fruit size and lowish acid. The Tardif de Janvier II also has medium fruit size and low acid and does not appear to be late maturing. Information on both selections is limited. Tardivo had poor production and medium small fruit size, good quality and green stylar ends, maturing mid May to mid June. Nour production was not good with poor fruit size. Fruit quality was good with similar maturity to Tardivo. Rind colour is retarded and the selection does not look promising. Final evaluations are necessary on some of the selections.

Mandarins: The objective of the mandarin hybrid project is to find high quality, well coloured, seedless selections with good production and fruit size, with special emphasis on extending the soft citrus season earlier and particularly later. M37 had good quality, maturing in June. The Murcott x Clem semi commercial block bore its first small crop with large fruit size, quite acceptable quality and seedy fruit, maturing late June. Bay Gold had good production and fruit size but does not look promising in the cooler production areas due to high acid. Hadas had good production and fruit size but consistently high acid like an Ellendale. Winola had poor production and medium fruit size and excessive acid. Cami had fair yields, good fruit size and high acid. Empress mandarin had poor production and fruit size and does not look promising. Some selections need to be evaluated further.

Navels: The aim is to find cultivars to spread the navel season both earlier and later and to minimise production peaks, also with more advanced rind colour, particularly at the commencement of the season and with improved fruit set potential in the desired fruit size range. Fukumoto is early maturing, similar, to slightly earlier than Lina/Newhall, developing a deep orange/red rind colour. There appears to be no direct evidence of incompatibility of Fukumoto on citrange hybrid rootstocks although Swingle citrumelo and Koethan citrange had some indications of possible abnormality. More clarity is needed on rootstock choice. Maturity of Letaba Early appears to be later than Lina/Newhall. Atwood had slightly later maturity than the Lina/Newhall and has delayed rind colour. Dream matures after Lina/Newhall and can be considered as a mid maturing selection. Cliff Early looks promising as an early maturing navel. Fenix and Sundays River Early do not appear to be early maturing and Krajewski Early not so early. Washington (CFB material) performed well with good production, fruit size and quality. Santa Catarina 1 and 3 are large and vigorous trees and bore few fruit. Cambria (CFB material) had good production and fruit size, round to elongated fruit shape and good quality but acid can tend to get low. Summer Gold is late maturing. Renken Late, Coetzee Late and Mouton Late 1 and 2 improved over last season and warrant further evaluations. The Witkrans (old selection) has similar characteristics to Royal Late. Glenora Late is late maturing and a vigorous tree and had good quality but some seed in a mixed block. A comparison between the Autumn Gold, Powell, Chislett and Californian Lane Late showed little difference between the selections. Fruit size tends to be on the large side in Citrusdal. Juice percentage can be low on Rough lemon/Rangpur lime rootstocks on sandy soil. Further evaluations of all the selections are necessary as some of the trees are still young.

Midseasons: The aim is to find midseason selections suitable to the colder areas with larger fruit size, pigmented flesh and seedless. Tarocco production varied between sites and fruit size good. Quality was generally good but can be masked by high acid which could delay harvest. Tarocco Gallo and 57/1E/1 both had reasonable production with acceptable to good fruit size. Both had high acid. Gallo colour was behind Tarocco. All three older Tarocco selections have thorns of varying degrees and appear to lessen with ageing. There were only slight difference between the three commercially planted Maltaise selections. Maltaise Half II was slightly ahead of Maltaise Half, while Maltaise Barlerin the highest acid. Of concern with all three are the high acid levels. Raratonga had acceptable production, good fruit size, fair quality and tart. The trees are vigorous with large thorns. Further evaluations on all the Tarocco selections, Raratonga, Clara, Tacle and commercial Maltaise are necessary.

Valencias: The aim of the Valencia project is to find early, mid and late maturing Valencia selections that are seedless, have large fruit size and with improved fruit set ability compared to the existing range of selections. Various new selections were evaluated at the CFB. Limpopo seedless is the earliest to mature with acceptable quality off young trees and seedless without cross pollination. G5 also seedless where not cross pollinated and acceptable to good quality. Portsgate had borderline quality and not outstanding in any way except for virtual seedlees in a mixed block. McClean Seedless looks similar to Valencia Late with fairly high acid and virtually seedless. Rietspruit had smallish fruit size, poor quality, high acid and odd seed. Bend 8A2 production and quality was poor with smallish fruit size and virtually seedless where no cross pollination. Delicia had good production and fruit size, meeting standards in August. Kleinhans was characteristic of the selection.

Knysna area: The purpose of the trial is to find suitable, high quality, especially late maturing soft citrus cultivars for the Knysna area. Young Aoshima had poor quality. Bay Gold does not look promising due to high acid levels even when overmature. Sweet Spring bore a good crop of good fruit size but lack flavour. The Nouvelle was riddled with A*lternaria brown spot*. Kiyomi had excessive acid levels and performs better, although not outstanding in the Heidelberg area. Thoro Temple is not outstanding in any way and not recommended. The late maturing CELL, Clementarde and Clemlate clementine selections are similar and have certain production drawbacks, including small fruit size and low acid levels. Evaluations to continue on the satsuma and mandarin hybrids.

Projekopsomming: Kaapstreek

Satsumas: Die doel van hierdie projek is om geskikte, hoë gehalte seleksies te vind wat vroeg in die seisoen opkleur en die vroeë mark kan binnedring, asook om produksie pieke, beide vroeër en later, te voorkom. Satsuma x Nova lyk belowend as ’n vroeë seleksie. Primosole word vroeg ryp met wisselende potentiaal op hierdie vroeë stadium. Die kommersiële Dobashi Beni bome is nog nie in produksie nie. ’n Nuwe, enkele proef met al die laatrypende Satsuma seleksies is uitgelê.

Clementines: Die doel van die projek is om ’n breë verskeidenheid kultivars met hoër gehalte, goeie skilkleur en met goeie vruggrootte te verskaf om die pieke in die middel Clementine seisoen af te plat deur die oestyd beide vroeër en veral later te verleng. Clemenpons word vroeg ryp, het goeie gehalte, medium vruggrootte,

wisselende boomgrootte en galle op die stam. Die uitwerking van die galle in die langtermyn moet vasgestel word. Tardif de Janvier I het goed gedra maar met medium vruggrootte en suur aan die laer kant. Tardif de Janvier II het ook matige vruggrootte met laer suur gehad en lyk nie asof dit laat ryp word nie. Inligting op albei seleksies is beperk. Die Tardivo het swak produksie met medium tot klein vruggrootte, goeie gehalte, groen blomente en word tussen mid Mei tot mid Junie ryp. Nour produksie was swak met swak vruggrootte. Eetgehalte was goed en rypwording soortgelyk aan Tardivo. Skilkleur is vertraag en die seleksie lyk nie belowend nie. Finale evaluasies op sekere van die seleksies is nodig.

Mandaryne: Die doel van die mandaryn projek is om hoër gehalte, goed gekleurde, saadlose mandaryn seleksies te ondersoek. Hulle moet ook goeie produksie en vruggrootte hê en die sagte sitrus seisoen kan versprei, beide vroeër en veral later. M37 het goeie gehalte en word in Junie ryp. Die semi kommersieële Murcott x Clem boord het begin dra. Die vrugte was groot met aanvaarbare vruggehalte, vol saad en word laat Junie ryp. Bay Gold het goeie produksie en vruggrootte gehad maar lyk nie belowend in die koeler gebiede nie weens hoër suur. Hadas het goeie produksie en vruggrootte gehad maar gereelde hoër suur soos Ellendale. Winola het swak produksie, medium vruggrootte en hoër suur gehad. Cami het matige produksie, goeie vruggrootte en hoër suur gehad. Empress mandaryn het swak produksie en vruggrootte gehad en lyk nie belowend nie. Van die seleksies moet verder evalueer word.

Nawels: Die doel van die proef is om kultivars te bekom om die seisoen vroeër en later te versprei en produksie pieke te verminder, asook om seleksies to vind met meer gevorderde vrugkleur, veral aan die begin van die seisoen en vrugte te kry met verbeterde vrugset wat binne die gesogte vruggrootte reeks val. Fukumoto word vroeg ryp, dieselfde tyd tot effens voor Lina/Newhall en ontwikkel 'n diep oranje-rooi skilkleur. Daar blyk geen duidelike tekens van onverenigbaarheid op citrange x onderstamme nie, maar Swingle citrumelo en Koethan citrange wys moontlike abnormaliteite aan. Meer duidelikheid word benodig oor onderstam keuse. Letaba Early word effens later ryp as Lina/Newhall. Atwood word effens later as Lina/Newhall ryp en skilkleur is vertraag. Dream word later as Lina/Newhall ryp en kan as 'n midseisoen seleksie beskou word. Cliff Early lyk belowend as 'n vroeër nawel. Fenix en Sundays River Early lyk nie asof hulle vroeg ryp word nie en Krajewski Early is nie so vroeg nie. Washington (SGB materiaal) het goed gevaar, met goeie produksie, vruggrootte en gehalte. Cambria (SGB materiaal) het goeie produksie en vruggrootte gehad, beide ronde en langwerpige vrugte met goeie gehalte maar suurvlakke kan neig om laag te wees. Summer Gold word laat ryp. Renken Late, Coetzee Late en Mouton Late 1 en 2 het beter gevaar as die vorige seisoen en moet verder evalueer word. Glenora Late is 'n groeikragtige boom en word laat ryp met goeie gehalte maar vrugte in 'n gemengde blok het pitte in gehad. 'n Vergelyking tussen Autumn Gold, Powell, Chislett en Californian Lane Late het min verskille tussen hulle getoon. Vruggrootte op Citrusdal is geneig om effens groot te wees. Sap vlakke op growweskilsuurlemoen en Rangpur lemmetjie onderstamme op sanderige grond kan laag wees. Verdere evaluasies van al die seleksies is nodig aangesien van die bome nog jonk is.

Midseisoene: Die doel van die proef is om midseisoen seleksies, wat beter in die koeler streke sal aard in terme van vruggrootte, gepigmenteerde vleis en saadloosheid, te vind. Tarocco produksie het tussen die verskillende gebiede gewissel en vruggrootte was goed. Gehalte was oor die algemeen goed maar die hoë suur kan neig om die geur te verbloem en dalk oes te vertraag. Tarocco Gallo en 57/1E/1 het albei redelike produksie met aanvaarbare tot goeie vruggrootte gehad, maar hoë suur. Gallo vrugkleur was later as Tarocco. Al drie ouer Tarocco seleksies is doringagtig in meedere of mindere mate en die dorings verminder sodra die bome verouder. Daar was min verskille tussen die drie verskillende kommersieële Maltaise seleksies. Maltaise Half II het effens voor Maltaise Half ryp geword en Maltaise Barlerin die hoogste suur. Die hoë suur vlakke is 'n bekommernis. Raratonga het aanvaarbare produksie en goeie vruggrootte gehad en redelike gehalte maar suur. Die bome is groeikragtig en doringagtig. Verdere evaluasies op al die Tarocco seleksies, Raratonga, Clara, Tacle en kommersieële Maltaise is nodig.

Valencias: Die doel van die Valencia proef is om vroeër, mid en laat seleksies met groot vruggrootte, saadloos en verbeterde vrugset as alternatiewe vir die huidige seleksies te soek. Verskeie nuwe seleksies was by die SGB geevalueer. Limpopo Seedless is die eerste seleksie om ryp te word. Die bome is baie jonk en het aanvaarbare gehalte gehad en saadloos waar daar geen kruisbestuiwing is. G5 is ook saadloos met aanvaarbare tot goeie gehalte. Behalwe vir amper saadloosheid in 'n gemengde blok, is die Portsgate nie uitstaande nie en het grenslyn gehalte. McClean Seedless lyk soos Valencia Late met taamlike hoër suur en is amper saadloos. Rietspruit het kleinerige vruggrootte, swak gehalte, hoër suur en enkele pitte gehad. Bend 8A2 se produksie en gehalte was swak met kleinerige vruggrootte en amper saadloos onder geen kruisbestuiwing. Delicia het goeie produksie en vruggrootte gehad en uitvoer standaarde in Augustus behaal. Kleinhans het sy tipiese eienskappe getoon. Evaluasies moet voortgaan.

Knysna area: Die doel van die proef is om geskikte, hoër gehalte, veral laat mandaryn kultivars te vind vir die Knysna area. Jong Aoshima het swak gehalte gehad. Bay Gold lyk nie belowend nie weens hoë suur al is die vrugte oorryp. Sweet Spring het goed gedra maar 'n tekort aan smaak. Nouvelle was vrot van *Alternariabruinvlek* gewees. Kiyomi het hoë suur gehad en het beter in die Heidelberg area gepresteer. Thoro Temple het geen uitstaande eienskappe vertoon en word nie aanbeveel nie. Die laat Clementarde, CELL en Clemlate clementine seleksies is eenders en het sekere produksie nadele, insluitend klein vruggrootte en lae suurvlakke. Evaluasies op die satsuma en mandariyn kruisings moet nog voortgaan.

6.3.2 **Project summary: Inland areas**

Clementines: A trial was laid out to ascertain whether certain Clementine mandarins could be produced commercially for export in the intermediate and cool inland citrus production areas of the country in accordance with market needs, as well as finding superior selections in terms of internal fruit quality, colour and fruit size. The first selection to harvest was Ain Toujdate, with only the juice content on SC below export minimum. The trees produced a good crop with damage to the branches because of the heavy crop loads. There was less cross pollination between the selections and less seeds per fruit this season.

Mandarins (Burgersfort): To find suitable Mandarin selections for the hot, intermediate and cool inland citrus production areas to fill the early, mid and late season gap. Primsole was the earliest selection to mature this season. Unfortunately this selection seems to develop major problems in the cooler areas. Granulation, large fruit size, coarse rinds and low juice content occurred in this area. The Orighstad area may be more suitable to test Primosole. Hadass will be evaluated in Swaziland at Tambuti Estate the next season when the trees produce sufficient fruit for evaluations. Cami produced good yields and internal quality this season. The fruit was sweet with a nice juicy taste.

Mandarins (Marble Hall): Primosole is not sutable for the Marble Hall area because of too high heat units. Orighstad and other cooler areas may be more appropriate for this selection. Hadas is also not sutable for this area because of too low heat units. Hot areas may be more appropriate like Swaziland.

Navels (Burgersfort): To optimise profitability by improving productivity (fruit set and size); pack out percentage (creasing and oleo resistance, smaller navel ends to counter mealy bug, *Alternaria* infection, less wind prone – time of flowering and inside bearing), fruit quality (rind colour early in the season, juice quality, granulation, low acidity) and extend the harvest and marketing season (early- mid and late maturing selections). Most of the selections evaluated did not comply with the minimum export standards. Low acid levels seem to be one of the common problems and reasons for this scenario. The trial will be evaluated for one more season that might foresee some explanations.

Navels (Marble Hall): All the selections evaluated in this trial did not comply with the minimum export standards. The large quantities of rainfall measured late in the season might be part of the problem for the decrease in fruit quality. The trial will be evaluated for one more season.

Valencias (Onderberg): To optimise profitability by improving internal fruit quality (seedlessness, fibre toughness, juice quality); improving productivity and fruit size distribution (count 48, 56 and 72); improving rind colour, external appearance and peelability; extending the picking period by developing early (June / mid July) and late maturing selections (for hot areas). There was good potential between most of the selections that were evaluated. Alpha and Turkey were the best selections for this season. Ruby Valencia seemed to have an off season, producing small green fruit up to harvest time. There are still no signs of incompatibility with Turkey on CC, and the bud union looks healthy.

Valencias (Swaziland): The trees are still young and this was the first evaluation conducted. The production and quality of the fruit will improve by time, including the average fruit size. Alpha seems promising, with most of the other selections not complying with the minimum export standards.

Lemons: To develop cold hardy, thornless, seedless (with acceptable fruit size) lemon selections which are compatible with a wider rootstock range; to extend the picking period to ensure continuity of supply from March to September, i.e. early and late maturing selections; to reduce the problems associated with protracted flowering; to maintain high fruit quality (colour, rind thickness, juice content). Tree characteristics and performance of new cultivars were compared with the commercially grown Eureka in light of the above objectives. Villafranca still produce the lowest seed count per fruit followed by Verna. We determined the production per tree for this season. Limoneira produced the best yield on the trees with 151kg, followed by Fino 49. Evaluations will stop. Eureka SL (ARC) remains the best seedless lemon selection available, keeping in mind that Limoneira produced the best crop on the trees with high numbers of seed per fruit.

Projekopsomming: Binnelandsestreek

Clementines: 'n Proef is saamgestel om te bepaal of sekere Clementine manderyne kommersïeel vir uitvoer in die intermediêre en koel binnelandse sitrusproduserende streke van die land met betrekking tot markbehoeftes, geproduseer kan word. Daar word gesoek na uitstaande seleksies met uitstekende interne vruggehalte, eksterne vrugkleur en vruggrootte verspreiding. Hierdie seisoen was Ain Toujdate weer eerste gereed om geoes te word, met slegs SC se sap vlakke onder die minimum uitvoer vereiste. Die bome het oor die algemeen baie goed gedra, en baie takke het gebreek of geskeur onder die oes lading. Kruisbestuiwing was laer en daar het dus minder sade per vrug voorgekom.

Mandaryne (Burgersfort): Geskikte Mandaryn seleksies vir die warm, intermediêre en koel binnelandse sitrusproduserende streke moet gevind word om die vroeë-, middel- en laatseisoen leemtes te vul. Primosole was die vroegste gereed vir oes, maar die seleksie ondervind heelwat probleme in hierdie area. Granulasie, groot vruggrootte, baie growwe vrugte en lae sap volume was van die ergste probleme wat hier opgeduik het. Orighstad sal moontlik `n beter klimaat wees vir die aanplant van Primosole. Hadas word tans in Swaziland by Tambuti Estate ge-evalueer en die bome behoort in die volgende seisoen heelwat vrugte te produseer. Cami het hierdie seisoen baie goed gevaar met goeie interne kwaliteit en produksie. Die vrugte he took 'n soet, sappige smaak opgelewer.

Mandarins (Marble Hall): Primosole word nie vir die Marble Hall area aanbeveel nie, want die hitte eenhede van hierdie area is te hoog. Orighstad area en ander koeler gebiede kan oorweeg word. Hadas word ook nie vir hierdie area aanbeveel nie, want vir hierdie seleksie is die hitte eenhede weer te laag. Warmer gebiede kan oorweeg word soos Swaziland ens. Roma lyk die belowendste van die ander seleksies wat in hierdie proef ge-evalueer is. Die vruggrootte en interne kwaliteit voldoen aan die nodige vereistes.

Nawels (Burgersfort): Winsgewendheid moet verhoog word deur boomproduksie (oes- en vruggrootte), pakpersentasie (kraakskil en oleo weerstand, kleiner nawelente om witluis teë te werk, *Alternaria*-besmetting, windbestandheid, blomdatum, en binnedrag) en vruggehalte (skilkleur vroeg in die seisoen, sapgehalte, granulasie, lae suur) te verbeter, asook om die oes- en bemarkingseisoen (vroeë-, middel- an laatrypwordende seleksies) te verleng. Meeste van die seleksies wat hierdie seiseon ge-evalueer is, het nie aan al die uitvoer standaarde voldoen nie. Lae suur vlakke by die vrugte was 'n algemene voorkoms gewees. Die proef sal nog een seisoen ge-evalueer word wat moontlike oplossings kan uitlig.

Nawels (Marble Hall): Al die seleksies wat in hierdie proef ge-evalueer word het nie aan die minimum uitvoer standaarde voldoen nie. Die groot hoeveelheid reën wat laat in die seisoen ontvang is, kan moontlik bydra tot die afname in kwaliteit van die vrugte. Die proef sal nog vir een jaar ge-evalueer word, waarna geldige gevolgtrekkings gemaak kan word

Valencias (Onderberg): Winsgewendheid moet verhoog word deur interne gehalte (saadloosheid, veselsterkte, sapgehalte) te verbeter; beter boomproduksie en vruggrootteverspreiding (telling 48, 56 en 72); beter skilkleur, uitwendige voorkoms en skilbaarheid; verleng plukseisoen deur vroeg- (Junie/middel Julie) en laatrypwordende seleksies (vir warm streke). Meeste van die seleksies toon baie potensiaal in hierdie proef. Dis veral Alpha en Turkey wat besonders goed gevaar het hierdie seisoen. Ruby Valencia het moontlik 'n af seisoen gehad, met klein groen vrugte tot en met oes. Daar word nog geen tekens van onverenigbaarheid opgemerk by Turkey op CC nie, en die entlas verbinding lyk gesond.

Valencias (Swaziland): Die bome is nog jonk en is vir die eerste keer ge-evalueer. Die produksie en kwaliteit van die vrugte sal nou begin toeneem, insluitend die algemene vruggroote. Alpha het klaar belowend gevaar, met meeste van die ander seleksies wat nog nie aan die uitvoer standaarde voldoen het nie

Suurlemoene: Kouegeharde, doring- en saadlose suurlemoenseleksies (met aanvaarbare vruggrootte), wat met 'n wye reeks onderstamme verenigbaar is, moet ontwikkel word. Die oesseisoen moet verleng word om aaneenlopende produksie van Maart tot September te verseker, aangesien dit vroeë en laatrypwordende seleksies insluit. Probleme wat met lang blomtyd ge-assosieer kan word, moet verminder word. Goeie vrugkwaliteit (kleur, skildikte, sapinhoud) moet behou word. Die boomeienskappe en prestasie van nuwe kultivars moet met die kommesieel gekweekte Eureka vergelyk word om te bepaal of hulle aan bogenoemde doelwitte voldoen. Villafranca produseer steeds die laagste saad telling per vrug, gevolg deur Verna. Die produksie per boom is vir hierdie seisoen bepaal. Limoneira het die beste ous op die bome geproduseer met 151kg, gevolg deur Fino 49. Evaluasies sal nou op heirdie proef gestaak word. Eureka saadloos (LNR) bly steeds die beste saadlose suurlemoen seleksie tans beskikbaar, maar Limoneira produseer 'n hoër oest met heelwat saad.

6.3.3 **Evaluation of Satsuma mandarins and Primosole in the Cape areas**
Experiment 57 by C J Alexander (Private Contractor)

Opsomming

Die doel van hierdie projek is om geskikte, hoë gehalte seleksies te vind wat vroeg in die seisoen opkleur en die vroeë mark kan binnedring, asook om produksie pieke, beide vroeër en later, te voorkom. Satsuma x Nova lyk belowend as 'n vroeë seleksie. Primosole word vroeg ryp met wisselende potentiaal op hierdie vroeë stadium. Die kommersiële Dobashi Beni bome is nog nie in produksie nie. 'n Nuwe, enkele proef met al die laatrypende Satsuma seleksies is uitgelê.

Introduction

The objective of the Satsuma project is to provide high quality, well coloured fruit early in the southern hemisphere marketing season, to capitalise on market opportunities between the late northern hemisphere season and early southern African citrus season and to overcome anticipated production peaks by extending the harvest season earlier. There is also a demand for later maturing selections.

Materials and methods

The trial trees are either planted or topworked within commercial orchards, or established on a semi-commercial scale, with Miho Wase or Owari as a control where possible. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape areas. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is also based on subjective tasting.

Fruit quality was compared with the following standards previously considered most acceptable by the market place, based on visual and organoleptic tests: 48% juice; 8.5% Brix or 9.0% TSS; 0.7 – 1.5% acid; 7.5:1 ratio; colour T3 of set 36 of CRI blemish standards; 0 seeds per fruit. A list of the selections and sites evaluated is given in Table 6.3.3.1 and internal quality tests in Table 6.3.3.2.

Table 6.3.3.1. Satsuma trial sites evaluated during 2006.

Selection	Area	Site	Plant Date	Root stock	No of trees
Satsuma x Nova	Addo	ITSC	2000	CC	3
Dobashi Beni	Wolseley	Whitebridge	2003	TC	Semi com t/w
Primosole	Uitenhage	CFB Psylla house	2003	SC	6
Primosole	Addo	ITSC			
Primosole	Stellenbosch	Slaley	1999	CC	3
Primosole	Citrusdal	Brakfontein	2001	CC	2

Results and Discussion

A discussion of each selection follows with a comparison of the selections and internal quality results presented in Tables 6.3.3.2 & 3 which need to be referred to when reading the text.

ITSC Satsuma x Nova. The yield was good with a good medium, to larger fruit size. Colour during the 2nd week of April was T5-6, the fruit firm with a flattish shape and a thinnish, fairly tight skin, fairly easily peeled with slightly open cores. The sugars were high (11.4% Brix and acid around 1.2%). Maturity after Miho Wase. The cultivar is protected and managed by Citrogold.

Dobashi Beni. This selection is renowned to have a deeper, redder rind colour than Owari. Initial observations on single trees have shown this, with other characteristics similar to Owari. An orchard was topworked to Dobashi Beni to provide information on a larger scale but the topworking was not too successful and only some young interplants were evaluated. These only bore the occasional fruit off odd trees. Not too much emphasis should be placed on the evaluation at this stage due to the erratic crop/set and young tree age. Next season should produce enough fruit for a meaningful evaluation. The Dobashi Beni is reported to perform well on C35 rootstock in California.

Primosole. An early maturing Italian hybrid of Miho Satsuma and Carvalhais (seedy Portuguese mandarin). The tree looks somewhat different to a Satsuma, a drooping habit when heavily laden with long, curled leaves. Yields on slightly older trees were good. Fruit size tends to be on the large side, Count 1XXX – 1 at Stellenbosch, larger than adjacent older Marisols and even more so than similar aged Clemenpons. Colour was variable on each tree but this could also be due to the fact that some fruit had already been picked. Colour not necessarily earlier than Marisol/Clemenpons - this needs to be established. The fruit has an unusual flavour, not outstanding, sometimes lacking sugars and flavour. Flesh colour is orange, some open cores, vesicles are large and can be ricey/dry. This can also be due to young tree age. Only one test was conducted, the acid exceeding 1%, which was similar to Marisol. Both selections had been partially picked. Sugars in the Western Cape were adequate. There was zero to odd seed, counts between zero to 0.1 seeds per fruit, adjacent Marisol similar.

The fruit had a flatter shape, better looking (attractive) in the Western Cape and firmer, but this may be due to tree age and management practices. The fruit looks more and feels more like a Satsuma (soft) the more mature it gets. The rind is smooth to slightly coarse. Peelability is fairly easy with some oil contamination (unique aroma). Creasing was observed. Maturity is early, varying per area but around late March, to first two weeks in April. Due to the fact that both adjacent Marisol and Clemenpons had been partially picked on evaluating in Stellenbosch, one cannot say whether it is in fact earlier than the other two selections or not. The cultivar is protected and managed by Citrogold.

Imamura. There should be a few trial trees coming into production in the Wolseley area next season.

Late selections. A trial has been planted in the Paarl area, which includes the following selections: Dobashi Beni, Ohtsu, Imamura, Ueno, Aoshima and Owari as control. These should bear some fruit in 2008 or 2009.

Conclusions

Satsuma x Nova. Looks promising and needs to be established in other areas as a potential early Satsuma.

Dobashi Beni. There were only odd fruit of both Dobashi Beni and Owari. The trees are still too young and small to produce any meaningful results. Next season should produce enough fruit for a proper evaluation.

Primosole. Yields were generally good with large fruit size and acceptable quality. The fruit appeared better in the Western Cape, but not necessarily due to climate. It has a lot of Satsuma traits, more apparent as the fruit matures. At some sites the fruit appeared to have no potential, at others possible potential as an early, large fruited variety, but tends to be dry/ricey, which could be related to tree age. The cultivar is early maturing. There is too little information available to make recommendations and further evaluiations are necessary.

Future evaluations

Evaluate Dobashi Beni when semi commercial block comes into production and late selections when the new trial comes into production. Continue evaluations with Primosole.

Table 6.3.3.2. Comparison of the production, colour, quality, maturity, comments and seed of various Satsuma selections at different sites in the Cape areas during 2006.

Selection	Date	Site	Root stock	Yield	Fruit Size	Colour	Taste	Test	Maturity	Comment	Ave seed
Satsuma x Nova	12/4	ITSC	CC	Good	Medium+	5-6	Good	11.4% Brix, ± 1.2% acid	After Miho Wase	Firm	
Dobashi Beni	24/5	Whitebridge	TC	Odd fruit	Variable, medium large	Variable set,T1-2 & later	Sufficient acid	± 8% Brix	Possibly a few days later than Owari	Smaller fruit firm, trees still young	
Owari (control)	24/5	Whitebridge	TC	Odd fruit	Variable medium large	Variable set, T1 & later	Sufficient acid, small fruit tart	± 10% Brix	± Peak	Fruit firm, trees still young	
Primosole	11-23/4	CFB	SC	Fair	Medium large	1-2 and 1	Unusual flavour, lacks quality		Mid-late March	Creasing, flat shape	0
Primosole	12/4	ITSC		Good – excellent	Medium large – large	4-7	Poor	8.9% Brix	Over-mature	Satsuma like, better externally than CFB. Ricey/dryish	
Primosole	18/4	Slaley	CC	Fair-excellent, some fruit picked	Med large – large (1-1XXX 71.5mm)	2-6	Varies, fairly good sugars, generally high acid	Good, ratio between Marisol and Clemenpons	1-2 weeks to go, but earlier fruit probably picked		0.1
Primosole	24/4	Brakfontein	CC	Few fruit	Medium large - large	5	Fair – good, sufficient sugars and acid. Ricey looking.		Past by 2 weeks	Look similar to Stellenbosch fruit	0

Table 6.3.3.3. Internal fruit quality data for Satsuma mandarins (and control Clementines) for Stellenbosch, Western Cape tested during the 2006 season.

Selection	Rootstock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Average Seed
Primosole	CC	Slaley	18/04	2-4	1X	58.4	10.8	1.15	9.4	0.1
Clemenpons	CC	Slaley	18/04	3-5	2	59.4	10.8	0.99	10.9	0
Marisol	CC	Slaley	18/04	3-5	2	65.1	10.3	1.19	8.7	0

6.3.4 **Evaluation of Clementine Mandarins in the Cape areas**
Experiment 63 by C J Alexander (Private Contractor)

Opsomming

Die doel van die projek is om 'n breë verskeidenheid kultivars met hoër gehalte, goeie skilkleur en met goeie vruggrootte te verskaf om die pieke in die middel Clementine seisoen af te plat deur die oestyd beide vroeër en veral later te verleng. Clemenpons word vroeg ryp, het goeie gehalte, medium vruggrootte, wisselende boomgrootte en galle op die stam. Die uitwerking van die galle in die langtermyn moet vasgestel word. Tardif de Janvier I het goed gedra maar met medium vruggrootte en suur aan die laer kant. Tardif de Janvier II het ook matige vruggrootte met laer suur gehad en lyk nie asof dit laat ryp word nie. Inligting op albei seleksies is beperk. Die Tardivo het swak produksie met medium tot klein vruggrootte, goeie gehalte, groen blomente en word tussen mid Mei tot mid Junie ryp. Nour produksie was swak met swak vruggrootte. Eetgehalte was goed en rypwording soortgelyk aan Tardivo. Skilkleur is vertraag en die seleksie lyk nie belowend nie. Finale evaluasies op sekere van die seleksies is nodig.

Introduction

The objective of the project is to find suitable superior Clementine selections to help flatten the existing midseason production peaks by extending the harvest season both earlier and particularly later in accordance with market needs.

Materials and methods

The trial trees are either planted or topworked within commercial orchards, or established on a semi commercial scale, using Marisol, Nules or Clemlate as controls where possible. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape areas. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is also based on subjective tasting. Fruit quality was compared with the standards previously considered most acceptable in the market place: 48% juice; 9.5% TSS; 0.7 – 1.5% acid; 8.0:1 ratio; colour T3 of set 36; seed maximum average 3.0 seeds per fruit. A list of selections and sites evaluated is given in Table 6.3.4.1.

Table 6.3.4.1. Clementine trial sites evaluated during 2006.

Selection	Area	Site	Plant Date	Root stock	No of trees
Clemenpons	Uitenhage	CFB Block 7	1999	RL	2
Clemenpons	Patensie	Tierhok			comm
Clemenpons	Stellenbosch	Slaley	1999	CC	4
Tardif de Janvier I	Uitenhage	CFB Psylla house	1999	TC	1
Tardif de Janvier I	Buffeljagsrivier	Sovereign	1999	SC	4 topwork
Tardif de Janvier II	Uitenhage	CFB Block 4	2001	CC	4
Tardif de Janvier II	Uitenhage	CFB Psylla house	2001	CC	4
Tardivo	Uitenhage	CFB Block 7	1999	TC	
Tardivo	Buffeljagsrivier	Sovereign	1999	SC	10 topwork
Tardivo	Citrusdal	Brakfontein	1998	CC	5 topwork
Nour	Uitenhage	CFB Block 8	1999	SC	2
Nour	Buffeljagsrivier	Sovereign	1999	SC	7 topwork
Nour	Citrusdal	Brakfontein	1988	CC	5 topwork
Marisol (control)	Stellenbosch	Slaley	1992	CC	commercial
Nules (control)	Buffeljagsrivier	Sovereign	1990	TC	3
Clemlate (control)	Buffeljagsrivier	Sovereign	1999	SC	3 topwork

Results and Discussion

A discussion of each selection follows with rootstock, production, fruit size, fruit colour, maturity, comment and average seed presented in Table 6.3.4.2 for early maturing selections and Table 6.3.4.3 for later selections and internal quality results in Table 6.3.4.4 which need to be referred to when reading the text. The Clemenpons was evaluated as a potential early maturing clementine, Tardif de Jan I & II are unknown at this stage and Tardivo and Nour as potential late maturing selections.

Clemenpons. Generally good yields between the sites, although some fruit picked which commenced on 12 April. Fruit size tends to be on the small side (medium small) although some trees have less, larger, greener fruit (and smaller galls). Fruit shape was fairly round and smooth to slightly pebbly, with an occasional nipple. Colour (3rd week April) varied between sites (some fruit picked) T1-3, T4 and T5-6. There were some bunches of green fruit at Stellenbosch. The fruit is fairly easily peeled and oily with an orange flesh colour and cores between closed and open. Fruit was fairly easily picked at Stellenbosch with similar to even slightly later colour than older Marisol. The quality at Stellenbosch and Patensie was good but poor at the CFB (low sugars and acid). The tests at Patensie and Stellenbosch were good with high juice levels and good to high sugars and good acid levels (just below 1.0%), too few fruit at the CFB. Marisol at Stellenbosch had 0,5% lower sugars and 0,2% higher acid with a somewhat lower ratio. There were odd seed at Patensie. Trees have variable vigour and galls on the stems. It appears that the better coloured fruit tend to have more galls (not noted for CFB). Odd brown spots were observed on the navel ends at the CFB. Maturity is around 2nd to 3rd week of April.

Tardif de Janvier I. There were no fruit at Buffeljagsrivier, a good crop at the CFB with medium fruit size and colour T1 on 6 June. Fruit shape is mainly round with a smooth to finely pebbled rind and oily on peeling. Flesh colour is deep orange with an open core, quality fair with low acid and probably good at peak maturity, but now past. The test had very high sugars and just acceptable acid (psylla house) and seedless. Stems are black.

Tardif de Janvier II. Evaluated in the psylla house and open block at the CFB. The yield was fair with mainly medium to larger fruit size. The colour in the psylla house was earlier, T 1-3 versus T3-4 for the open ground trees. Fruit shape is round to flattish with a smooth to pebbly rind, easily peeled and oily with deep orange flesh and open core. The taste was fair with low acid, poorer quality than T. de Jan I and past peak maturity. The sugars were good to high, but acid levels in both tests too low. Seedless in non pollinated blocks and stems black.

Tardivo. Yield was poor with medium small fruit size. The quality was good with high sugars and good acid levels. The fruit was less raggy and softer than Nour in Citrusdal. The fruit has green stylar ends, while the Nour generally has a lighter rind colour. The Tardivo colouring is more consistent. The fruit is virtually seedless in the absence of cross pollination. Maturity variable between sites, mid May to mid June.

Fruit shape is round, but with nipples in Citrusdal (76%), a smooth to pebbly rind (smoother than Nour), difficult to peel, oily and brittle. The trees are flat, squat and dense with a very dark stem.

Nour. Yields poor to fair with mainly medium and smaller fruit size. Fruit shape is generally round, 55% with nipples in Citrusdal, a smooth to very pebbly rind and very oily on peeling. Seedless in absence of pollinators. Eating quality generally good but can be a bit dry. Maturity around end May to mid June, but rind colour may be retarded. The trees are vigorous and dense with long and sometimes wavy leaves and a dark to black stem.

Conclusions

Clemenpons. Good yields and generally medium small fruit size. Fruit is early maturing around 2nd - 3rd week of April. Good quality and seedless to odd seed. Of concern is the variation in tree size and galls. Further evaluations are necessary. Clemenpons is a protected selection.

Tardif de Janvier I. Good yield but medium fruit size. High sugars and lowish acid, probably matures at the end of May. Limited information. Evaluations to continue.

Tardif de Janvier II. Fair yield with medium to slightly larger fruit size. Good sugars, but unacceptably low acid, probably maturing late May to early June. Due to the limited information, no recommendations can be made and it does not appear to be late maturing. Evaluations to continue.

Tardivo. Production was poor with medium small fruit size. Fruit quality is good and maturity mid May to mid June. Green stylar ends are prevalent. Evaluate one more year.

Nour. Production was generally poor to fair, fruit size mainly medium and smaller. Fruit quality was generally good with good tests and good acid levels, although the fruit can be a bit dry. Maturity around end May to mid June, but rind colour may be retarded, with green stylar ends. The Nour does not look a promising late Clementine selection. Evaluate one more year.

Table 6.3.4.2. Comparison of the production, fruit size, rind colour, maturity, comment and average seed of various early maturing Clementine selections at the different trial sites on different dates in the Cape areas during 2006.

Area	Selection	Root stock	Production	Fruit Size	Date, Colour transparency	Maturity	Comment (ave seed)
Uiten-hage	Clemenpons	RL	Poor - good	Medium small	**23 April** T4		Poor, low TSS and acid. (±3)
Patensie	Clemenpons	SC?	Good, variable, some fruit picked	Generally medium small	**21 April** T5-6	Peak	Good quality, greener fruit have less galls. (0–0.8)
Stellen-bosch	Clemenpons	CC	Good - excellent, variable	Variable, generally medium small up to medium large. Mainly calibre 2-3 (58.3mm)	**18 April** T1-3, 5-6, 7	Good quality, better than Marisol. Peak to 1 week to go.	Good quality, superior to Marisol. Galls. (0)

Table 6.3.4.3. Comparison of the production, fruit size, rind colour, maturity, comment and average seed of various mid and late maturing clementine selections at the different trial sites on different dates in the Cape areas during 2006.

Area	Selection	Root stock	Production	Fruit Size	Date, Colour transparency	Maturity	Comment (ave seed)
Uiten-hage	Tardif de Janvier I	TC	Good	Medium	**6 June** T1	End May	Lowish acid but past peak. (0)
	Tardif de Janvier II	CC	Fair	Medium and larger	**6 June** T1-3 and 3-4	Probably end May, early June	Fair, good sugars, acid too low. (1 and 1.8)
	Tardivo	TC	Poor	Medium small	**6 June** T5-6, **19 July** T1-3	2 weeks short of peak in early June.	Good quality, split fruit. (4.8)
	Nour	CC	Poor	Small	**6 June** T5-6, **19 July** T1	Mature around mid June, but colour poor	Good quality, high sugar and fair acid. (0.9 – 2.2)
* Buffel-jags-rivier	Tardif de Janvier I	SC	Zero				
	Tardivo	SC	Zero				
	Nour	SC	Fair	Medium and slightly larger	**25 May** T3, all green stylar ends	Probably end May but difficult to judge because dryish. Fruit look slightly old	Good quality, slightly dry. (2.8)
	Clemlate (control)	SC	Good	Small - medium small	**25 May** T5-6, all green stylar ends	Not yet mature, probably towards early June	Good eating quality, high acid. (5.4)
	Nules (control)	SC	Good	Medium and slightly larger	**25 May** T1-2, 3-4, some light green stylar ends	Peak	High quality. (0.3)
Citrus-dal	Tardivo	CC	Zero to poor	Medium small Calibre 1-3 mainly 2-3 (59.6mm)	**15 May** T6-7, some green stylar ends	Mature around mid May, picks easily	Good quality, less rag and less acid than Nour. 12% light oleo. (0.2)
	Nour	CC	Poor-zero	Variable, medium Calibre 1-3 (60.7mm)	**15 May** T6-7 87.5% green stylar ends	Around end May	Fair quality, good test, good acid. No oleo. (0)

* Note – the site at Buffeljagsrivier tends to get waterlogged during heavy rains.

Table 6.3.4.4. Internal fruit quality data of the various Clementine selections for the Eastern and Western Cape during the 2006 season.

Selection	Root stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Clemenpons		Tierhok	21/04	5-6	2	60.5	11.9	0.86	13.9	0.8
Clemenpons		Tierhok	21/04	5-6	3	61.1	12.6	0.94	13.4	0.1
Clemenpons	CC	Slaley	18/04	3-5	2	59.4	10.8	0.99	10.9	0
Tardif de Jan I	TC	CFB	06/06	1	1	59.0	12.5	0.77	16.2	0
Tardif de Jan II Ps	CC	CFB	06/06	1-3	1X	49.4	12.8	0.57	22.5	0
Tardif de Jan II B4	CC	CFB	06/06	3-4	1	60.0	11.2	0.69	16.2	1.8
Tardivo	TC	CFB	06/06	5-6	2	59.0	12.6	0.86	14.7	4.8
Tardivo	TC	CFB	19/07	1-3	3	59.3	12.1	0.91	13.3	1.3
Tardivo	CC	Brakfontein	15/05	6-7	2	60.7	11.8	1.15	10.3	0.2
Nour	SC	CFB	06/06	5-6	3	56.1	12.9	0.93	13.9	2.2
Nour	SC	CFB	19/07	1	3	53.5	13.7	1.04	13.2	0.9
Nour	SC	Sovereign	25/05	3	1	53.4	13.5	1.12	12.1	2.8
Nour	CC	Brakfontein	15/05	6-7	2	56.9	11.1	1.14	9.7	0
Marisol	CC	Slaley	18/04	3-5	2	65.1	10.3	1.19	8.7	0
Nules	SC	Sovereign	25/05		2	57.7	13.8	0.88	15.7	0.3
Clemlate	SC	Sovereign	25/05	5	3	48.5	13.3	1.43	9.3	5.4

Future evaluations

Continue evaluations on most selections.

6.3.5 Evaluation of Mandarin hybrids in the Cape areas

Experiment 73 by C J Alexander (Private Contractor)

Opsomming

Die doel van die mandaryn projek is om hoër gehalte, goed gekleurde, saadlose mandaryn seleksies te ondersoek. Hulle moet ook goeie produksie en vruggrootte hê en die sagte sitrus seisoen kan versprei, beide vroeër en veral later. M37 het goeie gehalte en word in Junie ryp. Die semi kommersieële Murcott x Clem boord het begin dra. Die vrugte was groot met aanvaarbare vruggehalte, vol saad en word laat Junie ryp. Bay Gold het goeie produksie en vruggrootte gehad maar lyk nie belowend in die koeler gebiede nie weens hoër suur. Hadas het goeie produksie en vruggrootte gehad maar gereelde hoër suur soos Ellendale. Winola het swak produksie, medium vruggrootte en hoër suur gehad. Cami het matige produksie, goeie vruggrootte en hoër suur gehad. Empress mandaryn het swak produksie en vruggrootte gehad en lyk nie belowend nie. Van die seleksies moet verder evalueer word.

Introduction

The objective of the mandarin hybrid project is to find high quality, well coloured, seedless selections with good production and fruit size, with special emphasis on extending the soft citrus season earlier and particularly later.

Materials and methods

The trees were either planted or topworked within commercial orchards where possible (to prevent cross pollination), or established on a semi commercial scale. Comparisons were made with a range of existing commercial selections or Clementines where possible. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape areas. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is also based on subjective tasting. Fruit quality was compared with the standards for Mandarins: 50% juice; 8.5% Brix; 0.7 – 1.5% acid; 7.5:1 ratio; colour T3 of set 36; seed maximum average 3.0 seeds per fruit. A list of selections and sites evaluated is given in Table 6.3.5.1.

Table 6.3.5.1. Mandarin hybrid trial sites evaluated during 2006.

Selection	Area	Site	Plant Date	Root stock	No of trees
ITSC M37	Fort Beaufort	Baddaford	1998	TC	4
Murcott x Clem	Uitenhage	CFB Block 8	1999	TC	2
Murcott x Clem	Kirkwood	Kirkwood	2003	CC	semi com
Bay Gold	Uitenhage	CFB Block 8	1999	TC	2
Bay Gold	Clanwilliam	Jansekraal	2001	CC	48 semi com
Hadas	Uitenhage	CFB Block 8	1999	TC	2
Winola	Uitenhage	CFB Block 8	1997	SC	2
Cami	Uitenhage	CFB Block 8	1999	SC	2
Empress Mandarin	Uitenhage	CFB Block 8	1997	TC	3
Gold Nugget	Uitenhage	CFB Psylla house	2004	CC/SC	3
Nules (control)	Uitenhage	CFB Block 4	2001	SC	3

Results and discussion

A discussion of each selection follows. Various tables also need to be referred to when reading the text. Tables 6.3.5.2 & 3 are a comparison of the various selections at different sites and internal quality data is presented in Table 6.3.5.4.

ITSC M37. Trees carried a mainly good crop of medium fruit size, colour transparency T1 and odd T3 on 12 June. Fruit shape is slightly flat with a smooth rind. Internal colour is deep orange with closed cores and a good rind thickness. Peelability is difficult and oily, very juicy and messy to eat. The quality was excellent with high sugars and acid still a bit high, but not tart. The test was very good. The fruit was firm with signs of creasing, picking fairly easily and externally at peak, to one week to go in mid June. The tree size was medium large and seedy under cross pollination.

Murcott x Clementine. Good yields and fruit size at the CFB, mainly T1-2 on 6 June and fair quality. Overmature in late July. At Kirkwood the trees carried their first small crop, variable, zero to good with large fruit size and out of season fruit. The rind colour was T2-4 on 13 June with a deep colour beginning to show through. The fruit shape is flattish with a fairly smooth rind. The fruit is firm and easily peeled with a thin, oily rind and deep orange, slightly coarse flesh and open cores. Fruit size was between counts 1X – 1XXX, mainly 1XX averaging 74.1mm. The fruit is seedy in a solid block, averaging 8.0 seeds/fruit with the following breakdown: 12% 0-3 seeds, 60% 4-9 seeds, 28% 10-16 seeds/fruit. Quality was good and slightly tart although the sugars could be higher, about 2 weeks short of peak maturity. There were odd mealy bug.

Bay Gold. Good yield and large fruit size. The colour was good on 6 June, T1-3 and looking overnature and slightly puffy a week later. The quality was poor with just acceptable sugars and high (just acceptable) acid resulting in a low ratio. The fruit has an obovoid to pyriform shape and a smooth rind, open core and orange, sometimes dry flesh and variable seed numbers. There was some stylar end split in April. Due to the poor quality (high acid) the trees at Clanwilliam have been removed.

Hadas. Good production and fruit size. The fruit was pale and picked easily in late July, too tart with excessive acid, even though the sugars were high. Fruit shape is typical Ellendale, fairly flat, smooth and some fruit with a tiny navel. Fairly easily peeled but very oily with orange flesh and seedy in a mixed block. The fruit quality improved by mid August when the acid had dropped to 1.38%, the test unacceptable in July (high acid) and acceptable in August. Some wind blemish. The features are typical of an Ellendale. Maturity around early August.

Winola. Production and fruit size was poor, colour almost acceptable in June. The eating quality only became acceptable in late July due to the high acid, even though sugars were high. The tests in July and August had unacceptably high acid. Fruit shape is flattish with a smooth rind. Difficult to peel, oily and messy on peeling in June. Zero to odd seed in a mixed block. Maturity around July/August.

Cami. The yields were fair with medium large fruit size, variable coloured fruit on the tree in June. The fruit had high acid and sugars and tart on eating. The acid level was still 1.32% after mid July, hardly dropping over a 4½ week period. The fruit has an Imperial like flavour and easily peeled in July but oily. Fruit shape is roundish with a smooth, thin rind. There was some split fruit in June. The trees are vigorous with a willowy shape opening under a heavy crop, the thin branches prone to sunburn and appear to lose leaves

with a heavy crop. A lot of probably wind blemish, alternaria? Externally the fruit was overmature after mid July and picked easily, but still tart, although the sugars were high. Seedy in a mixed block.

Empress mandarin. Production was poor with small fruit size. Colour on 6 June was T6-7 and 2-4 in late July. Fruit quality was variable in July, lacking sugars to sweetish to tart. The fruit picked reasonably easily. Fruit shape was round to flat with shoulders and a smooth rind. Peelability in July was fairly easy but very oily and the rind sometimes braeaking up. Flesh colour was a good orange with open cores and seedy in a mixed block. The test had fair sugars and extremely high acid in early June, dropping drastically in 4½ weeks. Maturity difficult to judge, probably July. Trees are upright and narrow with a noticeably benched bud union on Troyer and severe on Swingle.

Gold Nugget: First few fruit borne of medium fruit size and colour T1 on 27 July. Fruit looks similar to an Empress mandarin with a flat to sunken stylar end and nipple on the stem end. The rind is smooth. The tree is tall and vigorous with some thorns. There were too few fruit to test.

Table 6.3.5.2. Comparison of production, fruit size, colour, maturity, comment and average seed of various mandarin hybrids evaluated at the CFB, Uitenhage during 2006.

Selection	Production	Fruit size	Date, Colour transparency	Maturity	Comment	Ave * seed
Murcott x Clem	Good	Medium large and smaller	**6 June** 1-2 to 4 **19 July** 1	Mid to end June	Fair quality	10.3 - 10.8
Bay Gold	Good	Medium large - large	**6 June** 1-3	Probably early June but acid too high	Poor quality, fair sugars but acid too high. Unacceptable test, fruit dry	4.5
Hadas	Good - excellent	Mainly medium large	**19 July** 1-3, yellow orange **17 Aug** 1	Probably early August	High sugars but too tart, fair in mid August	10.8 - 12.3
Winola	Poor	Medium	**6 June** 3-4 **17 Aug** 1	Probably mature in July/August	Poor quality in June due to high acid, high sugars	0.4 – 0.7
Cami	Fair	Medium large	**6 June** 2-3 **19 July** 1	Externally mature in early/mid July, internally early August	Fair in June (tart) good but slightly tart in July, Imperial like flavour. Good test, but high acid.	7.3 - 7.8
Empress mandarin	Poor - zero	Small	**6 June** 6-7 **19 July** 2-4	Mature Internally in June except acid, probably only externally in July	Variable quality in July. Acid too high in June.	5.9 - 6.4
Nules (control)	Good	Medium large	**6 June** 1	Mature in early June	Good quality, test has high sugars and borderline (low) acid	3.0

* Average seed counts from internal quality tests.

Table 6.3.5.3. Comparison of production, fruit size, colour and comment of various mandarin hybrids evaluated at ITSC, Addo on 12 April 2006. These are not discussed further.

Selection	Production	Fruit Size	Colour	Comment
Satsuma x Nova	Good	Good, medium to larger	5-6	Good quality and firm (11.4 Brix and 1.2% acid.). A flattish shape with slightly open cores and fairly tight, thinnish rind and fairly easily peeled. Potential as an early selection after early satsumas.
J8 O7	Excellent	Medium small	4, excellent deep orange/red, shiny	Acceptable quality, not outstanding (11.2-11.9 Brix). Doesn't taste as good as it looks. Difficult to peel, closed cores, excellent flesh colour. Tough segment walls and seedy.

Conclusions

ITSC M37. Production was good but fruit size only medium. Fruit quality was good, but messy on peeling and seedy under cross pollination. Mature in mid June. No further evaluations.

Murcott x Clementine. Considering the tree age, the trees bore well with large fruit size. The cultivar is seedy and quality not outstanding although tests are good, which is consistent with previous observations. Evaluations to continue.

Bay Gold. Production was good with good fruit size. Quality was poor due to mediocre sugars and high acid levels. Maturity is estimated around early June, but the acid is still high. The selection does not look promising and evaluations to continue for one more season.

Hadas. Production and fruit size was good but eating quality poor due to high acid. Maturity around early August. The selection has typical Ellendale features and is probably best suited to hotter areas. The selection is protected. Evaluate for one more season.

Winola. Production and fruit size was poor and the fruit had excessively high acid on Swingle. Virtually seedless in a mixed block and maturity around July/August. Probably suited to warmer climates. The selection is protected. Further evaluations are necessary.

Cami. Yields were fair with medium large fruit size. The fruit has high sugar and high acid levels. The fruit matured externally by mid July, but not internally and may be suited to warmer areas. The fruit had a lot of blemish. The selection is protected. Evaluate for one more season.

Empress mandarin. Production and fruit size was poor and variable eating quality in July. Probably matures in July. The fruit shows no outstanding features and does not look promising. Evaluations to continue for another season.

Gold Nugget: First few fruit were borne and look in some ways like an Empress mandarin. Too few fruit to evaluate meaningfully. The selection is protected. Further evaluations are necessary.

Table 6.3.5.4. Internal fruit quality data of mandarin hybrid selections for the Eastern and Western Cape during the 2006 season.

Selection	Root stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
ITSC M37	TC	Baddaford	12/06	1	2	61.1	14.3	1.05	13.6	8.1
ITSC H25	BN/RL	ALG	11/07			51.1	10.6 B	1.03	10.3	
ITSC M37	BN/RL	ALG	11/07			58.5	11.8 B	0.83	14.2	
ITSC H36	BN/RL	ALG	11/07			51.0	11.3 B	0.76	14.9	
Murcott x Clem	TC	CFB	06/06	3-4	1X	53.9	10.7	1.13	9.5	10.8
Murcott x Clem	TC	CFB	19/07	1	1XX	55.4	11.7	1.13	10.4	10.3
Murcott x Clem	CC	Kirkwood	13/06	3-4	1XX	57.4	13.4	1.19	11.3	7.9
Bay Gold	TC	CFB	06/06	1-2	1XX	50.4	9.6	1.43	6.7	4.5
Hadas	TC	CFB	19/07	1	1	56.2	12.6	1.64	7.7	12.3
Hadas	TC	CFB	17/08	1	1X	57.8	12.8	1.38	9.3	10.8
Winola	SC	CFB	06/06	3-4	1	58.2	11.9	2.15	5.5	0.5
Winola	TC	CFB	19/07	1	2	55.1	13.5	1.77	7.6	0.7
Winola	SC	CFB	17/08	1	1XX	58.3	12.8	1.54	8.3	0.4
Cami	SC	CFB	06/06	3-4	1	59.8	12.1	1.38	8.8	7.8
Cami	SC	CFB	19/07	1	1	62.1	14.3	1.32	10.8	7.3
Empress Man	TC	CFB	06/06	6-7	3	54.1	10.1	1.82	5.5	5.9
Empress Man	TC	CFB	19/07	2-3	1	53.0	10.9	0.99	11.0	6.4
Nules (control)	SC	CFB	14/06	1	1X	53.1	12.6	0.78	16.2	3.0

Future research

Continue evaluations.

6.3.6 **Evaluation of navel oranges in the Cape areas**
Experiment 74 by C J Alexander (Private Contractor)

Opsomming

Die doel van die proef is om kultivars te bekom om die seisoen vroeër en later te versprei en produksie pieke te verminder, asook om seleksies to vind met meer gevorderde vrugkleur, veral aan die begin van die seisoen en vrugte te kry met verbeterde vrugset wat binne die gesogte vruggrootte reeks val. Fukumoto word vroeg ryp, dieselfde tyd tot effens voor Lina/Newhall en ontwikkel 'n diep oranje-rooi skilkleur. Daar blyk geen duidelike tekens van onverenigbaarheid op citrange x onderstamme nie, maar Swingle citrumelo en Koethan citrange wys moontlike abnormaliteite aan. Meer duidelikheid word benodig oor onderstam keuse. Letaba Early word effens later ryp as Lina/Newhall. Atwood word effens later as Lina/Newhall ryp en skilkleur is vertraag. Dream word later as Lina/Newhall ryp en kan as 'n midseisoen seleksie beskou word. Cliff Early lyk belowend as 'n vroeër nawel. Fenix en Sundays River Early lyk nie asof hulle vroeg ryp word nie en Krajewski Early is nie so vroeg nie. Washington (SGB materiaal) het goed gevaar, met goeie produksie, vruggrootte en gehalte. Cambria (SGB materiaal) het goeie produksie en vruggrootte gehad, beide ronde en langwerpige vrugte met goeie gehalte maar suurvlakke kan neig om laag te wees. Summer Gold word laat ryp. Renken Late, Coetzee Late en Mouton Late 1 en 2 het beter gevaar as die vorige seisoen en moet verder evalueer word. Glenora Late is 'n groeikragtige boom en word laat ryp met goeie gehalte maar vrugte in 'n gemengde blok het pitte in gehad. 'n Vergelyking tussen Autumn Gold, Powell, Chislett en Californian Lane Late het min verskille tussen hulle getoon. Vruggrootte op Citrusdal is geneig om effens groot te wees. Sap vlakke op growweskilsuurlemoen en Rangpur lemmetjie onderstamme op sanderige grond kan laag wees. Verdere evaluasies van al die seleksies is nodig aangesien van die bome nog jonk is.

Introduction

The aim of the navel project is to find cultivars that can spread the navel season both earlier and later so as to minimise production peaks and also find selections with more advanced rind colour, particularly at the commencement of the season as well as improved fruit set potential with fruit in the desired size range.

Materials and methods

The trees were either planted or topworked within commercial orchards, or established on a semi commercial scale. Tuligold, Lina, Newhall, Palmer, Royal Late and Lane Late navels were used as controls. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape areas. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is based on subjective tasting. Fruit quality was compared with the following standards previously considered acceptable by the market place (higher standard for Navelates in brackets): 48% juice; 8.5% (10.0%) TSS; 0.6 – 1.5% (0.8 – 1.50%) acid; 7.5:1 (9.0:1) ratio; colour T3 (T2) of set 34; 0 seeds per fruit.

A list of selections and sites evaluated during 2006 is given in Table 6.3.6.1.

Table 6.3.6.1. Navel trial sites evaluated during 2006.

Selection	Area	Site/ Orchard	Plant Date	Root Stock	No of trees
Fukumoto	Sunland	Woodridge	2000	CC	2 topwork
Fukumoto	Heidelberg	Kruisrivier	2001	C35	4
Fukumoto	Citrusdal	ALG	2002	RL	20 topwork
Letaba Early	Sunland	Woodridge	2000	CC	2 topwork
Letaba Early	Heidelberg	Kruisrivier	2001	C35	7
Atwood	Sunland	Woodridge	2000	CC	2 topwork
Atwood	Heidelberg	Kruisrivier	2001	C35	5
Dream	Sunland	Woodridge	2000	CC	3 topwork
Dream	Heidelberg	Kruisrivier	2001	C35	5
Cliff Early	Fort Beaufort	Riverside	1996	TC	4
Fenix Early	Addo	ITSC	1999	SC	2 topwork
Krajewski Early	Addo	ITSC	1999	CM	2 topwork
Mistkraal Early	Addo	ITSC	2000	MxT	2 topwork
Sundays River Early	Addo	ITSC	1999	VA	2 topwork
Washington	Addo	Willowtree	1998	CC	commercial

Washington	Patensie	Ripplehill	1999	RL	commercial
Washington	Fort Beaufort	Riverside	2000	TC	commercial
Santa Catarina 1 & 3	Addo	ITSC			2
Cambria	Uitenhage	CFB Block 7	1999	CC	2
Cambria	Patensie	Patensie Acht	2000	SC **	commercial
Autumn Gold	Fort Beaufort	Baddaford	1998	TC	10
Autumn Gold	Citrusdal	ALG	1995	RL	Semi com
Autumn Gold	Citrusdal	ALG	1995	RPL	Semi com
Autumn Gold	Citrusdal	Hexrivier	1997	RPL	semi comm
Powell	Fort Beaufort	Baddaford	1998	TC	5
Powell	Citrusdal	ALG	1995	RL	Semi com
Powell	Citrusdal	Hexrivier	1997	RPL	semi comm
Chislett	Fort Beaufort	Baddaford	1998	TC	4
Chislett	Citrusdal	ALG	1995	RPL	5
Chislett	Citrusdal	Hexrivier	1997	RPL	semi comm
Summer Gold	Uitenhage	CFB Psylla	1999	TC	1
Coetzee Late Navel	Citrusdal	Hexrivier	1997	RPL	9 topwork
Renken Late Navel	Citrusdal	Hexrivier	1997	RPL	8 topwork
Mouton Late Navel 1	Citrusdal	Hexrivier	1997	RPL	4 topwork
Mouton Late Navel 2	Citrusdal	Hexrivier	1997	RPL	2 topwork
Glenora Late	Uitenhage	CFB Block 4	2001	CC	2
Glenora Late	Clanwilliam	Jansekraal	2002	Val/RL	9 topwork
Witkrans (old selection B13)	Clanwilliam	Jansekraal	2002	Val/RL	8 topwork
Tuligold	Citrusdal	ALG	1998	RL	17
Lina (control)	Heidelberg	Kruisrivier	2001	C35	5
Lina (control)	Citrusdal	ALG	1997	RL	commercial
Newhall (control)	Sunland	Woodridge	1999	CC	commercial
Newhall (control)	Citrusdal	ALG	1997	RL	commercial
Californian Lane Late (control)	Uitenhage	CFB Block 4	2001	CC	2
Californian Lane Late (control)	Citrusdal	Hexrivier	1997	RPL	semi comm
Californian Lane Late (control)	Clanwilliam	Jansekraal	2002	Val/RL	semi co top
Royal Late	Uitenhage	CFB Block 4	2001	SC	2
Royal Late (control)	Clanwilliam	Jansekraal	2002	Val/RL	semi co top

** SC because on replant soil.

Results and discussion

A discussion of each selection follows with internal quality results presented in Table 6.3.6.20 that need to be referred to when reading the text. A comparison of the various early navel, midseason and late maturing navel selections is presented in the following tables and text.

Comparison of early navel selections on Carrizo citrange at Woodridge, Addo on 21 April and 4 May.

Table 6.3.6.2. Comparison of production, fruit size, colour, quality and maturity of early navel selections evaluated at Woodridge, Sunland (Addo) during 2006.

Selection	Yield	Fruit size	Colour	Taste	Test	Estimated maturity
Fukumoto	Fair	Medium large	**21 April** T5 **4 May** T3-4	Fair, fair sugars and low acid in April and May	Navelate standards in April except colour, juice too low in May	Early May
Letaba Early	Fair	Medium large	**21 April** T6-7 **4 May** T6-7	Fair sugars and acid in April & May	Close to Navelate standards in April and May (low juice and ratio & colour)	3rd week May to later
Atwood	Fair	Large	**21 April** T7 **4 May** T6	Fair sugars and acid in April & May	Good sugars, highish acid, too low juice & colour	3rd week May to later

Dream	Fair	Large	**21 April** T7 **4 May** T6	Fair, low sugars and acid	Juice too low, colour poor, highish acid	3rd week May to later
Newhall (control)	Good	Medium large	**21 April** T5 **4 May** T4	Fair sugars and acid in April. Started orchard harvest on 1 May	Juice and colour too low in April, Navelate except colour in May, highish acid	Mid to 3rd week May

All had low juice except the first Fukumoto and later Newhall test. On average Atwood had the highest sugars and Dream the lowest, Fukumoto the lowest acid, Atwood and Newhall the highest. Fukumoto had the highest ratio, Dream and Newhall the lowest. Fukumoto had the earliest colour followed closely by Newhall. All had round fruit except Letaba Early, which was round and slightly elongated. Letaba Early and Atwood had some split fruit. Fukumoto and Newhall were easier to peel than the others. Fukumoto was the earliest to mature followed by Newhall. The other selections are not so early maturing. Dream and Atwood were however internally earlier than Palmers at the CFB (all similar rind colour).

Comparison of early navel selections on citrange C35 at Kruisrivier, Heidelberg on 25 May.

Table 6.3.6.3. Comparison of production, fruit size, colour, quality and maturity of early navel selections evaluated at Kruisrivier, Heidelberg during 2006.

Selection	**Yield**	**Fruit Size**	**Colour 25 May**	**Taste**	**Test**	**Estimated maturity**
Fukumoto	Fair	Medium large and large	T1-3. Colourbreak late April	Fair, similar to more variable than Lina. Some slightly sweeter. More raggy	Good test except juice too low	2nd - 3rd week May
Letaba Early	Zero-poor	Medium large-large	T5-6, some 3-4. 5-6 later set?	Poor-fair. Tender. Consistent, tasteless, sufficient sugars, low acid	Juice too low, poor colour, borderline sugars	Peak but colour is poor
Atwood	Good	Medium + - med large	T5-6	Poor-fair. Tender. Consistent, lacks sugars, acid ok. Poorest of all	Juice too low, poor colour, borderline sugars to ok	Peak but colour is poor. One fruit had 1 seed, the rest 0
Dream	Good	Medium + - med large	T5-6	Fair, slightly variable, some poor, low sugars to slightly sweeter	Good test. One fruit had 3 seeds, the rest 0	Peak to end May/early June
Lina (control)	Variable, fair-good	Medium – med large	T3-4, green stem ends	Fair, lacks sugars, sufficient acid	Good test, meets Navelate standards	2nd – 3rd week May

The area had a lot of rain in April/May. All the selections had good internal colour, Fukumoto and Lina deeper and all had halos in the flesh to varying degrees. Lina had the finest flesh. The Fukumoto core was slightly more open (others closed) and Letaba Early the thickest rind. Fukumoto appeared to be more juicy than Lina. Fukumoto had variable fruit shape, Lina typically elongated, Letaba Early and Dream some slightly elongated fruit. Atwood was round. Fukumoto had deep orange red rind colour coming through, Lina also but all Lina fruit had green stem ends. Fukumoto was unattractive due to the coarse rind and ribbing and Dream due to the green rind colour, ribbing and pebbly stem ends. Fukumoto had the most pebbly rind (slight), Lina the smoothest. Only Dream and Lina had acceptable juice levels, Fukumoto the highest sugars and ratio and Letaba Early slightly lower acid. Atwood and Dream had odd seed. Fukumoto and Lina were the earliest to mature, Dream the latest, 2-3 weeks later. Only Atwood and Deam had some light oleo. All selections had ribbing, up to 7% not exportable, Lina and Letaba Early the least and Fukumoto the most followed by Dream. Lina had the most protruding navels (all exportable) followed by Fukumoto, Dream and Atwood the least and Lina virtually no malformed navel ends, while the others all had some and Dream a little more. Fukumoto had variable, small trees like Lina, Dream the largest followed by Atwood. There were

no signs of incompatibility on the Fukumoto bud union. Letaba Early had some leaf drop. Atwood fruit picked easily.

Table 6.3.6.4. Percentage fruit per count of various early navel selections measured at Kruisrivier, Heidelberg on 26 May, 2006.

Selection	Average fruit size (mm)	Approx. count	Percentage fruit per count						% fruit in counts 56-72
			>=40	48	56	64	72	88	
Lina	77.6	64/72		4	18	26	44	8	88
Dream	80.5	64	2	8	36	32	18	4	86
Atwood	82.3	56	2	22	34	36	6		76
Fukumoto	83.8	56	15	10	40	33	2		75
Letaba Early	90.8	40	60	35	5				5

Due to the poor crop, Letaba Early had large fruit size. Fukumoto had slightly larger fruit than Atwood and Lina the smallest with most fruit falling into the popular counts. The trees are still young.

It is interesting to note the presence of seed in Atwood and Dream. There are some mandarins in an adjacent row. At Woodridge where the trees are planted in a solid block, there is no seed.

Comparison of early navel selections on Rough lemon at ALG, Citrusdal on 15 May, 2006.

Table 6.3.6.5. Comparison of production, fruit size, colour, quality and maturity of early navel selections evaluated at ALG, Citrusdal during 2006.

Selection	Yield	Fruit size	Colour 15 May	Taste	Test	Estimated maturity
Fukumoto	Fair-good	Medium large	T3 and 5-6, 3 getting deep colour	Fair, not too much flavour, slightly raggy	Navelate standards	Mature
Tuligold	Good, even	Medium-medium large, even	T4-5	Fair, fair sugar and acid. Tender	Acceptable, meets navel standards	Mature
Lina	Good-excellent, even	Medium, even	T5	Fair, lacks flavour, tender	Like Tuligold, but unacceptable due to low juice	Mature
Newhall	Fair-good	Medim large and large	T2-3 and 4-5	Good, fairly tender	Navelate standards except for low juice	Mature to 1 week to go

Note, the Fukumoto are younger topworked trees.

There was a general improvement in internal quality over last season, with an increase in juice levels, sugars and acid. Newhall had by far the best quality, followed closely by Fukumoto; Lina and Tuligold similarly poorer. All juice percentages were borderline. There was little difference in maturity with Newhall slightly ahead.

Table 6.3.6.6. Percentage fruit per count of various early navel selections and Fukumoto on various rootstocks measured at ALG, Citrusdal on 15 May, 2006.

Selection	Average fruit size (mm)	Approx. count	Percentage fruit per count						% fruit in counts 56-72
			>=40	48	56	64	72	88/105	
Tuligold	77.5	64/72			20	26	44	10	90
Lina	80.0	64		8	24	44	24		92
Newhall	82.6	56	12	10	34	32	12		78
Fukum RL	78.5	64	8	4	14	20	38	16	72

Selection	Average fruit size (mm)	Approx. count	Percentage fruit per count						% fruit in counts 56-72
			>=40	48	56	64	72	88/105	
Fukum BC	76.2	72	2		12	24	40	22	76
Fukum C35	77.9	64/72		6	16	30	38	10	84
Fukum KC	77.7	64/72		2	12	44	30	12	86
Fukum SC	77.9	64/72		12	12	26	30	20	68

Comparing the various navel selections (and Fukumoto on Rough lemon), Newhall had by far the largest fruit size (22% larger than count 56), Lina the most fruit in the popular counts (92%), Fukumoto spread right across the size range and Tuligold the smallest, but with 90% in the popular range. Oleocellosis was evaluated four days after harvest. The fruit was not treated in a packhouse. All selections had some oleo. Newhall was the worst affected with 13% not exportable, Tuligold 10%, Lina 5% and Fukumoto all exportable. Newhall had some small split navel ends. Tuligold had slightly more open cores than the others but yellow flesh and Newhall the best (orange) flesh colour. Fukumoto had most fruit T3 and better (84%) although Newhall with 82% had more T1-2 fruit. Newhall developed a deep orange/red colour sooner than Fukumoto. Lina was behind with 34% and Tuligold 10%. However Newhall and Fukumoto had 50% of the fruit with green stem ends, Lina 74% and Tuligold none. Newhall had the most ribbing (plate 22 of Outspan colour plates), 18% not exportable and Fukumoto 2%. Tuligold had little ribbing, followed by Lina.

There were differences in navel ends between the selections. Tuligold had few protruding navel ends, but a lot of malformed navel ends (12% of fruit with malformed navels not exportable). Lina had quite a lot of protruding navels (10% of those not exportable) and few malformed navels. Newhall and Fukumoto were similar with a lot of protruding navel ends (75% of all the fruit) and some malformed navels.

Tuligold had a round shape, the others round and elongated to varying degrees. Newhall had the most pebbly fruit and Fukumoto the coarsest flesh. Tuligold had the most sunburn (more sparse trees).

A summary of the comparison of various early navel selections at various sites during May, 2006.
Note that these are generalisations and do not take tree age, rootstock, etc.

Atwood – The yield was fair-good with variable medium large fruit size. Taste was generally fair, meeting navel standards. Fruit shape is round with few protruding and malformed navel ends. Maturity 3rd-4th week May to later.

Fukumoto – The yield was fair with with medim large and larger large fruit size. Taste was fair, slightly raggy and generally up to Navelate standards. Fruit shape varied and navel ends sometimes flat. Fruit had protruding and malformed navel ends and some ribbing. Maturity around 2nd week May.

Letaba Early – Yields varied form zero-fair, fruit size medium large and larger. The taste was fair meeting different standards. The fruit shape is round to slighty elongated with a few protruding and malformed navel ends. Maturity 3rd-4th week May to later.

Dream – Yields were fair-good with variable medium large fruit size. The taste was fair, meeting navel standards. The fruit shape is round to some elongated fruit with some ribbing, some malformed navel ends and few protruding navels. Maturity 4th week May to early June.

Lina – Yields were good with mainly medium to larger fruit size. The taste was fair and navel to Navelate quality. The fruit shape is elongated with closed protruding navel ends and little ribbing. The fruit had the finest flesh. Maturity 2nd-3rd week May.

Newhall – Yields were good with medium large and larger fruit size. The taste was fair-good, meeting Navelate standards. Navel ends were closed to protruding and with some malformed navels. Fruit shape is slightly elongated with ribbing. Maturity 2nd-3rd week May.

Tuligold – The yield was good with medium large-large fruit size. The taste was fair and tender, meeting navel standards. Fruit shape is round with malformed malformed navel ends. Maturity 2nd week May to later.

Overall, Fukumoto matures at the same time to just ahead of Lina and Newhall. Tuligold the same time to later than Lina and Newhall. Atwood and Letaba Early at least 1-2 weeks later than Fukumoto and Dream a

week later still. Fukumoto generally had the earliest fruit colour and Dream the latest. Fukumoto and Newhall develop a deeper rind colour earlier than the other selections.

Observation of Fukumoto on various rootstocks at ALG Boerdery, Citrusdal.
Trees were planted in December, 1997 and Fukumoto navel budded to rootstock shoots during 2002.

Some cuts were made over the bud union on some of the trees on 10 October to see if there was a brown ring on the wood. Results and observations of the evaluations are given in Tables 6.3.6.7. to 6.3.6.9.

Table 6.3.6.7. Comparison of yield, fruit size, fruit colour and quality of Fukumoto navel on different rootstocks at ALG Boerdery, Citrusdal on 15 May, 2006.

Rootstock	Yield	Fruit size	Colour	Quality/test
Rough lemon (row B)	Fair-good	Medium large	T5-6 some T3, developing deep colour	Fair, not too much flavour, slightly raggy. Navelate quality. Mature
Benton citrange	Fair-good	Variable medium to large	Tree 1, T2-3 Tree 2, T6	Fair-good. Sweetish, sufficient acid. Slightly raggy. Navelate quality. Mature
Citrange C35	Good-excellent	Medium - medium large	T5-6, some T3 (deep colour)	Fair. Lacks flavour and sugars, slightly tart. Navelate quality except low juice. Mature
Koethen citrange	Fair-good	Medium	T2-3, more consistent good colour	Good quality, slightly higher acid. Best test, Navelate quality, except low juice. Peak to 1 week to go
Swingle citumelo	Variable, zero-good	Medium – medium large	T3, developing deep colour	Good quality, slightly acid, could be lower. Tender. Navelate quality. Mature

Yields improved dramatically over last season and rind colour slightly later. Benton had the thinnest rind followed by Koethan and Rough lemon the thickest. Swingle had the deepest orange flesh colour, Rough lemon the palest.

Table 6.3.6.8. Percentage fruit per count of various early navel selections and Fukumoto on various rootstocks measured at ALG, Citrusdal on 15 May, 2006.

Rootstock	Average fruit size (mm)	Approx. count	Percentage fruit per count					% fruit in counts 56-72
			>=48	56	64	72	88/105	
Rough lemon	78.5	64	12	14	20	38	16	72
Benton citrange	76.2	72	2	12	24	40	22	76
Citrange C35	77.9	64/72	6	16	30	38	10	84
Koethan citrange	77.7	64/72	2	12	44	30	12	86
Swingle citrumelo	77.9	64/72	12	12	26	30	20	68

Rough lemon had the largest fruit size, Swingle the widest range but the least in the popular counts and Benton a tendency to smaller fruit size. Koethan and C35 had most fruit in the popular range. All rootsocks showed signs of oleo, Benton the worst with 8% non exportable fruit, followed by C35. Koethan and C35 had the least fruit with green stem ends. Swingle again had good deeper rind colour.

Table 6.3.6.9. Evaluation of bud unions of Fukumoto navel on different rootstocks at ALG Boerdery, Citrusdal on 4 October, 2006.

Rootstock/ row/no of trees	Tree condition	Tree size	Blossom	Bud union	Sprouts/photo numbers	Cut through bud union
Rough lemon B 13	Flushing	Medium large and large	Mainly medium, also light. Balloon and mainly full bloom	Smooth	None seen	
Rough lemon (end	Slight flush	Small, medium,	Light and heavy, balloon and full	Smooth	502, 503	1 cut, clean but tiny knobs on rootstock,

Rootstock/ row/no of trees	Tree condition	Tree size	Blossom	Bud union	Sprouts/photo numbers	Cut through bud union
of row C, near windbreaks) C 7		medium+	bloom			showing under bark on wood
Benton citrange B 2	Flushing	Medium large	Not as heavy as SC. Full bloom and mainly balloon stage	Smooth?/ smoothish	Shoots just above bud union, next to cut. 491, 492, 493	1 cut, only indent in wood.
C 35 C 3	Flushing	Medium – medium+	1 x heavy, full bloom. 2 x medium, balloon and full bloom	Slight bench	Tree 2, sprouts. 497, 498, 500	± 25 mm away from sprouts, "green shoots" on wood under bark at bud union.
Koethan citrange C 1	Flushing	Med – medium large	Medium+, full bloom and mainly balloon	Slight bench	None	
Koethan citrange (trees further down the row near oak tree) C 6	Flushing, not as well as others, probably because of oak tree	Variable, small, medium, medium+	1x heavy, full bloom and balloon. 5 x mainly light, balloon	Slight bench	None	2 trees cut, completely clean, no ridge, nothing
Swingle B 14	Flushing	Variable, med - large	Heavy, balloon and mainly full bloom	Severe bench	Photo 479	2 trees cut. Tree no 6 heavy bench, not sure if a brown ring, probably a shadow on the bench

There was no direct evidence of an incompatibility in terms of a brown ring at the bud union.

Cliff Early. A bud sport of Palmer navel origin. The fruit were evaluated too late, on 12 June, colour T1 and overmature and some creasing but still attractive. The fruit shape is round to slightly elongated and fairly firm with a smooth and thin rind. Even at this late stage the cores were closed. The fruit is seedless with orange, slightly coarse textured flesh. The taste was now poor to fair and lacking flavour but still retaining acid. Most navel ends were very small to closed with the occasional malformed or protruding navel end. The tree carried a fair crop of good, medium large fruit size. The test was still good, meeting Navelate standards except for low juice. The tree is medium large and fairly vigorous. Maturity is estimated around mid to late April.

Fenix Early. The young topwork trees had a poor crop of medium fruit size, colour T7 on 12 April. The taste was sweetish and lacking acid although the Brix was 11%. The fruit is round to flattish with small to closed navel ends. Internal colour is pale and cores closed. It does not look like an early maturing selection although the acid level is low and the rind colour is delayed.

Krajewski Early. The young topworks had a poor to fair crop of good, medium large fruit size and colour T7-8 on 12 April. The taste was good with Brix levels between 9.8-11.6%. The flesh colour was slightly orange and the juice fairly free and cores closed. Navel ends varied between closed to closed protruding to slightly open. The trees are fairly large. Internal maturity appears ahead of rind colour.

Mistkraal Early. The young topwork trees had a zero to poor crop and rind colour T7 on 12 April. Even though the sugars were good, 10.1% Brix and fairly acid the fruit was poor. It does not appear early maturing like last season. The trees also look unhappy (like the parent tree).

Sundays River Early. The young topwork trees had a poor crop of medium large fruit size, rind colour T7-8 on 12 April. The sugars were not high (9.8%Brix) and highish acid (Volckameriana rootstock) and far from mature. The flesh was pale and cores closed. The navel ends are small to closed, the occasional closed protruding navel end and occasional smaller, flat fruit. It does not appear early maturing, similar maturity to Palmer.

Washington. Commercial orchards were evaluated at Addo, Patensie and Fort Beaufort. Data is presented in the relevant tables.

The trees at Addo bore a good crop of medium large fruit size. Fruit color on 1 June was T1-3 (partially picked). The quality was fair, at peak maturity. Fruit tests were acceptable to good, except for a low juice percentage. Tests done earlier on 9 May were very good with high sugars and relatively high acid levels.

Table 6.3.6.10. Packhouse data for Washington navels harvested at Willowtree Farm, Addo. Trees were planted in 1998 on Carrizo citrange rootstock (5.5 x 2.5m).

Year	Tons /ha	Export % (Grade)		Over size	Percentage fruit per count in grade 1 only								% fruit in counts 56-72
		1	1&2		36	40	48	56	64	72	88	105	
2002	11	44	66										
2003	25	26	42	(1)	12	24	24	20	12	8			40
2004	27	38	57	(1)	11	13	24	18	18	13	3		49
2005	29	48	72		4	12	19	17	21	19	6	2	57
2006	26	54	76		2	6	9	32	20	22	7	2	74

2006. Fruit colour was good, skin texture good and a lot of windblemish due to lack of winbreaks. The fruit was harvested between 25 May and 6 June as the fruit was slightly earlier this year.

The yield over the past four years has stabilised but there has been a shift in fruit size from larger to smaller fruit, increasing the percentage of fruit in the more popular counts.

The trees at Patensie had an excellent yield with very good medium large to large fruit size. Fruit colour on 15 June was T1-T4 (54% T1, 20% T2, 23% T3 and 3% T4), some fruit with slightly green stem ends. The quality was fair to good, not outstanding with good acid levels, but sugars could be higher. Except for the low juice, the test met Navelate standards. There was no creasing and the fruit could be harvested or hung for a week or two.

Table 6.3.6.11. Packhouse data for Washington navels harvested at Ripplehill, Patensie, 2006. Trees were planted in 1999 on Rough lemon.

Year	Yield (t/ha)	Percentage fruit per count (all fruit)							% fruit in counts 56-72
		40	48	56	64	72	88	105	
2006	72.1	1	8	37	23	21	7	3	81

The trees at Fort Beaufort carried a good crop of medium large to large fruit size with clean, firm, good looking fruit. Rind colour on 12 June was orange, T1. The fruit was good quality with a good sugar/acid balance, even though the area had had heavy rain early in the year. The test was good just missing Navelate standards due to low juice. A test done three weeks later was also good, while old Palmer trees nearby had higher sugars and slightly lower acid. Peak maturity was about 3rd week June.

Table 6.3.6.12. Packhouse data for Washington navels harvested at Riverside Farm, Fort Beaufort. Trees were planted in 1999 on Troyer citrange roostsock (5 x 3m).

Year	Tons /ha	Percentage packed fruit per count *									% fruit in counts 60-72	Comment
		36	50	55	60	65	72	88	105	125		
2002	14											1st crop
2003	25											
2004	36											
2005	24											Severe hail
5/7/2006	53	11	18	21	22	15	9	3	1	0.5	46	Light frost

* Note. There were some fruit larger than count 36 not recorded.

The Washington's at the three sites overall had a good to excellent crop of good fruit size, sometimes on the large side. Fruit colour was generally good sometimes with green stem ends. Fruit shape is mostly round, some slightly elongated with a fairly smooth rind. Navel ends varied, closed to open, up to 63% of the fruit with varying degrees of malformed navel ends (all exportable), 37% protruding (all exportable) and 46% ribbing (3% not exportable). Flesh colour varied between the areas, deep orange in Fort Beaufort and pale in Patensie. Eating quality varied from fair to good, and tests mainly Navelate standards except for low juice. Maturity varied between areas from early to late June.

Santa Catarina 1 and 3. The trees of both selections are large and vigorous and bore a few fruit of large fruit size. The fruit colour was T8 on 12 April and far from mature.

Cambria. Seven year old trees on Carrizo at the CFB had a fair crop of variable, medium fruit size, colour mainly T1 on 26 July. Fruit quality was fair to mainly good and mature. The test was excellent. Royal Late trees on Swingle had a poor crop, medium to medium large fruit size, colour T2-4 and mature. Fruit quality was good, meeting Navlate standards, but Cambria was better. Cambria cores were slightly open, Royal Late closed. Fruit shape was similar with both selections having slightly elongated fruit, some Cambria with high shoulders. Both had smooth rinds and mostly closed to small navel ends. Royal Late had some stylar end split.

Commercial six year old trees at Patensie had a good crop of variable medium large fruit size, colour T1-3 on 26 July. The quality was good.with good sugars and sufficient acid. The tests were very good, easily meeting Navelate standards although the acid levels not high. Fruit shape varied with round and elongated fruit, some with high shoulders with closed to closed protruding navel ends. Rinds were smooth, average to slightly thick. The cores were closed with a fine, orange flesh. Peak maturity (due to harvest the next week).

It appeared that there was a tree and quality difference between the rounder and elongated fruit. Trees with rounder fruit had a slightly lighter crop, but there was little difference in the tests to quantify the assumption that the rounder fruit had higher sugars. Of the single tests done, the rounder fruit did have fractionally higher sugars, lower acid and higher ratio.

Comparison of late navel selections in the Eastern Cape during 2006

At the CFB, all selections were mature by 3rd-4th week July. Summer Gold has a round shape, pebbly rind and small to very small navel ends. There was some creasing. At Fort Beaufort, all selections were round, Autumn Gold occasionally slightly elongated. Little difference in terms of other characteristics.

Table 6.3.6.13. Navels at Uitenhage and Fort Beaufort

Selection, Rootstock Year planted	Yield	Fruit size	Colour	Taste	Test
Uitenhage – 19 or 26 July (and 17 August)					
Glenora Late SC. 2001	Fair	Variable medium and large	**July** T1	Good, good sugars, slight rag	Good, Navelate quality except odd seed. Mature to 1 week too early. Past peak in August.
Summer Gold. TC. 1999	Fair	Medium large-large	**July** T1-3, slightly green stem ends	Good	Navel standards, acid lowish, high sugars. Mature
Californian Lane Late CC. 2001	Fair	Large	**July** T1 some T2	Good, good sugar/acid balance	Navel standards in July and August. Peak in July, better than Royal Late, past peak in August
Fort Beaufort – 12 June					
Autumn Gold	Poor – fair, (shaded)	Good, medium large	T3-4, some T2. Some green stylar ends	Good, slightly more tart. Raggy	Navelate standards except seed. Mature 3rd – 4th week June
Powell	Variable, fair	Medium large, some large	T3-4, some T2, odd T5	Good. Slightly raggy	Navel quality except juice. Autumn Gold better. Mature internally in June
Chislett	Too variable in yield, size and colour to evaluate				

Comparison of Australian Late navel selections in the Western Cape during 2006

Table 6.3.6.14. Comparison of production, fruit size, colour and quality of Australian Late navel selections evaluated in the Western Cape at ALG and Hexrivier, Citrusdal during 2006.

Selection /rootstock	Yield	Fruit size	Colour	Taste	Test
ALG – 7 August					
Autumn Gold. Rough lemon	Excellent	Medium and medium large	T1-2	Poor, tasteless, raggy	Navelate except low juice. Past peak by 1 week
Autumn Gold. Rangpur lime	Excellent	Medium – medium large	T1-2	Poor, tasteless, raggy	Navelate except very low juice. Past peak by 1-2 weeks
Powell. Rough lemon	Excellent	Medium large	Mainly T1. T2+3	Poor, tasteless, raggy	Navelate except very low juice. Past peak by 1-2 weeks
Chislett. Rangpur lime	Excellent	Medium and medium large	Mainly T1. T2-3	Poor, tasteless, raggy	Navelate except low juice. Past peak by 1-2 weeks
Hexrivier – 3 July (harvested early due to potential theft). All on Rangpur lime.					
Autumn Gold	Good-excellent	Medium large-large	T3-4	Good. Fairly sweet, sufficient acid, slightly raggy	Navelate standards
Powell	Good-excellent	Medium large	T2-3	Fair-good. Lacks flavour, sufficient sugars, slightly tart. Raggy	Navelate standards
Chislett	Good-excellent	Mainly large and medium	T2-3	Good. Lacks flavour, sufficient sugars and acid. Raggy	Navelate standard except low juice
Californian Lane Late	Good-excellent	Medium large-large	T3-4	Fair-good. Could have higher sugars, slightly tart. Slightly raggy	Navelate standards

Table 6.3.6.15. Percentage fruit per count of various late navel selections on Rough lemon or Rangpur lime at ALG, Citrusdal on 7 August, 2006.

Selection	Average fruit size (mm)	Approx. count	Percentage fruit per count						% fruit in counts 56-72
			40	48	56	64	72	88	
Autumn Gold (RL)	79.9	64	4	6	24	42	18	6	84
Autumn Gold (RPL)	78.9	64	2	10	14	40	22	12	76
Powell (RL)	82.2	56	4	22	36	20	16	2	72
Chislett (RPL)	79.2	64	2	8	22	26	34	8	82

All had good fruit size, Powell the largest. Autumn Gold (RL) and Chislett the most fruit in the popular counts.

Table 6.3.6.16. Percentage fruit per count of various late navel selections on Rangpur lime at Hexrivier, Citrusdal on 3 July, 2006.

Selection	Average fruit size (mm)	Approx. count	Percentage fruit per count						% fruit in counts 56-72
			<=40	48	56	64	72	88	
Autumn Gold	80.9	64	8	6	24	42	16	4	82
Powell	83.2	56	8	14	46	26	6		78
Chislett	82.9	56	8	22	28	38	4		70
Californian Lane	81.6	56/64	6	10	34	32	16	2	82

The Australian late navels generally had good fruit size, tending to slightly large fruit, Powell and Chislett the largest.

A summary of a comparison of various Australian Late navel selections in Citrusdal during 2006
The differences, if any, between the various selections at the two sites are highlighted.

- Tree size: Chislett the largest, Powell smallest.
- Yield: All equally good.
- Fruit size: The fruit size of all the selections is good to a little on the large side. There were some differences between selections, but overall not huge. Powell on Rough lemon had slightly larger fruit than Chislett, then Lane Late. Autumn Gold had the smallest. Trees on Rangpur lime had slightly smaller fruit size compared to Rough lemon.
- Colour: There were slight differences, but variation. Autumn Gold and Lane Late possibly slightly later. The Autumn Gold was the best looking fruit with odd green stem ends and a good orange rind colour. Powell were fairly attractive with a good, deep rind colour. Lane Late had some green stem ends and Chislett was the poorest looking with odd green stem ends but developing a deeper rind colour.
- Fruit shape: Little difference, all generally round, Lane Late the least round.
- Rind texture: Rinds tend to be coarser in Citrusdal. There was little difference between the selections, all slightly pebbly.
- Navel ends: With all selections there were roughly twice as many protruding navel ends of varying degrees compared to malformed navel ends. Californian Lane Late and Chislet had the most protruding navel ends while Autumn Gold and Powell the most malformed navel ends. At one site Autumn Gold had up to 15% fruit with non exportable malformed navel ends. Autumn Gold and Chislett had more internal navels.
- Split fruit: At one site all selections had some tiny splits on the navel end, Autumn Gold the least and Powell/Lane Late similarly the most.
- Rind thickness: No noticeable differences.
- Internal colour: All good to excellent colour, a small percentage of ricyness with Chislett.
- Taste and internal quality: Irrespective of the tests the taste at ALG was poor, lacking flavour. At Hexrivier, Autumn Gold and Chislett were good, the others fair to good. At both sites all tests met Navelate standards except at ALG where all had low juice. All had some rag, Powell and Chislett more so.
- Maturity: Around 2nd -4th week July.

Evaluation of late maturing Renken Late, Coetzee Late, Mouton Late 1 and Mouton Late 2 navels at Hexrivier, Citrusdal

The trees are planted in poor, sandy soil and the trees have not performd well in the past, generally producing poorer external and internal quality fruit. The trees are improving with age and producing better fruit giving a better indication of their worth. Comments on the evaluations are presented in the tables below.

Table 6.3.6.17. Evaluation of tree size, yield, fruit size, colour and quality of various late maturing navel selections on Rangpur lime rootstock at Hexrivier, Citrusdal on 3 July, 2006.

Selection	Tree size	Yield	Fruit size	Colour	Taste/ test/ maturity
Renken Late	Medium, some thorns	Excellent	Variable, medium large	T1-2	The eating quality was good, consistent, could have slightly higher sugars, sufficient acid. Raggy. Easily meets Navelate standards
Coetzee Late	Medium small, not too healthy	Fair	Medium large-large	T2-4	The eating quality was good, could have slightly higher sugars, sufficient acid. Better than Renken. Not raggy. Easily meets Navelates standards
Mouton Late 1	Sparse	Fair	Variable. Medium-medium large	T1-3	The eating quality was fair, lacking sugars and slightly acid. Tender and juicy. Easily meets Navelate standards
Mouton Late 2	Small. Regrowth at bud union. Odd thorns	Fair-good	Variable. Medium large	T3-4	The eating quality was fair to good, lacking flavour, sufficient sugars and slightly tart. Very raggy. Except for borderline juice, easily meets Navelate standards

Table 6.3.6.18. Percentage fruit per count of various local late navel selections on Rangpur Lime at Hexrivier, Citrusdal on 3 July, 2006.

Selection	Average fruit size (mm)	Approx. count	Percentage fruit per count						% fruit in counts 56-72
			<=40	48	56	64	72	88	
Renken Late	78.2	64		8	14	32	38	8	84
Coetzee Late	84.4	56	20	22	27	24	7		58
Mouton Late 1	80.5	64	7	9	27	29	24	4	80
Mouton Late 2	80.9	64		15	26	36	23		85

The selections had good fruit size with Coetzee late considerably larger than the others. The other selections had a good percentage falling into the popular counts.

Renken Late. The fruit is quite attractive with odd green stem ends and firm. Fruit shape is is round with a slightly pebbly and a thinnish rind. The internal colour was good with coarse flesh and slightly open cores. The navel ends were small to closed but with quite a lot of malformed navels and some slight stylar end splitting. Fruit slightly earlier maturing than the Australian Lates. Slightly smaller fruit size this year.

Coetzee Late. The fruit was not attractive due to a sometimes pebbly rind. The fruit shape is round with a variable pebbly to smoothish and thinnish rind. There were some coarse stem ends and ribbing, 2% not exportable. Generally small navel ends, some small protruding and virtually no malformed navel ends. The fruit is hard and picks fairly easily. Internal colour was a deep orange with slightly open to closed cores. Mature end June/early July.

Mouton Late 1. The fruit is round to elongated with a slight shoulder, slightly pebbly to coarse rind and stem end and an average to thickish rind. Internal colour was deep orange with slightly open cores. The fruit was overmature with an orange red rind colour and unattractive. There were some protruding navel ends and few malformed navels. Picks easily. There was quite a bit of oleo, 11% not exportable.

Mouton Late 2. The fruit developes a good deep rind colour but with some green stem ends and ribbing, 2% not exportable. The fruit shape is round with some flattened stylar ends and retained styles, a slightly pebbly rind and hard. Internal colour was orange, coarse flesh with tiny, aborted seeds and closed cores. There were less protruding than malformed navel ends, 5% and 17% not exportable respectively. There was some fruit drop.

Comparison of Witkrans, Royal Late, Glenora Late and Californian Lane Late in the Western Cape during 2006

The topworked trees in the Clanwilliam area produced their second small crop with an improvement on last seasons first few fruit. All trees are growing vigorously. Results therefore to be read with caution.

Table 6.3.6.19. Comparison of production, fruit size, colour, quality and maturity of late navel selections evaluated at Jansekraal, Clanwilliam on 7 August, 2006.

Selection	Yield	Fruit size	Colour	Taste/Test	Maturity
Witkrans (old selection)	Variable, fair	Medium–medium large. Mainly counts 64/72	Mainly T1	Disappointing, lack flavour, slightly better than Royal Late. The test was good just meeting Navelate standards (borderline acid)	Past peak maturity by 1 week.
Royal Late (control)	Variable, fair-good	Medium–medium large. Mainly Counts 56-72	T1	Disappointing, lack flavour. Navelate quality, slightly higher acid than Witkrans, high juice	Mature to just past.
Glenora Late	Poor	Medium large–large. Mainly 40-56	T1	Good, slightly raggy. Lane Late slightly better. Navelate quality	Peak maturity
Californian Lane Late (control)	Even, fair-good	Medium large-large. Largest. Mainly 40-56	T1	Good, sweet. Navelate quality, lower acid than Glenora Late	Peak maturity

Witkrans. This is the older Witkrans selection. The tree is fairly vigourous like Royal Late. The fruit picks easily, like Royal Late. The fruit is developing a deep orange red rind colour, smooth and has an elongated

fruit shape, similar but not identical to and rounder than Royal Late. Most fruit have high, coarse shoulders. The flesh colour is deep orange with slightly open cores and a good rind thickness. Most navel ends are closed protruding.

Glenora Late. The tree is large, vigorous and thorny. The fruit is not overly attractive due to its rind coarseness, although variable and odd ribbing. The Lane Late is slightly pebbly and not coarse. The frut is firm and shape round to slightly flat with a coarse stem end. Navel ends are variable but generally on the small side, to closed with some protruding and malformed to a small degree (Lane Late more protruding navel ends). The rind thickness is not thick and the flesh a good orange colour, slightly coarse and wide open cores (Lane Late slightly open).

Conclusions

Fukumoto. The yield was fair with with medim large and larger large fruit size. Taste was fair, slightly raggy and generally up to Navelate standards. Fruit shape varied and navel ends sometimes flat. Fruit had protruding and malformed navel ends and some ribbing. Maturity around 2nd week May.

Observations of trees in Citrusdal showed no direct evidence of incompatibility although Swingle citrumelo and Koethan citrange had some indications of possible abnormal development. More clarity is needed as to what rootstock to use. As the trees are still young and vigorous further evaluations are necessary before any recommendations can be made.

Letaba Early. Yields varied from zero to fair, fruit size medium large and larger. The taste was fair, meeting different standards. The rind colour is slightly delayed. The fruit shape is round to slighty elongated with a few protruding and malformed navel ends. Maturity 3rd-4th week May to later. As the trees are still young, further evaluations are necessary before any recommendations can be made.

Atwood. The yield was fair to good with variable medium large fruit size. Taste was generally fair, meeting navel standards. Fruit shape is round with few protruding and malformed navel ends. Maturity 3rd-4th week May to later. The fruit matures internally before the rind has adequately coloured up. As the trees are still young, further evaluations are necessary before any recommendations can be made.

Dream. Yields were fair to good with variable medium large fruit size. The taste was fair, meeting navel standards. The fruit shape is round with some elongated fruit, some ribbing, malformed navel ends and few protruding navels. Maturity 4th week May to early June. This selection could be considered to be more a mid maturing navel. As the trees are still young, further evaluations are necessary before any recommendations can be made.

Cliff Early. The selection is early maturing, and was evaluated 6-8 weeks too late. It looks promising as an early maturing navel. Further trial plantings need to be established once virus free material becomes available. Further evaluations needed.

Fenix Early. This selection does not appear to be early maturing. Further evaluations are necessary.

Krajewski Early. This selection does not appear to be so early maturing and rind colour delayed. Evaluations will be continued on virus free trees when they are in production.

Mistkraal Early. There is too little data available to draw any conclusions. Further evaluations are necessary.

Sundays River Early. This selection does not appear to be early maturing. Further evaluations are necessary.

Washington. Production was good, with good to large fruit size. Colour was good and mainly Navelate quality except for low juice. The Washington can be considered for commercial planting although the fruit tends to be on the large side and quality not always good on young trees. More commercial production data needed.

Santa Catarina 1 and 3. The trees of both selections are large and vigorous and bore a few fruit. The selecton is not early maturing and further evaluations are necessary.

Cambria. Production in the commercial block was good with good fruit size, good quality and tests, although acid levels need to be monitored. The fruit has an elongated and round fruit shape, maturing around mid to

late July. Further evaluations of trees from Foundation Block material are necessary before recommendations can be made. The selection is protected.

Summer Gold. The yield was fair with a good fruit size and some green stem ends. The fruit is late maturing with acceptable qualityand lowish acid. Evaluations to continue.

Renken Late Navel. Production was excellent with good fruit size and good quality. Attractive and earlier than Californian Lane Late. It is worth pursuing as a slightly later maturing midseason navel, but is raggy and has some fruit split. Further evaluations are necessary.

Coetzee Late Navel. Production was fair with fairly large fruit size and good quality. The fruit was not very attractive. Further evaluations are necessary.

Mouton Late Navel 1. Production was fair with fairly good fruit size and a good quality test. The fruit was unattractive and overmature by early July. Pursue for one more season then scrap if poor.

Mouton Late Navel 2. The production was fair to good, with good fruit size. The fruit lacked flavour but had a good test. There were quite a few non exportable fruit due to unacceptable navel ends. Further evaluations are necessary.

Witkrans (old selection). The trees are still young and vigorous and only produced their second small, fair crop. The fruit size was good and met Navelate quality standards but had disappointing flavour. It has similar but not identical characteristics to Royal Late. Further evaluations are necessary. The selection is protected and is superseded by a new, improved selection.

Glenora Late. The tree is vigorous with a poor to fair yield and fruit size medium to too large. Quality is good. There were odd seed at the CFB. Maturity end July/early August. Further evaluations are necessary. The selection is protected.

Comparison of various Australian Late navel selections at Citrusdal.
There were no major differences between the selections. Chislett had larger tree size. All had equally good yields. Fruit size of all the selections is on the large side, Powell slightly larger and Autumn Gold the smallest. Autumn Gold had the most attractive fruit followed by Powell. All had more protruding than malformed navel ends. The tests were all good except for low juice at the one site although they also lacked flavour. A good quality-inducing rootstock should improve flavour and juice percentages. Maturity is around mid July to later July. These Australian selections should be suitable for planting in the Eastern and Western Cape areas, but attention should be paid to excessive fruit size and low juice levels. Autumn Gold, Powell and Chislett are protected. Further evaluations are necessary to confirm the results presented here.

Future evaluations

Evaluate all sites and selections, as some of the trees are still young and only just come into production.

Table 6.3.6.20. Internal fruit quality data for navel orange selections for the Eastern and Western Cape areas during the 2006 season.

Selection	Root stock	Site	Date harvest	Colour	Count	Juice %	* TSS %	Acid %	Ratio	Ave. Seed
Fukumoto	CC	Woodridge	21/04	5	56	53.7	10.6	1.17	9.1	0
Fukumoto	CC	Woodridge	04/05	3-4	64	45.9	11.5	1.15	10.0	0
Fukumoto	C35	Kruisrivier	25/05	2-3	56	46.4	11.0	1.06	10.4	0
Fukumoto	RL	ALG	16/05			48.3	10.5 B	0.97	10.8	
Fukumoto	C35	ALG	16/05			46.4	10.4 B	1.05	9.9	
Fukumoto	BC	ALG	16/05			51.7	10.3 B	1.15	9.0	
Fukumoto	KC	ALG	16/05			46.9	11.9 B	1.04	11.4	
Fukumoto	SC	ALG	16/05			48.1	11.7 B	1.02	11.5	
Letaba Early	CC	Woodridge	21/04	6-7	56	47.8	10.6	1.16	9.1	0
Letaba Early	CC	Woodridge	04/05	6	64	47.6	10.9	1.23	8.8	0
Letaba Early	C35	Kruisrivier	25/05	6	48	47.2	9.2	0.98	9.4	0
Atwood	CC	Woodridge	21/04		64	46.5	11.4	1.28	8.9	0

Selection	Root stock	Site	Date harvest	Colour	Count	Juice %	* TSS %	Acid %	Ratio	Ave. Seed
Atwood	CC	Woodridge	04/05	6	56	47.9	11.6	1.25	9.3	0
Atwood	C35	Kruisrivier	25/05	5-6	56	47.5	9.4	1.01	9.3	0.1
Dream	CC	Woodridge	21/04	7	48	46.9	10.3	1.16	8.9	0
Dream	CC	Woodridge	04/05	6	64	47.6	10.5	1.28	8.2	0
Dream	C35	Kruisrivier	25/05	5-6	64	48.1	10.3	1.07	9.6	0.3
Cliff Early	TC	Riverside	12/06	1	64	46.8	11.6	0.90	12.9	0
Tuligold	RL	ALG	16/05			49.0	9.2 B	0.89	10.3	
Lina	C35	Kruisrivier	25/05	3	64	50.9	10.6	1.06	10.0	0
Lina	RL	ALG	16/05			47.5	9.2 B	0.91	10.1	
Newhall	CC	Woodridge	21/04	5	56	47.8	10.6	1.25	8.5	0
Newhall	CC	Woodridge	04/05	4-5	64	48.0	11.6	1.28	9.1	0
Newhall	RL	ALG	16/05			47.5	11.5 B	0.98	11.7	
Washington	CC	Willowtree	09/05	2-3	large		11.8	1.25	9.4	
Washington	CC	Willowtree	09/05	2-3	small		12.5	1.45	8.6	
Washington	CC	Willowtree	01/06	2-3	56	46.9	9.4	0.88	10.7	0
Washington	CC	Willowtree	01/06	2-3	48	46.6	10.6	0.96	11.0	0
Washington	RL	Ripplehill	15/06	1-2	56	47.7	10.6	0.89	11.9	0
Washington	TC	Riverside	12/06	1	48	47.1	10.8	1.18	9.2	0
Washington	TC	Riverside	05/07				10.4	1.05	9.9	
Palmer		Riverside	05/07				11.7	0.99	11.8	
Cambria	CC	CFB	19/07	1		56.0	12.0	1.1	10.9	0
Cambria Long	SC	Patensie Acht	26/07	1-2	64/72	51.1	11.9 B	0.92	12.9	
Cambria Round	SC	Patensie Acht	26/07	1-2	56	53.3	12.1 B	0.88	13.8	
Autumn Gold	TC	Baddaford	12/06	2-3	64	52.0	11.4	1.05	10.9	0.7
Autumn Gold	RL	ALG	08/08			47.2	10.1 B	0.87	11.6	
Autumn Gold	RPL	ALG	08/08			44.2	10.2 B	1.06	9.6	
Autumn Gold	RPL	Hexrivier	03/07	2	56	51.9	11.2	1.06	10.6	0
Powell	TC	Baddaford	12/06	2-3	56	46.6	10.1	0.78	12.9	
Powell	RL	ALG	08/08			44.8	10.5 B	0.94	11.2	
Powell	RPL	Hexrivier	03/07	1-2	56	51.2	10.7	0.96	11.1	0
Chislett	RPL	ALG	08/08			46.6	10.1 B	0.99	10.2	
Chislett	RPL	Hexrivier	03/07	1-2	56	47.7	11.2	0.93	12.0	0
Summer Gold	TC	CFB	19/07	1	56	55.3	12.4	0.73	17.0	0
Renken Late	RPL	Hexrivier	03/07	1-2	64	52.8	11.8	0.92	12.8	0
Coetzee Late	RPL	Hexrivier	03/07	1-3	56	51.8	12.4	1.15	10.8	0
Mouton Late 1	RPL	Hexrivier	03/07	1-2	56	49.9	11.3	1.04	10.9	0
Mouton Late 2	RPL	Hexrivier	03/07	2	64	48.3	12.2	1.08	11.3	0
Glenora Late	SC	CFB	19/07	1	56	51.9	10.8	1.05	10.3	0
Glenora Late	SC	CFB	17/08	1	56	56.3	11.6	0.87	13.3	0.5
Glenora Late	LV/RL	Jansekraal	08/08			51.5	10.6 B	0.98	10.8	
Witkrans old sel	LV/RL	Jansekraal	08/08			53.6	11.1 B	0.81	13.7	
Cal. Lane Late	CC	CFB	19/07	1	48	52.3	10.2	0.76	13.4	0
Cal. Lane Late	CC	CFB	17/08	1	56	53.1	10.3	0.73	14.1	0
Cal. Lane Late	RPL	Hexrivier	03/07	2-3	56	53.5	11.5	1.03	11.2	0
Cal. Lane Late	LV/RL	Jansekraal	08/08			49.3	11.1 B	0.86	12.9	
Royal Late	SC	CFB	19/07	2-3	56	52.9	10.8	0.89	12.1	0
Royal Late	LV/RL	Jansekraal	08/08			62.7	10.8 B	0.86	12.6	

* TSS %. Values are recorded in % Total Soluble Solids (TSS). Figures with a “B” are % Brix values.

6.3.7 **Evaluation of Midseason oranges in the Cape areas**
Experiment 77 by C J Alexander (Private Contractor)

Opsomming

Die doel van die proef is om midseisoen seleksies, wat beter in die koeler streke sal aard in terme van vruggrootte, gepigmenteerde vleis en saadloosheid, te vind. Tarocco produksie het tussen die verskillende gebiede gewissel en vruggrootte was goed. Gehalte was oor die algemeen goed maar die hoë suur kan neig om die geur te verbloem en dalk oes te vertraag. Tarocco Gallo en 57/1E/1 het albei redelike produksie met aanvaarbare tot goeie vruggrootte gehad, maar hoë suur. Gallo vrugkleur was later as Tarocco. Al drie ouer Tarocco seleksies is doringagtig in meedere of mindere mate en die dorings verminder sodra die bome verouder. Daar was min verskille tussen die drie verskillende kommersieële Maltaise seleksies. Maltaise Half II het effens voor Maltaise Half ryp geword en Maltaise Barlerin die hoogste suur. Die hoë suur vlakke is 'n bekommernis. Raratonga het aanvaarbare produksie en goeie vruggrootte gehad en redelike gehalte maar suur. Die bome is groeikragtig en doringagtig. Verdere evaluasies op al die Tarocco seleksies, Raratonga, Clara, Tacle en kommersieële Maltaise is nodig.

Introduction

The aim is to find midseason selections suitable to the colder areas with larger fruit size, pigmented flesh and seedless.

Materials and methods

The trees were either planted or topworked within commercial orchards or established on a semi commercial scale where possible. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape areas. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is based on subjective tasting. Fruit quality was compared with the following standards: 48% juice; 8.5% Brix (9.0% TSS); 0.6 – 1.5% acid; 7.0:1 ratio; colour T3 of set 34; seed maximum average 6.0 seeds per fruit.

A list of selections and sites evaluated is given in Table 6.3.7.1.

Table 6.3.7.1. Midseason orange trial sites evaluated during 2006.

Selection	Area	Site	Plant Date	Root-stock	No of trees
Tarocco (older)	Adelaide	Saxfold Park	1996	TC	commercial
Tarocco (younger)	Adelaide	Saxfold Park	2002	TC	commercial
Tarocco	Ashton	Excelsior	2002	CC	T/w comm
Tarocco Gallo	Adelaide	Saxfold Park	2002	CC/SC	Semi comm
Tarocco 57/1E/1	Cookhouse	J&B Citrus	2002	CC	commercial
Maltaise Half (B)	Sunland	Junkyard	1998	SC	commercial
Maltaise Half II (A)	Sunland	Junkyard	1998	SC	commercial
Maltaise Barlerin (C)	Sunland	Junkyard	1998	SC	commercial
Raratonga	Uitenhage	CFB Psylla house	1999	TC	2
Clara	Uitenhage	CFB Psylla house	2004	RL/CC/SC	3
Tacle	Uitenhage	CFB Psylla house	2004	CC/SC	3
Tarocco Scire	Uitenhage	CFB Psylla house	2004	CC/SC	4
Tarocco Scire N	Uitenhage	CFB Psylla house	2004	RL/CC	3
Tarocco Tapi	Uitenhage	CFB Psylla house	2004	CC/SC	4

Results and discussion

A discussion of each selection follows with yield, fruit size, colour, estimated maturity and internal quality results for the various selections presented in Tables 6.3.7.2 and 3 which need to be referred to when reading the text. Tarocco with its various selections and Raratonga were evaluated as midseasons to determine their suitability to the Cape areas. The Clara and Tacle are new selections of unknown performance.

Tarocco. Trees at Adelaide had a heavy crop last year and also suffered frost damage which probably affected this season's crop. The older orchard trees are large, vigorous and dense. Production was poor

with very large fruit and the fruit not up to test (acid just too high) on 12 June. The younger orchard also had a poor crop of good fruit size and acceptable quality (acid 1.36%). The fruit had little blemish.

Production in Ashton was good to excellent although slightly variable and good fruit size, (also slightly variable), mainly between counts 56 and 88. Fruit colour in June was good, T1 mostly orange rinds although some slightly pale. On 15 June some fruit had a slight external blush. Quality was slightly variable, some fruit good, others slightly tart. The test on 25 May had unacceptably high acid and only dropping 0.14% over 3 weeks and acceptable in mid June, although still high (1.42%). In May the fruit had an excellent flesh colour and signs of pigmentation but not the greener fruit (probably a slightly later set). There was variable pigmentation in June, improving since May. What was noticeable in May was the difference in pigmentation for the better coloured versus later set (lack of pigmentation). Cores were closed to slightly open in June and average to fairly thin rinds for the better coloured fruit. There were odd split fruit in May and signs of creasing in May and June. There was no oleo in May and the fruit had quite a bit of wind blemish, a lot more than the other areas. Maturity around mid to 3rd week of June.

The fruit is tender and slightly soft, fairly easily peeled and not oily. Fruit shape in both areas is similar, round and some with shoulders and a generally smooth rind. The trees are large and vigorous with large thorns on the trunks, becoming less with few on the smaller twigs. The fruit is mainly borne lower down and inside the tree.

Tarocco Gallo. Trees had a variable, fair to good yield of good fruit size. The rind colour was later than Tarocco, T2-3 versus T1. The fruit lacked flavour and there were no signs of pigmentation. The quality was not acceptable on 12 June due to a low ratio (acid 1.46%). The fruit shape is round and also elongated, sometimes with shoulders, a smooth rind and sometimes borne in bunches and seedless. There were virtually no thorns on the stems and there was some iron deficiency. Maturity around mid to 3rd to 4th week of June.

Tarocco 57/1E/1. The crop was fairly good but variable between trees. Heavy bearers tended to bear fruit inside the canopy. The fruit size was acceptable, smaller than Taroccos at Adelaide and good colour. The eating quality was fair, acid masking the sugars resulting in a tart fruit. The fruit was soft with signs of creasing. The internal colour was orange, some fruit with pigmentation, starting at the stylar end. Cores were mostly closed with zero to odd seed. About at peak maturity on 12 June, but acid still excessive and not meeting standards. The trees are tall, large and vigorous with variable thorniness, the smaller twigs having no thorns.

Comparison of Maltaise selections planted commercially at Sunland. There are three orchards, Maltaise Half (Block B), Maltaise Half II (Block A) and Maltaise Barlerin (Block C). All had good yields, Barlerin slightly higher and Maltaise Half II slightly lower. Barlerin had the smallest fruit size (best crop) and Half II the largest. The majority of fruit of all selections fell between counts 64-88. Barlerin had the latest fruit colour, Half II the best (T1) while Half had paler orange fruit. The fruit was generally round except for Half II which had some slightly elongated fruit with shoulders and finer rind pebbles, the others pebbly. None had oleo. All were oily to very oily on peeling, Half II the easiest to peel (the others difficult). Barlerin and Half II had thinner stylar ends. Half II had the least (18%) “navel ends” (very small navels or closed protruding navels), Half (44%) and Barlerin (55%). Half II had pale orange flesh colour, Half a yellow colour and Barlerin a deeper yellow. All had fine flesh texture, closed cores and tender flesh but Half and Barlerin had tough segment walls. Half II had the best looking fruit and slightly softer while the others were reasonably firm. Half II was the ripest of the three, Half higher juice and sugars. Half and Barlerin had unacceptably high and very high acid levels respectively. Half II had the largest tree size and Half the smallest, medium large (could also be soil related). Half II had paler leaves and some leaf drop. All have a more open growth habit than navels. All are at or close to peak maturity but high acid is of concern (Half II acceptable) as the fruit may not hold physically should one wait for the acid level to drop sufficiently.

Raratonga. Production was fair with medium large to large fruit size, colour T1 on 14 June. Quality was fair with sufficient sugars, but slightly tart and seedless. The fruit had a unique taste. The test was very good with high sugars and high acid, easily meeting export standards. Last season the acid was considerably lower). The flesh was soft with tough segment walls. The fruit was not so easily peeled, extremely oily with an orange flesh. The fruit shape is round with the occasional neck and the rind thick and coarse with longitudinal indentations running the length of the fruit. The trees are large and vigorous with thorns. The bud union on Troyer had quite a noticable bench. Comparable Salustianas had less thorns and although fractionally later colour (T1-2 with slightly green stem ends) appeared similar to a week earlier and a better looking fruit. Raratonga had a better flavour.

Clara. Trees bore odd fruit at the CFB with medium large fruit size and a deep orange rind colour, T1 on 17 July. The fruit shape was round to Minneola like with a furrowed neck and slightly pebbly. Slightly puffy and look overmature. The trees are very vigorous and extremely thorny.

Tacle. Not yet in production. Extremely vigorous growth, deep green leaves and extremely thorny with long thorns.

Tarocco Scire. First few fruit of mainly medium large fruit size. Colour T1 and deep orange on 27 July. Fruit shape is round with a nipple like Tarocco, a smooth rind and soft. A nice looking fruit. There were signs of red pigmentation. A vigorous tree with deep green leaves.

Tarocco Scire N. Not yet in production. Extremely vigorous tree with large thorns.

Tarocco Tapi. Odd fruit of medium large fruit size. Colour T1 on 27 July with a round, Tarocco like shape and smoothish rind. Pigmented flesh. Extremely vigorous tree with large thorns.

Table 6.3.7.2. Yield, fruit size, fruit colour, estimated maturity, quality, comments and average seed of various midseason oranges evaluated during 2006.

Selection	Yield	Fruit size	Colour transparency	Estimated maturity	Quality / Comment / (average seed)
Sunland					
Maltaise Half	Good-excellent	Medium and larger	**13 June** T1, some 2-3. Palish orange	3rd – 4th week June, but acid may be too high	Fair, lacks sugars, slightly tart. Unacceptable test as acid too high
Maltaise Half II	Good	Medium-medium large	**13 June** T1	Mid June	Ripest of the three. Fair-good. Good sugars and acid, not as tart, sweetest. Unacceptable test as juice just too low (0)
Maltaise Barlerin	Mainly Excellent	Medium-medium small	**13 June** T1-3, some T4	3rd week June, but acid too high	Fair, lacks sugars, slightly tart. Unacceptable test as acid far too high (0)
Adelaide					
Tarocco (older orchard)	Poor	Good, large-extra large	**12 June** T1-2	3rd-4th week of June	Frost in 2005. Probabably good sugars, tart. No signs of pigmentation (0.2)
Tarocco (younger) C1	Poor	Medium large	**12 June** T1	3rd-4th week of June	Fairly high acid, odd signs of pigmentation (0.7)
Tarocco Gallo C3	Variable, fair-good	Medium large-large	**12 June** T2-3	3rd-4th week of June	Poorer flavour. Younger trees (0)
Cookhouse					
Tarocco 57/1E/1	Variable, fair-good	Medium-medium large	**12 June** T1 and 2-3	About peak maturity but acid unacceptably high	Fair, tart (0.3)
Ashton					
Tarocco	Good-excellent	Medium large, variable	**25 May** Smaller fruit T1-3, larger fruit T3-5 **15 June** T1	Mid to 3rd week of June	Slightly variable, good to tart (0-0.6)

Conclusions

Tarocco. Production varied between areas and fruit size was good. The quality is generally good but flavour masked by high acid. There was little external blush on the fruit and little pigmentation, but progressing during the season. Thorns can be problematic especially when the trees are young. Production of the Tarocco looks good in suitable areas where adequate internal pigmentation can be produced. However the acid levels are very high which could result in fruit not being exportable. Maturity around the 3rd to 4th week

of June. Note must also be taken if there are two sets that may influence flesh pigmnentation. Commercial orchards to be evaluated in the Eastern and Western Cape next season.

Tarocco Gallo. Production was reasonable with good fruit size. Rind colour was slightly behind Tarocco. There were no signs of flesh pigmentation. Quality was unacceptable due to a low ratio. The fruit was seedless and maturity around mid to 3rd to 4th week of June. Of concern is the high acid level. Further evaluations are necessary.

Tarocco 57/1E/1. The crop was fairly good and variable with acceptable fruit size. There was variable pigmentation and the acid level excessive resulting in a tart fruit and not meeting standards. The trees are vigorous with variable thorniness. The fruit matures around mid June, but acid still excessive which is a cause for concern. Further evaluations are necessary.

Comparison of Maltaise selections planted commercially at Sunland. There were only slight differences between the selections, mostly with the internal quality. All had fairly similar, good yields and medium fruit size, Barlerin slightly smaller size, slightly better yield and later colour. Half II had the least navel ends, the better looking fruit and earliest maturing. There were differences in internal quality that are consistent with the 2005 trend: Half had the highest juice and sugars, Half II the lowest acid and best ratio and Barlerin the highest acid and lowest ratio. Production and fruit size at this early stage are good for midseasons, but fruit size may become too small as the trees age. Of concern are the high acid levels as this may result in the fruit becoming overmature once the acid has dropped sufficiently for harvest. Evaluations to continue.

Raratonga. Production was fair with good fruit size. The quality was fair, high sugars and high acid resulting in a tart fruit. The Raratonga is similar to a week later than Salustiana and not as attractive, peak maturity about the beginning of June, but acid very high. The tree is vigorous with large thorns. Further evaluations are necessary.

Clara. Only odd fruit borne. Trees are extremely vigorous and thorny. The selection is protected. Evaluations to continue.

Tacle. Not yet in production. Extremely vigorous growth and extremely thorny. The selection is protected. Evaluations to continue.

Tarocco Scire. Odd fruit with signs of red pigmentation. A vigorous tree. Evaluations to continue.

Tarocco Scire N. Not yet in production. Extremely vigorous tree with large thorns. Evaluations to continue.

Tarocco Tapi. Odd fruit with pigmented flesh. Extremely vigorous tree with large thorns. Evaluations to continue.

Future evaluations

Continue evaluations of all the Tarocco selections, Raratonga, Clara, Tacle and commercial Maltaise planting.

Table 6.3.7.3. Internal fruit quality data of the various midseason orange selections for the Eastern and Western Cape during the 2006 season.

Selection	Root stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Tarocco (older)		Saxfold Park	12/06	1	64	53.8	10.8	1.51	7.2	0.2
Tarocco (young)		Saxfold Park	12/06	1	56	50.3	10.5	1.36	7.7	0.7
Tarocco	CC	Excelsior	25/05	2-3	72	51.7	12.6	1.56	8.1	0.6
Tarocco	CC	Excelsior	25/05	4-5	72	49.3	11.6	1.55	7.5	0
Tarocco	CC	Excelsior	15/06	1	72	54.0	12.9	1.42	9.1	0.5
Tarocco Gallo C3		Saxfold Park	12/06	1-3	56	52.2	10.1	1.46	6.9	0
Tarocco 57/1E/1	CC	J&B Citrus	12/06	1	64	55.2	11.4	1.78	6.4	0.3
Maltaise Half	SC	Junkyard	13/06	1	64	55.0	12.4	1.68	7.4	
Maltaise Half II	SC	Junkyard	13/06	1	72	47.9	11.6	1.36	8.5	0
Maltaise Barlerin	SC	Junkyard	13/06		64	49.1	11.6	1.83	6.3	0

Raratonga	TC	CFB	14/06	1	64	53.0	14.0	1.36	10.3	0

6.3.8 **Evaluation Valencia oranges in the Cape areas**

Experiment 77 by C J Alexander (Private Contractor)

Opsomming

Die doel van die Valencia proef is om vroeër, mid en laat seleksies met groot vruggrootte, saadloos en verbeterde vrugset as alternatiewe vir die huidige seleksies te soek. Verskeie nuwe seleksies was by die SGB geevalueer. Limpopo Seedless is die eerste seleksie om ryp te word. Die bome is baie jonk en het aanvaarbare gehalte gehad en saadloos waar daar geen kruisbestuiwing is. G5 is ook saadloos met aanvaarbare tot goeie gehalte. Behalwe vir amper saadloosheid in 'n gemengde blok, is die Portsgate nie uitstaande nie en het grenslyn gehalte. McClean Seedless lyk soos Valencia Late met taamlike hoër suur en is amper saadloos. Rietspruit het kleinerige vruggrootte, swak gehalte, hoër suur en enkele pitte gehad. Bend 8A2 se produksie en gehalte was swak met kleinerige vruggrootte en amper saadloos onder geen kruisbestuiwing. Delicia het goeie produksie en vruggrootte gehad en uitvoer standaarde in Augustus behaal. Kleinhans het sy tipiese eienskappe getoon. Evaluasies moet voortgaan.

Introduction

The aim is to evaluate early, mid and late maturing valencia selections in terms of their maturity, rootstock compatibility, colour, fruit size and seediness.

Materials and methods

The trees are planted at the CFB in the Eastern Cape and a suitable control included where possible. Field evaluations and laboratory analyses were conducted. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is also based on subjective tasting. Fruit quality was compared with the following Valencia standards previously considered acceptable by the market (Midknight in brackets): 50% (50%) juice; 9.5% (10.0%) TSS; 0.8 – 1.5% (0.8– 1.5%) acid; 8.5:1 (9.0:1) ratio; colour T3 of set 34; seed maximum average 9.0 seeds per fruit (Midknight 1.0).

A list of selections evaluated during 2006 is given in Table 6.3.8.1.

Table 6.3.8.1. Valencia orange selections evaluated at the CFB, Uitenhage during 2006.

Selection	Orchard	Plant Date	Root Stock	No of trees
Limpopo Seedless	Psylla house	2004	SC	2
G5	Psylla house	1999	TC	3
Portsgate	Block 7	1999	TC	2
McClean Seedless SL	Block 7	1999	SC	2
Rietspruit	Block 4	2001	SC	2
Bend 8A2	Psylla house	1999	TC/SC	2
Delicia	Block 7	1994	TC	1
Kleinhans	Block 8	1997	TC/SC	4
Valencia Late (control)	Block 4	2001	SC	2

Results and discussion

A discussion of each selection follows. Various tables also need to be referred to when reading the text. Table 6.3.8.2 is a comparison of the various selections at the CFB and internal quality data is presented in table 6.3.8.3. Limpopo Seedless was evaluated as a potential early valencia selection and the others as seedless, late or superior selections. Unfortunately all the selections are not all directly comparable with each other and are discussed according to their estimated order of maturity.

Limpopo Seedless. The trees are small and young, bearing their first crop. It has the lowest acid percentage of all the valencias evaluated. It just met valencia standards in July and juice too low in August (overmature). Seedless in the absence of cross pollination. The earliest maturing of the selections evaluated.

G5. Production was fair with medium fruit size and acceptable to good quality, although the sugars could be higher. The acid levels are not too high. The fruit is seedless. Maturity probably from early August.

Portsgate. A medium large, spreading tree. Producton was fair with medium fruit size. The fruit is hard but not raggy. The quality was unacceptable to mediocre.

McClean Seedless. Production was good with medium small fruit size, fair quality and meeting Midknight standards in August although the acid tended to be a little high. Virtually seedless. Fruit looks like a Valencia Late and matures from about mid August.

Rietspruit. An open, spreading tree. The fruit is coarse with thick rinds resulting in unacceptable to borderline juice. It has the highest acid levels (on Swingle) of the selections evaluated.

Bend 8A2. The trees are vigorous. Production was poor with medium small fruit size. Internal quality was poor, both tests with an unacceptably low ratio and low sugars in August. The acid level was too high in July and considerably lower in August although on a different rootstock. This difference is questionable as it also does not correspond with the taste. It would appear that the selection is late maturing, probably August or later.

Delicia. The trees are large with large, wavy leaves. The yield and fruit size were good and the quality just acceptable in mid August, acids tending to be on the high side. Eating quality was good but tart. Odd seeds. Maturity from mid August to later.

Kleinhans. The trees are planted next to a windbreak and subjected to stress. The internal quality results are typical for trees under stress. Elongated fruit shape is typical of the selection. The fruit had odd seed.

Valencia Late. Tall, vigorous trees. Poor production with medium large fruit size. Good quality in August. Typical of the selection.

Conclusions

Limpopo Seedless. Production was acceptable for such young trees and fruit size medium. Initial quality was acceptable. It is early maturing and seedless in the absence of cross pollination. Further evaluations are necessary.

G5. Production was fair with medium fruit size, acceptable to good quality and seedless. Further evaluations are necessary.

Portsgate. Production was fair with medium fruit size and borderline quality. Except for virtual seedlessness, nothing outstanding. Further evaluations are necessary.

McClean Seedless. Production was good with medium small fruit size, fair quality and meeting Midknight standards in August. Looks similar to a Valencia Late, although virtually seedless, maturing from about mid August with fairly high acid. Further evaluations are necessary.

Rietspruit. Production was fair to good with smallish fruit size. The quality was poor with high acids and borderline juice. Odd seed. Further evaluations are necessary.

Bend 8A2. Production was poor with medium small fruit size, poor quality, virtually seedless and late maturing. Further evaluations are necessary.

Delicia. Production and fruit size were good, tart and meeting standards in August. The characteristics are typical of the selection and virtually seedless.

Kleinhans. As the trees are planted next to a windbreak, the results are indicative of trees under stress and not too much emphasis should be laid on them. The fruit had zero to odd seed.

Future evaluations

Evaluate all new selections at the CFB and other sites when they come into production.

Table 6.3.8.2. Yield, fruit size, colour, quality, maturity, fruit characteristics and average seed of various Valencia selections evaluated at the CFB during 2006.

Selection/ Orchard/ Rootstock/Age	Yield	Fruit size	Fruit colour - transparency	Taste	Test	Estimated maturity	Fruit characteristics	Ave. Seed
Limpopo SL Psylla house SC 04	Fair-good	Medium-medium large	**19 July** 1 **17 Aug** 1	Fair, low acid	Acceptable in July, low Juice in August	Mature in July, overmature in mid August	Flattish fruit, smooth to pebbly rinds, slightly sunken navel	Zero
G5 Psylla house TC 99	Fair	Medium	**19 July** 2-3 **17 Aug** 1	Poor, low sugars and acid	July test meets valencia standards and August test just make Midknight standards	Mature from early August	Round to very slightly elongated, smooth to pebbly rind. A lot of chimeras	Zero
Portsgate Block 7 TC 99	Fair	Medium	**19 July** 1-3 **17 Aug** 1	Poor in July, fair in August, not raggy	Unacceptable in July (TSS and ratio) just acceptable in August	Mature around mid August	Round with a smoothish rind, a hard fruit but not raggy	0-0.1
McClean SL Block 7 SC 99	Good	Medium small	**19 July** 1-4, fairly pale orange **17 Aug** 1-2	Fair in July, slightly tart	Acid too high in July, just meets Midknight standards in August	Matures from about mid August	Round, smooth to slightly pebbly, slightly thick rind, odd out of season fruit, good orange flesh	0-0.1
Rietspruit Block 4 SC 01	Fair to good	Medium small	**19 July** 1-3 **17 Aug** 1	Poor, low sugars, high acid	Juice low to borderline, acid too high	Mature in August	Round, coarse and thick rinds	0.3-0.8
Bend 8A2 Psylla house TC/SC 99	Poor	Medium small	**19 July** 2-3 **17 Aug** 1-2	Poor, low sugars and acid in July	Unacceptable, low ratio due to high acid or low TSS	Probably August	Fairly round with a slightly flat stem end and smooth to pebbly rind	0-0.3
Delicia Block 7 TC 94	Good	Medium large	**19 July** 2-4 **17 Aug** 1-2	Fair, high sugars and fairly acid in July	Acid too high in July, just meeting valencia standards in August	Mature from mid August on	Round, smooth rind, flattish stem end, not raggy	0.2-0.3
Kleinhans Block 8 TC 97	Poor and medium to medium large. Windbreak effect		**19 July** 1		High sugars and excessive acid due to stress		Elongated, smooth rind	0.7-0.9
Valencia Late (control) Block 4 TC/SC 01	Poor	Medium large	**19 July** 1-3 **17 Aug** 1	Poor, low sugars and high acid	Low juice and ratio in July, meets Midknight standards in August, except seed	August	Round with a coarsish and thick rind. Odd leaf drop.	0.8-1.8

Table 6.3.8.3. Internal fruit quality data for Valencia orange selections at the CFB, Eastern Cape during the 2006 season.

Selection	Root-stock	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Limpopo SL	SC	19/07	1	72	50.2	9.8	1.11	8.8	0
Limpopo SL	SC	17/08	1	64	45.9	10.3	1.00	10.3	0
G5	TC	19/07	2-3	56	57.3	10.1	1.16	8.7	0
G5	TC	17/08	1	72	56.4	10.3	0.99	10.4	0
Portsgate	TC	19/07	1-3	72	51.4	9.3	1.20	7.8	0.1
Portsgate	TC	17/08	1	72	51.7	9.8	1.14	8.6	0
McClean SL	SC	19/07	1-4	72	53.0	10.6	1.52	7.0	0
McClean SL	SC	17/08	1-2	72	54.9	12.1	1.33	9.1	0.1
Rietspruit	SC	19/07	1-3	56	49.4	9.8	1.63	6.0	0.8
Rietspruit	SC	17/08	1	72	50.4	10.4	1.56	6.7	0.3
Bend 8A2	TC	19/07	2-3	72	54.3	9.8	1.51	6.5	0
Bend 8A2	SC	17/08	1-2	72	51.3	9.3	1.13	8.2	0.3
Delicia	TC	19/07	2-4	56	58.2	10.7	1.57	6.8	0.2
Delicia	TC	17/08	1-3	56	58.9	10.7	1.26	8.5	0.3
Kleinhans	SC	19/07	1	88	49.9	12.4	2.33	5.3	0.9
Kleinhans	TC	19/07	1	72	47.1	12.4	2.08	6.0	0.7
Valencia Late	SC	19/07	1-3	56	48.9	9.5	1.38	6.9	0.8
Valencia Late	TC	17/08	1	72	56.6	10.5	1.15	9.1	1.8

6.3.9 **Evaluation of existing cultivars at Lancewood, Knysna area**

Experiment CJA–1 by C J Alexander (Private Contractor)

Opsomming

Die doel van die proef is om geskikte, hoër gehalte, veral laat mandaryn kultivars te vind vir die Knysna area. Jong Aoshima het swak gehalte gehad. Bay Gold lyk nie belowend nie weens hoë suur al is die vrugte oorryp. Sweet Spring het goed gedra maar ’n tekort aan smaak. Nouvelle was vrot van *Alternaria* bruinvlek gewees. Kiyomi het hoë suur gehad en het beter in die Heidelberg area gepresteer. Thoro Temple het geen uitstaande eienskappe vertoon en word nie aanbeveel nie. Die laat Clementarde, CELL en Clemlate clementine seleksies is eenders en het sekere produksie nadele, insluitend klein vruggrootte en lae suurvlakke. Evaluasies op die satsuma en mandariyn kruisings moet nog voortgaan.

Introduction

The Knysna area produces mandarins for export, but due to climatic constraints the cultivar range is limited. The aim is to find suitable, high quality, especially late maturing soft citrus cultivars for the area.

Materials and methods

The trees are topworked or planted in the Rheenendal and Ruigtevlei areas. Field evaluations were conducted on the trees and fruit maturity based on subjective tasting. Fruit quality was compared with the following standards: Satsumas, Clementines and mandarins (differences between cultivars in brackets) – 48% juice (mandarins 50%); 8.5% Brix (Clementines 9.5%); 0.7-1.5% acid; 7.5:1 ratio (Clementines 8.0:1); colour set 3 of plate 36; seed maximum average 3.0 seeds per fruit (Satsumas 0). The selections and sites evaluated are presented in Table 6.3.9.1.

Table 6.3.9.1. Cultivar trial sites evaluated during 2006.

Selection	Area	Site	Plant Date	Rootstock	No of trees
Aoshima	Rheenendal	Candlewood	2003		5
Bay Gold	Rheenendal	Candlewood	2003	TC	5
Sweet Spring	Rheenendal	Candlewood	1995	TC	9
Nouvelle	Rheenendal	Candlewood	2001	SC	7 topwork
Kiyomi	Rheenendal	Candlewood	1995	CC	3
Thoro Temple	Rheenendal	Candlewood	1996	SC	2
CELL	Ruigtevlei	Rushmere	1996	X639	5
Clementarde	Ruigtevlei	Rushmere	1996	X639	4
Clemlate	Ruigtevlei	Rushmere	1988	X639	4
Thoro Temple	Ruigtevlei	Rushmere	1996	RL	15
Kiyomi	Kruisrivier	Heidelberg		CC	commercial
Thoro Temple	Kruisrivier	Heidelberg		CC	commercial

Results and discussion

A discussion of each selection follows with internal quality data presented in Table 6.3.9.3 that need to be referred to when reading the text. The Aoshima was evaluated for its potential as a late satsuma for the area, late Clementines and mandarin hybrids for extending the soft citrus season later.

Aoshima. The trees bore large quite blemished fruit. Fruit colour in late May was T4-6. The fruit quality was poor, and lacked taste with sufficient acid and no sugars. The test was poor due to unacceptably low sugars. The fruit has an excellent internal colour and average although slightly variable rind thickness. Even though the fruit has a slightly loose rind the fruit is nevertheless firm. On peeling some of the fruit still look immature with some albedo adhering to the segments. Fruit shape is fairly flat with a smoothish rind and 24% of the fruit having tiny, open navel ends. There were odd seed. 22% of the fruit had very light oleo. The fruit was possibly at peak maturity to a week to go, although not all cleanly peeled on 26 May.

Bay Gold. A sample was evaluated on 29 May, rind colour T1-2. The fruit had an attractive colour and appearance. The rind was smooth and thin, especially at the stylar end, breaking up and oily on peeling. Fruit quality was poor with high acid, probably masking the sugars and fruit already with large, dry looking vesicles. Externally the fruit looked at peak maturity. There had been some fruit split.

A further evaluation was done on fruit harvested on 5 July (waxed and cold stored) and evaluated a week later. The fruit size was good, variable and medium large. The rind colour was deep orange/red, very smooth, shiny and attractive. Fruit shape is obovoid to pyriform to flatter. The quality was fair, not too tart, probably good sugars but slightly masked. The test easily met clementine export standards. The fruit was soft and peelability now easy with little rind oil and some adhering albedo. The cores were open and flesh colour good, sometimes coarse, ricy and not juicy. There were odd seed. Maturity is difficult to estimate, probably late June, early July.

Sweet Spring. The trees had a good crop (estimate 60 kg/tree), with medium large to large fruit size. By 12 July the better coloured fruit, T1-3 had already been picked and those remaining T4-6. The fruit has a more yellow than orange rind colour. The quality was fair to good, slightly tart and fruit could have higher sugars and lacked flavour. Of the two tests done the better coloured fruit easily met export standards, the other fruit failing on colour and seed, while there was little difference between the sugars and acid. The fruit shape is round, some having a slight nipple to satsuma like and a smooth to pebbly rind. Peelability varied from fair to difficult and oily with a good to paler flesh colour. There were some seed. Hand pollination of a few fruit with Nules initiated 2-3 times more seed. Optimum maturity is around 3rd week of July to early August.

Nouvelle. The trees had severe *alternaria* on the fruit, also causing twig dieback.

Kiyomi. Production was poor with variable medium large to large fruit size. The colour on 12 July was T1-3 and yellow orange. The quality was not too bad, with fair and not excessive acid and sugars probably high. On testing the acid levels were too high. The fruit is round with shoulders, with a fairly smooth and good rind thickness, firm to slightly soft. The fruit picked easily, were reasonably peeled and slightly oily. The cores were closed and the flesh soft, deep orange, juicy and seedless. Maturity estimated around the end of July/early August, depending on the acid level.

A commercial planting evaluated at Heidelberg had an excellent crop of good, medium large fruit size, colour T1 on 11 July. Fruit quality was fair, a bit variable, sometimes slightly tart – of the best Kiyomis tasted. The test was good and the fruit seedless. The fruit was generally round with some necks and occasional very small navel ends and some with a sunken stylar end. The fruit was soft with open cores, thickish rind, juicy and signs of the skin parting from the segments. Around peak maturity.

Thoro Temple. The trees at Candlewood had a poor crop of good, medium large to large fruit size. The colour on 12 July was T1-3 (pale) on the northern side of the tree and orange T2-4 on the other side. The fruit was tart, probably with good sugars. The test was acceptable and acid near borderline (high). The fruit picked easily. Estimated maturity around end July, early August.

At Ruigtevlei the crops varied from fair to good with good, medium large to large fruit size. Fruit colour on 12 July was T1, paler fruit on the northern side. The fruit was tart and lacked flavour and not juicy (not dry). The test was acceptable with lower acid (sandy soil) than Rheenendal. Maturity is difficult to estimate, probably the end of July, although the fruit picks easily in mid July. There were a lot of out of season fruit.

The crop at Heidelberg was poor with medium large to large fruit size and colour T1 on 11 July. Quality was good to slightly tart and fruit not yet mature.

The fruit can be attractive and has a flattish shape, firm and smooth to slightly coarse (stylar end) and thin rind and the occasional closed navel. The fruit can be reasonable to very difficult to peel and very oily. There were some seed in the mixed blocks.

CELL, Clementarde and Clemlate. The trees are on a sandy, well drained slope and bore good to excellent crops of generally medium fruit size. Overall there was not much difference between the three selections, Clementarde with the most differences. All had green stylar ends, Clementarde the darkest. Eating quality was similarly good but not outstanding, Clemlate fractionally better and Clementarde possibly slightly earlier. Tests were similar and Clemlate overall slightly better in terms of juice, sugars and acid and the only one meeting standards (the others just failing due to low acid). All were at peak maturity. All were slightly pebbly, Clementarde fractionally more so. Clementarde was slightly easier to peel and there were signs of the rind parting from the flesh. Clementarde was seedless (Thoro Temple in the same row) and had the densest trees.

Table 6.3.9.2. Comparison of the yield, fruit size, rind colour, maturity and comment of three late clementine selections on X639 evaluated at Rushmere, Ruigtevlei on 12 July, 2006.

Selection	Yield	Fruit size	Colour	Maturity and comment
Clementarde	Good-excellent	Medium-medium+	T4. Dark stylar ends	Peak, possibly slightly earlier
CELL	Excellent	Medium	T4. Slightly deeper coloured (orange) stem ends	Peak. Slightly poorer than Clemlate
Clemlate	Good-excellent	Medium-medium small	Variable, T4-5 some T3	Peak. Good, not outstanding, good sugars and acid

Conclusions

Aoshima. The fruit was poor quality, colour and lacking sugars. Further evaluations are necessary.

Bay Gold. The fruit size was good. The fruit were externally mature in late May but acid levels still too high resulting in tart fruit. Met standards in early July. The selection does not look promising and evaluations to continue for one more year.

Sweet Spring. The trees carried a good crop of good fruit size. Eating quality was fair to good, slightly tart and lacking flavour. Although the fruit has some good characteristics the lack of flavour and pale colour are probably not widely acceptable to the overseas market. Maturity around late July to early August.

Nouvelle. Not recommended for the area due to the severe *alternaria*.

Kiyomi. Production at Rheenendal was poor and fruit size good. Due to the excessive acid levels it is not recommended in a cold climatic area. The quality at Heidelberg was considerably better although not outstanding. The cultivar will be monitored again next season.

Thoro Temple. The crops varied between sites with good fruit size. The fruit was generally tart although the tests were acceptable. There was a marked difference in fruit colour between the northern (paler) and southern sides of the tree. The cultivar does not exhibit any outstanding attributes and not recommended for planting.

CELL, Clementarde and Clemlate. None of the selections had outstanding performance in any way and were similar in most respects, i.e. production, fruit size, colour, quality and maturity. Clementarde was possibly slightly earlier with the darkest green stylar ends. Only Clemlate met export standards, the others with just insufficient acid. Should commercial planting be considered, low acidity and small fruit size can be limiting factors.

Future evaluations

Evaluate satsumas and mandarin hybrids for another season.

Table 6.3.9.3. Internal fruit quality data for various satsuma, clementine and mandarin hybrid selections for the Knysna area during 2006.

Selection	Root-stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Aoshima		Candlewood	26/05	4-5	1X	48.0	8.4	0.94	8.9	1.0
Bay Gold		Candlewood	05/07	1	1X	52.7	11.5	1.24	9.3	0.8
Sweet Spring	TC	Candlewood	12/07	1-3	1X	59.3	10.7	0.91	11.8	2.4
Sweet Spring	TC	Candlewood	12/07	4-5	1XX	56.1	10.5	0.94	11.2	4.0
Kiyomi	CC	Candlewood	12/07	2	1XX	57.1	11.4	1.73	6.6	0
Thoro Temple	CC	Candlewood	12/07	3-4	1XX	59.9	11.8	1.45	8.1	2.4
Clemtarde	X639	Rushmere	12/07	3	2	57.4	12.2	0.69	17.7	0
CELL	X639	Rushmere	12/07	4	2	59.3	12.5	0.68	18.4	0.3
Clemlate	X639	Rushmere	12/07	3	2	62.1	12.8	0.72	17.8	1.4
Thoro Temple	RL	Rushmere	12/07	1	1XX	60.4	10.3	1.22	8.4	1.3
Kiyomi	CC	Kruisrivier	11/07	1	1XX	56.4	10.7	1.09	9.8	0

6.3.10 **Evaluation of Clementine mandarins in the cool inland areas**
Experiment 72 by J.Joubert (CRI)

Opsomming

'n Proef is saamgestel om te bepaal of sekere Clementine manderyne kommersïeel vir uitvoer in die intermediêre en koel binnelandse sitrusproduserende streke van die land met betrekking tot markbehoeftes, geproduseer kan word. Daar word gesoek na uitstaande seleksies met uitstekende interne vruggehalte, eksterne vrugkleur en vruggrootte verspreiding. Hierdie seisoen was Ain Toujdate weer eerste gereed om geoes te word, met slegs SC se sap vlakke onder die minimum uitvoer vereiste. Die bome het oor die algemeen baie goed gedra, en baie takke het gebreek of geskeur onder die oes lading. Kruisbestuiwing was laer en daar het dus minder sade per vrug voorgekom.

Introduction

A trial was laid out to ascertain whether certain Clementine mandarins could be produced commercially for export in the intermediate and cool inland citrus production areas of the country in accordance with market needs, as well as finding superior selections in terms of internal fruit quality, colour and fruit size.

Materials and methods

Field evaluations and laboratory analysis were conducted on Ain Toujdate, L.L. and Sidi Aissa Clementine selections.

The minimum export requirements for the internal fruit quality of Clementines was compared during the 2006 season: 48% Juice; 9.5º Brix; 0.7% Acid (Min); 1.5% Acid (Max) Ratio 8.0:1; Colour T2 and 20% T3 of set 36.

Table 6.3.10.1. List of Clementine trial sites evaluated at Zalo Sitrus, Burgersfort area, during the 2006 season.

Selection	Rootstock	Tree Age	No. of trees
Ain Toujdate	CC, SC	1999	5 each
LL	CC, SC	1999	5 each
Sidi Aissa	CC, SC	1999	5 each

Results and discussion

Ain Toujdate
Yields on CC and SC was exceptionally good, with medium to large (count 3-1XX) fruit size. The juice content on CC was above 48% (49.3%), but unfortunately below 48% (46.7%) on SC at the time of harvest. Some of the branches on both rootstocks were damaged because of the heavy fruit load on the trees. The Brixº of the fruit on both rootstocks was impressively high (11.0-11.4º) and complied with the export standards. Externally the colour of the fruit on SC (T4-6) was 2 counts behind the CC (T2-3) fruit. Maturity of the fruit was middle to end of April.

L.L.
The internal quality of the fruit evaluated complied with the export standards, except for the acid content on CC (0.61%). The average juice content produced was impressive between 51.6 and 51.7%). Number of seeds per fruit decreased in comparison with the 2005 season from 8.25 to 4.83 (ave). There was some thrips damage on 50% of the fruit. The Maturity was estimated between the first and second week of May.

Sidi Aissa
Severe damage occurs on several of the trees bearing very heavy crops this season. Internally the fruit complied with the export standards. The juice content on CC was on the low side (48.7%), but still acceptable. This selection was also ready to harvest early in the season at the end of April with good external colour (T3-5). Fruit size varied from medium to large (3-1XX).

Conclusions and recommendations

Ain Toujdate was the first cultivars ready for harvesting, followed by Sidi Aissa and LL. The seed content for all the different selections decreased compared to the 2005 season. This was the last season for evaluations on this trial.

Table 6.3.10.2. Internal fruit quality data for Clementine mandarin selections at Zalo Sitrus (cool inland area) during the 2006 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix º	Acid %	Ratio	Ave. seed	Colour
Ain Toujdate	CC	06/04	3-1XX	49.6	11.1	0.72	15.42	12.1	T3-5
Ain Toujdate	CC	21/04	3-1X	49.3	11.4	0.81	14.07	6.2	T2-3
Ain Toujdate	SC	06/04	4-1XX	48.3	10.5	0.77	13.64	5.9	T5-6
Ain Toujdate	SC	21/04	3-1XX	46.7	11.0	0.81	13.58	6.9	T4-6
LL	CC	06/04	3-1XX	52.4	10.0	0.62	16.13	4.4	T7
LL	CC	21/04	3-1XXX	51.6	9.9	0.61	16.23	3.2	T5-6
LL	SC	06/04	3-1XX	45.1	10.2	0.62	16.45	9.0	T7-8
LL	SC	21/04	4-1X	51.7	10.1	0.73	13.84	2.7	T7-8
Sidi Aissa	CC	06/04	2-1XX	50.7	10.3	0.68	15.15	11.7	T4-6
Sidi Aissa	CC	21/04	2-1XX	48.7	10.6	0.73	14.52	10.6	T3-4
Sidi Aissa	SC	06/04	3-1XX	51.5	10.1	0.73	13.84	9.7	T4-6
Sidi Aissa	SC	21/04	2-1XX	50.7	10.3	0.77	13.38	9.0	T3-5

6.3.11 **Evaluation of Mandarin hybrids in the cool inland areas**
Experiment 73A by J.Joubert (CRI)

Opsomming

Geskikte Mandaryn seleksies vir die warm, intermediêre en koel binnelandse sitrusproduserende streke moet gevind word om die vroeë-, middel- en laatseisoen leemtes te vul. Primosole was die vroegste gereed vir oes, maar die seleksie ondervind heelwat probleme in hierdie area. Granulasie, groot vruggrootte, baie growwe vrugte en lae sap volume was van die ergste probleme wat hier opgeduik het. Orighstad sal moontlik 'n beter klimaat wees vir die aanplant van Primosole. Hadas word tans in Swaziland by Tambuti Estate ge-evalueer en die bome behoort in die volgende seisoen heelwat vrugte te produseer. Cami het hierdie seisoen baie goed gevaar met goeie interne kwaliteit en produksie. Die vrugte he took 'n soet, sappige smaak opgelewer.

Introduction

To find suitable Mandarin selections for the hot, intermediate and cool inland citrus production areas to fill the early, mid and late season gap.

Materials and methods

Field evaluations were conducted. Internal fruit analysis was conducted for the Burgersfort area during the 2006 season.

Table 6.3.11.1. List of mandarin selections evaluated during the 2006 season.

Selection	Site	Rootstock	Tree age	No. of trees
Bay Gold	Zalo Citrus	CC	2001	5
Cami	Zalo Citrus	CC	2001	3
Primasole	Zalo Citrus	CC	2001	5
Roma	Zalo Citrus	CC	2001	5

Results and discussion

Bay Gold
Trees were evaluated at Zalo Citrus (Burgersfort) in Mpumalanga during the 2006 season. The internal quality of the fruit looks promising with a high juice content (52.3%) and comply with the export standards. Fruit size varied from large to extra large (1XX-1XXX), with good yields produced on the trees. Maturity will be middle to end of May.

Cami
Cami produced an excellent yield at Zalo Citrus, with medium to large fruit size. The internal quality of the fruit complied with the export standards by the time of harvesting. The acid content was still on the high side (1.37%), but still acceptable. Cami produced between 17.7 and 23.3 seeds per fruit on average. Damage was caused to the trees because of the high yield production. Some of the branches were hanging on the ground with the intense fruit load. Maturity seems to be at the end of May.

Primosole
Primasole produced a low juice and acid content from early in the season. Most of the fruit granulated badly, because of the heat units in this area. External colour of individual fruit was uneven (green and orange spots). Primosole apparently requires a cooler area, e.g. Orighstad. Maturity end of March to the beginning of April (early).

Roma
The trees produced medium to large fruit size (1-1XX) with high seed counts between 22.1 and 25.8 seeds per fruit. Internally Roma qualified for exports, but the acid content (0.65%) with harvest might be on the low side. The fruit had a juicy sweet taste in comparison to the other selections. Maturity middle to the end of May.

Conclusions and recommendations

Primasole must be planted in a cooler, because of similar problems as the previous season. Fruit granulated and large fruit with very coarse rinds. The Orighstad area might be ideal. Hadas needs a warmer production

area to produce fruit with better internal quality and lower Acid%. Bay Gold and Roma produced a sour taste in the fruit and lasted up to harvest time. Cami produced a pleasant sweet taste with exceptional internal colour.

Table 6.3.11.2. Internal fruit quality data for Mandarin selections for the cool inland areas during the 2006 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix º	Acid %	Ratio	Ave. seed	Colour
Bay Gold	CC	06/04	1XX-1XXX	52.5	10.45	1.76	5.9	6.7	T6-7
Bay Gold	CC	21/04	1XX-1XXX	52.3	11.20	1.59	7.0	6.3	T5-6
Bay Gold	CC	31/05	1X-1XXX	53.2	11.50	1.1	10.5	5.0	T2-3
Cami	CC	06/04	3-1X	55.9	10.70	1.39	7.7	28.0	T7-8
Cami	CC	21/04	2-1XX	58.9	10.80	1.17	9.2	17.7	T5-6
Cami	CC	31/05	2-1XX	57.3	11.80	1.37	8.6	23.3	T1-2
Primasole	CC	06/04	1XXX	41.0	8.90	0.45	19.78	0.0	T5-6
Roma	CC	06/04	1-1XX	49.7	11.00	0.80	13.8	24.8	T4-7
Roma	CC	21/04	1-1XXX	51.7	11.40	0.74	15.4	25.4	T5-6
Roma	CC	31/05	1-1XX	54.2	11.45	0.65	17.62	22.1	T2-3

6.3.12 **Evaluation of Mandarin hybrids in the cool inland areas**

Experiment 73B by J.Joubert (CRI)

Opsomming

Geskikte Mandaryn seleksies vir die warm, intermediêre en koel binnelandse sitrusproduserende streke moet gevind word om die vroeë-, middel- en laatseisoen leemtes te vul. Primosole word nie vir die Marble Hall area aanbeveel nie, want die hitte eenhede van hierdie area is te hoog. Orighstad area en ander koeler gebiede kan oorweeg word. Hadas word ook nie vir hierdie area aanbeveel nie, want vir hierdie seleksie is die hitte eenhede weer te laag. Warmer gebiede kan oorweeg word soos Swaziland ens. Roma lyk die belowendste van die ander seleksies wat in hierdie proef ge-evalueer is. Die vruggrootte en interne kwaliteit voldoen aan die nodige vereistes.

Introduction

To find suitable Mandarin selections for the hot, intermediate and cool inland citrus production areas to fill the early, mid and late season gap.

Materials and methods

Field evaluations were conducted. Internal fruit analysis was conducted for the Marble Hall area during the 2006 season.

Table 6.3.12.1. List of mandarin selections evaluated during the 2006 season.

Selection	Site	Rootstock	Tree age	No. of trees
Bay Gold	Moosrivier Estate	CC	2001	9
Cami	Moosrivier Estate	CC	2001	5
Primasole	Moosrivier Estate	CC	2001	9
Roma	Moosrivier Estate	CC	2001	10

Results and discussion

Bay Gold

Trees were evaluated at Moosrivier Estate (Marble Hall) in Mpumalanga during the 2006 season. Internally the juice content (43.5%) and Brix º (9.3) was on the low side at the time of harvesting. There was quite a number of fruit with granulation problems, explaining the lower juice content. The fruit size looked promising and varied between count 1X and 1XXX. Maturity will be middle to end of May.

Cami
Trees were evaluated at Moosrivier Estate (Marble Hall) in Mpumalanga during the 2006 season. The fruit size varied from small, medium to large fruit (4-1XX), in comparison to the larger fruit produced at Burgersfort. The internal quality seems very promising with high juice (56.7%) and acid (1.07%) content, as well as Brix º (10.6) by the time of harvesting. Maturity seems to be at the end of May.

Primosole
Trees were evaluated at Moosrivier Estate (Marble Hall) in Mpumalanga during the 2006 season. Similar problems occur in comparison to the previous season with course rind externally, large fruit size and terrible granulation internally. This area is not suitable for Primasole production. Maturity end of March.

Roma
Roma produced better fruit on the trees compared to Bay Gold and Cami in this trial. The juice (46.4%) and acid (.82%) content seems low at the time of harvest, but the Brixº (12) looks promising. Fruit size varied from medium to large (3-1XXX). The last evaluation might be too late, explaining why the internal quality standards were too low. Maturity middle of May.

Conclusions and recommendations

Primasole must be planted in a cooler, because of similar problems as the previous season. Fruit granulated and large fruit with very coarse rinds. The Orighstad area might be ideal. Hadas needs a warmer production area to produce fruit with better internal quality and lower Acid%. Cami seems to be one of the best options for this area considering the results from the trial conducted.

Table 6.3.12.2. Internal fruit quality data for Mandarin selections for the cool inland areas during the 2006 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix º	Acid %	Ratio	Ave. seed	Colour
Bay Gold	CC	04/04	1X-1XXX	46.1	9.40	1.39	6.8	8.6	T8
Bay Gold	CC	24/04	1XX-1XXX	46.0	9.60	1.04	9.2	8.4	T7-8
Bay Gold	CC	17/05	1XX-1XXX	43.5	9.30	1.09	8.5	8.3	T3-5
Cami	CC	04/04	4 - 2	52.1	9.10	1.43	6.4	11.1	T7-8
Cami	CC	24/04	3 - 1	54.1	9.80	1.22	8.0	11.3	T8
Cami	CC	17/05	1-1XX	56.7	10.60	1.07	9.9	12.6	T3-5
Primasole	CC	04/04	1X-1XXX	41.0	8.20	0.57	14.4	2.9	T6-7
Roma	CC	04/04	3-1XX	50.3	10.40	0.75	13.9	16.1	T7
Roma	CC	24/04	2-1XX	48.6	10.50	0.74	14.2	14.0	T6-7
Roma	CC	17/05	2-1XXX	46.4	12.00	0.82	14.6	10.8	T2-4

6.3.13 **Evaluation of navels in the cool inland areas**
Experiment 74A by J. Joubert (CRI)

Opsomming

Winsgewendheid moet verhoog word deur boomproduksie (oes- en vruggrootte), pakpersentasie (kraakskil en oleo weerstand, kleiner nawelente om witluis teë te werk, *Alternaria*-besmetting, windbestandheid, blomdatum, en binnedrag) en vruggehalte (skilkleur vroeg in die seisoen, sapgehalte, granulasie, lae suur) te verbeter, asook om die oes- en bemarkingseisoen (vroeë-, middel- an laatrypwordende seleksies) te verleng. Meeste van die seleksies wat hierdie seiseon ge-evalueer is, het nie aan al die uitvoer standaarde voldoen nie. Lae suur vlakke by die vrugte was 'n algemene voorkoms gewees. Die proef sal nog een seisoen ge-evalueer word wat moontlike oplossings kan uitlig.

Introduction

To optimise profitability by improving productivity (fruit set and size); packout percentage (creasing and oleo resistance, smaller navel ends to counter mealybug, *Alternaria* infection, less wind prone – time of flowering and inside bearing), fruit quality (rind colour early in the season, juice quality, granulation, low acidity) and extend the harvest and marketing season (early- mid and late maturing selections).

Materials and methods

Field evaluations and laboratory analysis were conducted on Atwood, Autum Gold, Bahianinha, Cara Cara, Dream, Fukumoto, Powel Summer and Tule Gold selections at Zalo Citrus (Burgersfort), a site in the cool inland production area.

Internal quality data were compared with the minimum average export requirements for navels: 48% Juice; 9.7% TSS; 1.5% (Max) - 0.68% (Min) % Acid; 8:1 Ratio; Colour set 34 no.T3 shipped at 11°C.

Table 6.3.13.1. List of Navel selections evaluated at Zalo Citrus (Burgersfort) during the 2006 season.

Selection	Rootstock	Tree age	No. of trees
Atwood		2001	5
Autumn Gold	CC	1999	5
Autumn Gold	SC	1999	5
Bahianinha	SC	2001	4
Cara Cara		2001	8
Dream		2001	9
Fukumoto		2001	3
Powel Summer	CC	1999	5
Powel Summer	SC	1999	5
Tule Gold		2001	9

Results and discussion

Atwood
Atwood produced a good yield on the trees, with a large fruit size (64-40). The internal quality complied with the export standards, but there was a delay in the external colour (T5-6) of the fruit and a slightly low acid content (0.70%) by the time of harvest. Maturity end of April.

Autumn Gold (Late maturing navel)
Autumn Gold produced a good yield both on SC and CC, with medium to large fruit size (count 72-40).The external colour of the fruit was similar when harvested, between T1 and T3. Internally SC produced a better quality fruit in comparison to CC, with higher juice, Brix and acid content. Maturity seems to be middle to end of June.

Bahianinha
Bahianinha produced an excellent yield on the trees, with a medium to large fruit size (count 88-56). The internal quality complies with the export standards with high juice (55.5%) and Brixº (11) levels. There was a slight delay in external colour by the time of harvest (T5-6). Maturity might be middle of May.

Cara Cara
Trees were evaluated at Zalo Citrus (Burgersfort) in Mpumalanga during the 2006 season. The trees produced an excellent fruit size between count 72 and 40. The internal quality for this season barely meets the export requirements. The Brixº (9.3) declined, as well as the acid content (0.74%) of the fruit evaluated by the time of harvest. Maturity middle of May.

Dream
Trees were evaluated at Zalo Citrus (Burgersfort) in Mpumalanga during the 2006 season. There was a delay in the external colour of the fruit by the time of harvest. The acid content (0.79%) tested already on the low side, but the juice content (51.7%0 and Brixº (9.9) complied with the export standards. Internally the fruit produced an excellent uniform bright yellow colour. Maturity middle of May.

Fukumoto
The trees produced a good yield with medium to large fruit size (count 64-40). The internal quality did not comply with the export minimum with very low acid content (0.52%). At the time of harvesting the external colour was also delayed between T4 and 5. Maturity middle to end of April.

Powel Summer (Late maturing Navel)
Powel Summer produced a good yield and medium to large fruit size (count 88-40) on both CC and SC for this season. Internally the fruit comply with all the export standards on both CC and SC. SC produced an impressively high Brixº (12.5) by the time of harvesting. Maturity seems to be middle to end of June.

Tule Gold
Trees were evaluated at Zalo Citrus (Burgersfort) in Mpumalanga during the 2006 season. Approximately 40% of the fruit indicated signs of sunburn. In comparison to the other selections in this trial, the trees grow slower and are smaller in size. Internally there was also a problem with the acid content testing on the low side (0.69%) by the time of harvest. Maturity middle of May.

Conclusions and recommendations

There seems to be a general problem with the acid content in regards to most of the navel selections evaluated in this trial. There was a decreased in the acid content in comparison to the previous seasons. The incompatibility status of Fukumoto on CC and SC remains experimental, with no changes in the condition of the trees at Zalo Citrus. The green tips above the bud union remains more or less the same size and don't seem to increase in numbers. Evaluations will continue for one more season.

Table 6.3.13.2. Internal fruit quality data for Navel selections at Zalo Citrus (Burgersfort), a cool inland production area, during 2006.

Selection	Root-stock	Date harvested	Count	Juice %	Brix º	Acid %	Ratio	Ave. seed	Colour
Atwood	CC	06/04	64-48	49.3	9.80	0.69	14.20	0.0	T6-7
Atwood	CC	21/04	56-40	51.2	10.00	0.70	14.29	0.0	T5-6
Autum Gold	CC	31/05	64-40	49.4	10.20	0.82	12.44	0.0	T1-3
Autum Gold	CC	27/06	64-48	51.7	10.90	0.78	13.97	0.0	T1-3
Autum Gold	SC	31/05	72-48	52.3	11.80	0.96	12.29	0.0	T1-3
Autum Gold	SC	27/06	72-48	53.7	12.10	0.91	13.30	0.0	T1-3
Bahianinha	SC	06/04	88-56	53.0	10.20	1.02	10.00	0.0	T6-7
Bahianinha	SC	21/04	88-64	55.5	11.00	0.92	11.96	0.6	T5-6
CaraCara	CC	06/04	72-40	50.7	9.10	0.79	11.52	0.0	T1-2
CaraCara	CC	21/04	72-40	51.6	9.30	0.74	12.57	0.0	T1-2
Dream	CC	06/04	64-40	50.7	10.75	0.82	13.11	0.0	T7
Dream	CC	21/04	64-48	51.7	9.90	0.79	12.53	0.0	T6
Fukumoto	CC	06/04	64-40	48.4	9.10	0.54	16.85	0.0	T5-6
Fukumoto	CC	21/04	56-40	46.5	9.50	0.52	18.27	0.0	T4-5
Powel Summer	CC	31/05	64-56	52.8	11.10	1.00	11.10	0.0	T1-3
Powel Summer	CC	27/06	88-48	54.0	11.50	0.93	12.37	0.0	T1-2
Powel Summer	SC	31/05	72-48	49.2	11.90	0.83	14.34	0.3	T1-2
Powel Summer	SC	27/06	64-40	52.3	12.50	0.87	14.37	0.0	T1-2
Tule Gold	CC	06/04	72-56	54.5	8.70	0.73	11.92	0.0	T6-7
Tule Gold	CC	21/04	72-40	54.0	9.10	0.69	13.19	0.0	T6-7

6.3.14 **Evaluation of navels in the intermediate inland area**
Experiment 74B by J. Joubert (CRI)

Opsomming

Winsgewendheid moet verhoog word deur boomproduksie (oes- en vruggrootte), pakpersentasie (kraakskil en oleo weerstand, kleiner nawelente om witluis teë te werk, *Alternaria*-besmetting, windbestandheid, blomdatum, en binnedrag) en vruggehalte (skilkleur vroeg in die seisoen, sapgehalte, granulasie, lae suur) te verbeter, asook om die oes- en bemarkingseisoen (vroeë-, middel- an laatrypwordende seleksies) te verleng. Al die seleksies wat in hierdie proef ge-evalueer word het nie aan die minimum uitvoer standaarde voldoen nie. Die groot hoeveelheid reën wat laat in die seisoen ontvang is, kan moontlik bydra tot die afname in kwaliteit van die vrugte. Die proef sal nog vir een jaar ge-evalueer word, waarna geldige gevolgtrekkings gemaak kan word.

Introduction

To optimise profitability by improving productivity (fruit set and size); packout percentage (creasing and oleo resistance, smaller navel ends to counter mealybug, *Alternaria* infection, less wind prone – time of flowering and inside bearing), fruit quality (rind colour early in the season, juice quality, granulation, low acidity) and extend the harvest and marketing season (early- mid and late maturing selections).

Materials and methods

Field evaluations and laboratory analysis were conducted on Atwood, Cara Cara, Dream, Fukumoto and Tule Gold selections at Moosrivier Estate (Marble Hall), an intermediate production area.

Internal quality data were compared with the minimum average export requirements for navels: 48% Juice; 9.7% TSS; 1.5% (Max) - 0.68% (Min) % Acid; 8:1 Ratio; Colour set 34 no.T3 shipped at 11°C.

Table 6.3.14.1. List of Navel selections evaluated at Moosrivier Estate (Marble Hall) during 2006.

Selection	Rootstock	Tree age	No. of trees
Atwood	CC	2001	5
Cara Cara	CC	1997	Semi-Com.
Dream	CC	2001	9
Fukumoto	CC	2001	3
Tule Gold	CC	2001	9

Results and discussion

Atwood
Atwood produced an average yield with medium to large fruit size (count 72-40). Internally the low acid (0.59%) and juice (48.2%) content by the time of harvest didn't comply with the minimum export standards. Atwood had the best taste (sweet) in comparison to Dream and Fukumoto. Maturity middle of May.

Cara Cara
A semi-commercial orchard was evaluated at Moosrivier Estates (Marble Hall) in Mpumalanga during the 2006 season. Internally the fruit developed some granulation problems, with a very low acid (0.45%) and juice (47.5%) content by the time of harvest, similar to Atwood. Cara Cara produced an excellent fruit size this season between count 64 and 40. Maturity seems to be middle of May.

Dream
Dream produced a medium to large (count 64-40) fruit size on the trees with an average yield. The juice (46.5%) and acid (0.60%) content was below the export standard by the time of harvest. Early in the season when there was a delay in the external colour (T5-6), the internal quality complied with the minimum export standards. Degreening the fruit at this point in production might be a very valuable decision. Maturity seems to be end of April.

Fukumoto
The trees produced a large fruit size from count 64 to 40. There was a delay in the external fruit colour (T4-6) by the time of harvesting. The internal quality did not meet the export standards with low juice (42.8%) and acid (0.40%) content, as well as low Brix^{o} (8.7). Maturity middle of April.

Tule Gold
Internally Tule Gold produced fruit that did not comply with the export standards. The juice (47.3%) and acid (0.54%) content in the fruit tested, was below the export minimum. The fruit size seems promising and measured between count 72 and 40. Maturity middle of May.

Conclusions and recommendations

The production and internal quality improved in comparison with the 2005 season, but all of the selections did not comply with the export specifications. Professional irrigation practices and control of the quantity of water applied was very difficult because of late rainfall in the season. This factor does have a direct impact on the internal quality of the fruit produced. Evaluations will continue.

Table 6.3.14.2. Internal fruit quality data for Navel selections in the intermediate inland area (Moosrivier Estate, Marble Hall) during the 2006 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix º	Acid %	Ratio	Ave. seed	Colour
Atwood	CC	04/04	88-48	52.7	9.50	0.67	14.18	0.1	T7
Atwood	CC	24/04	64-40	49.6	9.70	0.66	14.70	0.8	T5-6
Atwood	CC	17/05	72-40	48.2	10.70	0.59	18.14	0.9	T1-4
CaraCara	CC	24/05	56-40	50.1	9.60	0.46	20.87	0.0	T1-2
CaraCara	CC	17/05	64-40	47.5	10.40	0.45	23.11	0.0	T1-2
Dream	CC	04/04	64-48	48.2	9.00	0.63	14.29	0.0	T6
Dream	CC	24/04	72-48	49.2	9.60	0.65	14.77	0.1	T5-6
Dream	CC	17/05	64-40	46.5	9.60	0.60	16.00	0.1	T1-3
Fukumoto	CC	04/04	64-40	45.1	8.30	0.50	16.60	0.8	T6
Fukumoto	CC	24/04	56-40	42.8	8.70	0.40	21.75	0.2	T4-6
Tule Gold	CC	04/04	88-56	35.7	9.20	0.52	17.69	0.0	T6
Tule Gold	CC	24/04	72-40	50.2	9.90	0.55	18.00	0.0	T6-7
Tule Gold	CC	17/05	64-48	47.3	10.20	0.54	18.89	0.1	T2-4

6.3.15 **Evaluation of Valencia selections in the inland areas (Onderberg)**

Experiment 75A by J. Joubert (CRI)

Opsomming

Winsgewendheid moet verhoog word deur interne gehalte (saadloosheid, veselsterkte, sapgehalte) te verbeter; beter boomproduksie en vruggrootteverspreiding (telling 48, 56 en 72); beter skilkleur, uitwendige voorkoms en skilbaarheid; verleng plukseisoen deur vroeg- (Junie/middel Julie) en laatrypwordende seleksies (vir warm streke). Meeste van die seleksies toon baie potensiaal in hierdie proef. Dis veral Alpha en Turkey wat besonders goed gevaar het hierdie seisoen. Ruby Valencia het moontlik ’n af seisoen gehad, met klein groen vrugte tot en met oes. Daar word nog geen tekens van onverenigbaarheid opgemerk by Turkey op CC nie, en die entlas verbinding lyk gesond.

Introduction

To optimise profitability by improving internal fruit quality (seedlessness, fibre toughness, juice quality); improving productivity and fruit size distribution (count 48, 56 and 72); improving rind colour, external appearance and peelability; extending the picking period by developing early (June / mid July) and late maturing selections (for hot areas).

Materials and methods

Field evaluations and laboratory analysis were conducted on Alpha (Rietspruit), Glen Ora Late, Maritz Early, McClean SL, Midknight, Ruby Valencia and Turkey (control) at Esseleen Nursery, Malelane.

Table 6.3.15.1. Internal fruit quality data was compared with the minimum export requirements for Valencia types.

Variety	% Juice	% TSS	Min % Acid	Max % Acids	Ratio	Colour
Valencia	48	9.75	0.68	1.6%	8.5:1	Colour plate 3 of set no. 34
Midknight	52	10.5	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34
Delta Seedless	52	10.5	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Interim internal fruit quality standards.

Table 6.3.15.2. List of Valencia selections evaluated at Esselen Nursery (Malelance) during 2006.

Selection	Rootstock	Tree Age	No. of trees
Alpha (Rietspruit)	CC	1996	1
Glen Ora Late	CC	2000(Top)	3
Maritz Early	CC	2002	1
McClean SL	TB		1
Midknight	C35	1998/2003(Top)	1
Ruby Val	CC		1
Turkey	C35	1998	1

Results and discussion

Alpha
Alpha produced a good yield with to medium to large (count 72-40) fruit size on the trees. The rind texture of the fruit was smooth and fairly thin, but peels quite easy. Internally Alpha produced fruit that comply with all the export minimum standards by the time of harvest (juice 59.8%, Brix º 11, acid 1.05%). Maturity seems to be middle of June.

Glen Ora Late
Trees were evaluated at Esselen nursery (Malelane) in Mpumalanga during the 2006 season. The trees produced an excellent yield with a medium to large fruit size (count 64-48). Internally the fruit comply with all the export standards. The seed content was very low at 0.1 seed per fruit. Maturity end of June.

Maritz Early
There was a large variation in fruit size from small to large (count 88-48). Maritz Early produced a poor yield on the trees and there was not enough fruit to conduct a second evaluation. The internal quality looks promising and complies with all the minimum export standards. Maturity middle to end of June.

Mc Clean SL
There was not enough fruit to do the evaluations.

Midknight
Midknight produced a very good yield with large to very large fruit size (count 56-36). Internally the juice content (59.6%) complies with the export standards, but unfortunately not the Brixº (8.7) and acid content (0.84%). Maturity middle of June.

Ruby
The tree produced small green fruit with a pale yellow internal colour. There were no sample fruit available to do the evaluations. The acid contends remained very high, as well as the intense external green colour of the fruit.

Turkey
Turkey produced an excellent crop on the trees with good internal quality. The fruit complied with all the export standards. Internally the Brixº was impressively high at 11.4. There were no incompatibility signs visible at the bud union between Turkey and Carizzo citrange. Maturity end of May.

Conclusions and recommendations

All the selections evaluated complied with the export standards, except for Midknight with a low Brixº. The maturity time of the fruit for this season was 2 to 3 weeks earlier in comparison to the previous season. Evaluations will continue.

Table 6.3.15.3. Internal fruit quality data for Valencia orange selections at Esselen Nursery (Malelane) during the 2006 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix º	Acid %	Ratio	Ave. seed	Colour
Alpha	CC	01/06	72-40	59.9	10.40	1.04	10.0	5.0	T1-3
Alpha	CC	22/06	72-48	59.8	11.00	1.05	10.5	7.0	T1-2
Glenora Late	CC	22/06	64-48	58.9	10.00	1.32	7.6	0.1	T5
Maritz Early		01/06	88-48	50.9	9.10	1.27	7.2	0.4	T2-3
Midknight	C35	01/06	56-36	59.8	8.80	0.90	9.8	0.2	T2-3
Midknight	C35	22/06	56-40	59.6	8.70	0.84	10.4	0.2	T1
Turkey	CC	01/06	88-56	56.7	11.40	0.94	12.1	9.1	T1-2
Turkey	CC	22/06	72-40	57.4	11.20	0.76	14.7	7.4	T1

6.3.16 **Evaluation of Valencia selections in the hot inland areas (Swaziland)**

Experiment 740A by J. Joubert (CRI)

Opsomming

Winsgewendheid moet verhoog word deur interne gehalte (saadloosheid, veselsterkte, sapgehalte) te verbeter; beter boomproduksie en vruggrootteverspreiding (telling 48, 56 en 72); beter skilkleur, uitwendige voorkoms en skilbaarheid; verleng plukseisoen deur vroeg- (Junie/middel Julie) en laatrypwordende seleksies (vir warm streke). Die bome is nog jonk en is vir die eerste keer ge-evalueer. Die produksie en kwaliteit van die vrugte sal nou begin toeneem, insluitend die algemene vruggroote. Alpha het klaar belowend gevaar, met meeste van die ander seleksies wat nog nie aan die uitvoer standaarde voldoen het nie.

Introduction

To select Valencia cultivars with improved and consistent productivity, fruit size, rind colour, peelability and internal fruit quality (seedlessness, ratio), and extended harvest period (both earlier and later maturity). To describe the cultivar characteristics of new Valencia cultivars and to determine the climatic suitability of these cultivars in a hot production region.

Materials and methods

Field evaluations and laboratory analysis were conducted on Alpha (Rietspruit), Delta, McClean SL, Mouton Early, Portsgate, Ruby Valencia and Tambuti Early at Tambuti Esatate, Swaziland.

Table 6.3.16.1. Internal fruit quality data was compared with the minimum export requirements for Valencia types.

Variety	% Juice	Brixº	Min % Acid	Max % Acids	Ratio	Colour
Valencia	48	9.75	0.68	1.6%	8.5:1	Colour plate 3 of set no. 34
Delta Seedless	52	10.5	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

Results and discussion

Alpha

Alpha produced a poor to average yield on the trees with medium to large fruit size (count 88-56). The trees are situated close to the fence, and theft might be a good possibility. There was some red scale present on the fruit by the time of harvest. The internal quality complies with the export standards, and the juice content (56.1%) was promising. Alpha produced a nice round fruit shape, with medium course external rind. Maturity Middle of June.

Delta

Trees were evaluated at Tambuti Estate (Swaziland) during the 2006 season. Delta produced a poor to average crop on the trees. The reason for this might have been high temperatures during fruit set periods on the trees. The acid content (0.77%) was below the minimum export requirements for Deltas. Maturity end of June, first week of July.

McClean SL
McClean SL also produced a poor to average yield on the trees, with medium to large fruit size (count 88-48). Internally the acid content (0.66%) of the fruit tested very low by the time of harvesting, although the juice content (57%) seems promising. Evaluations will continue. Maturity middle of June.

Mouton Early
There was a delay in the external colour of the fruit by the time of harvest. The internal quality analysis proved that the fruit was over matured internally, while the external colour was still between T5 and 6. Large numbers of red scale was counted on the rind of the fruit. Maturity seems to be first week of June.

Portsgate
Portsgate produced a very low acid content (0.65%) early in the season, while the external colour was between T5-6. The Brixº (9.6) was also a border case and just below the export minimum standards. Production on the trees was good, with medium to large fruit size (count 88-56). Maturity middle of June.

Ruby Valencia
In comparison with the trees at Esselen nursery, these trees produced an average to good crop with medium to large fruit size (count 88-56). The average number of seeds per fruit varied between 4.9 and 6.2. The internal quality seems promising, with only the Brixº (9.5) not complying with the export standards. Maturity end of June.

Tambuti Early
Trees were evaluated at Tambuti Estate (Swaziland) during the 2006 season. The juice contends (56.9%) comply with the export standards, but the Brixº (9.6) and acid content (0.87%) was on the low side. The external colour was between T3-4 by the time of harvesting. Maturity seems to be first to second week of June.

Conclusions and recommendations

Please bear in mind that 2006 was the first season to evaluate the trees. The production and fruit size will increase on the trees. Evaluations will continue.

6.3.17 Evaluation of Lemon selections in the inland areas

Experiment 79 by J. Joubert (CRI)

Opsomming

Kouegeharde, doring- en saadlose suurlemoenseleksies (met aanvaarbare vruggrootte), wat met 'n wye reeks onderstamme verenigbaar is, moet ontwikkel word. Die oesseisoen moet verleng word om aaneenlopende produksie van Maart tot September te verseker, aangesien dit vroeë en laatrypwordende seleksies insluit. Probleme wat met lang blomtyd ge-assosieer kan word, moet verminder word. Goeie vrugkwaliteit (kleur, skildikte, sapinhoud) moet behou word. Die boomeienskappe en prestasie van nuwe kultivars moet met die kommesieel gekweekte Eureka vergelyk word om te bepaal of hulle aan bogenoemde doelwitte voldoen. Villafranca produseer steeds die laagste saad telling per vrug, gevolg deur Verna. Die produksie per boom is vir hierdie seisoen bepaal. Limoneira het die beste ous op die bome geproduseer met 151kg, gevolg deur Fino 49. Evaluasies sal nou op heirdie proef gestaak word. Eureka saadloos (LNR) bly steeds die beste saadlose suurlemoen seleksie tans beskikbaar, maar Limoneira produseer 'n hoër oest met heelwat saad.

Introduction

To develop cold hardy, thornless, seedless (with acceptable fruit size) lemon selections which are compatible with a wider rootstock range; to extend the picking period to ensure continuity of supply from March to September, i.e. early and late maturing selections; to reduce the problems associated with protracted flowering; to maintain high fruit quality (colour, rind thickness, juice content).

Materials and methods

Field evaluations were conducted on Eureka SL (ARC) as control, Eureka SL (Israel), Fino 49 & 95, Genoa, Limoneira 8A, Lisbon (control), Verna and Villafranca SL on various rootstocks.

Table 6.3.17.1. List of lemon cultivars evaluated at Tekwane (Karino area) during the 2006 season.

Selection	Rootstock	Tree Age	No. of trees
Eureka SL (ARC) *	RL	2000	1
Eureka SL (Israel)	RL	1998	4
Fino 49	RL	1998	3
Fino 95	RL	1998	4
Genoa	RL	1998	4
Limoneira 8A	RL	1998	2
Lisbon	RL,SO	1998	2,2
Verna	RL	1998	4
Villafranca	RL	1998	2

* Esselen Nursery, Malelane

Results and discussion

Eureka SL (ARC)
Good production of seedless fruit at Esselen Nursery.

Tekwane Estates
This season Villafranca produced 0.2 seeds/fruit, followed by Verna with 2.3 seeds per fruit. Limoneira produced the highest seed count per fruit (18.4 seeds/fruit). Lisbon on SO produced the highest juice content (36.5%) followed by Villafranca (36.4%) and Fino 95 (36.3%). The lowest juice content analysed for this trial was Fino 49 with 31.5%.

This season the best crop was set by Limoneira on RL with 151.3 kg/tree, followed by Fino 49 (116.5kg/tree) and Eureka SL (IR) with 103.3 kg/tree. The lowest crop was set on Verna with 46.5 kg/tree.

Conclusions and recommendations

Limoneira produced an excellent crop this season with high juice content. The selection produced a highest number of seeds per fruit. Villafranca and Verna had relatively low seed count compared to the other lemon cultivars.

Table 6.3.17.2. Internal fruit quality data for Lemons from Tekwane Estate (Karino) on 7 April 2006.

Selection	Root-Stock	Juice %	Ave. seed
Eureka seedless(IR)	RL	31.9	12.6
Fino 49	RL	31.5	16.1
Fino 95	RL	36.3	18.2
Genoa	RL	34.9	17.0
Lisbon	RL	34.0	17.9
Lisbon	SO	36.5	14.0
Limoneira	RL	34.6	18.4
Verna	RL	32.1	5.1
Villafranca	RL	36.4	0.2

Table 6.3.17.3. Fruit size distribution of Lemons on different rootstocks at Tekwane Estate (Karino) during the 2006 season.

Cultivar	Rootstock	Kg/tree
Eureka seedless(IR)	RL	103.3
Fino 49	RL	116.5
Fino 95	RL	57.1
Genoa	RL	76.6
Lisbon	RL	73.8
Lisbon	SO	60.3
Limoneira	RL	151.3
Verna	RL	46.5
Villafranca	RL	74.2

6.4 PROJECT: ROOTSTOCK EVALUATION

6.4.1 Project summary

Commercial rootstock choice is relatively limited, and the best available rootstock option is seldom ideal in addressing all the site limitations and production and marketing requirements. The development of a new rootstock is inherently a long and involved process, and it is unlikely that any new rootstock will have all the desirable attributes.

One of the prime objectives of rootstock evaluation is to find reliable size-controlling rootstocks coupled with attributes such as good yield of marketable fruit size and internal fruit quality, pest and disease tolerance or resistance, and adaptability to a wide range of scion cultivars and soil types.

The rootstock research efforts of the 1980s and 1990s led to considerable changes in rootstock use from almost exclusively being rough lemon to Carrizo and Troyer citranges and Swingle citrumelo rootstocks. Yet, there still remains an acute need to seek out, evaluate and commercialise new generation rootstocks.

Projekopsomming

Die oogmerk van die onderstamprojek is om ’n bron van sitrusonderstamme te evalueer i.t.v. die produksie van hoë kwaliteit vrugte, asook siektebestand- en verenigbaarheid met verskillende grond- en klimaatstoestande regoor Suidelike Afrika. Oesopbrengs per hektaar moet geoptimaliseer word deur van bostam/onderstamverenigbarheid, tuinboukundige prestasie te verbeter en beter interne kwaliteit, vruggrootte en produksie te induseer. Dit is daarom noodsaaklik dat produsente die beste moontlike keuse maak wanneer ’n onderstam gekies word, aangesien dit direkte invloed op beleggingsopbrengs sal hê.

Rootstock evaluation guidelines

Overall evaluation objectives: Rootstock selection and evaluation serves two principle purposes, namely,

1. to minimise the effects of site limitations (soil type, irrigation water quality, disease presence), and
2. to enhance yield, fruit size and fruit quality.

Rootstock choice should aim to capitalise on any favourable soil or environmental factor and/or offset the effects of any limiting factors.

However, not all rootstocks can or will be evaluated under all possible conditions, and a degree of risk in rootstock choice will probably always exist. Therefore, the role of rootstock evaluation is to screen new rootstocks to identify their strengths and weaknesses, and thereby minimise the potential commercial risks involved in their commercial use.

Once initial screening has been conducted, citrus producers will be encouraged to further evaluate the performance of the most promising rootstocks on a semi-commercial basis.

Guiding principles for rootstock evaluations

1. Rootstock evaluations will be conducted in the major citrus-producing regions for each cultivar group using a mainline commercial cultivar (since rootstock evaluation is largely not dependent on climate). Information generated from such evaluations will, by necessity, be extrapolated to other regions or sites and to other similar cultivars.

2. Screening-type trials will be conducted with the emphasis on potential performance in terms of scion compatibility, canopy development, productivity (annual and cumulative yield potential, production efficiency), product quality (fruit size distribution, rind colour and texture, internal fruit quality), general susceptibility to rind disorders, pest and disease susceptibility, and general soil suitability.
3. A single, mainline scion cultivar on numerous rootstocks will be used, and factorial type experiment designs will be avoided. The commercial or standard scion-rootstock combination will serve as the control for comparison purposes.
4. Suitable nursery and grower cooperators are essential.
5. Use budded trees of the same (or similar) age.
6. Replicate 6 to 10 times.
7. Randomisation is not required in a screening type trial.
8. Site selection: uniform soil type, not near a windbreak, but preferably within a commercial orchard.
9. Conduct soil and site analysis, including chemical, physical and disease status of soil.
10. When the trial is discontinued, then a motivation is needed for further evaluation, e.g. to evaluate a few of the most promising rootstocks every two years, and orchard maps must be retained for subsequent evaluation and long-term performance and evaluation of disease tolerance.

Abbreviations used in text

SYMBOL	ROOTSTOCK
1. AT	Australian trifoliate
2. BC	Benton citrange
3. C	Calamandarin
4. CA	C. amblycarpa
5. CC	Carrizo citrange
6. CM	C. macrophylla
7. ChM	Changsa mandarin
8. CLM	Cleopatra mandarin
9. CO	C. obovoideae
10. C32	C32 citrange (trifoliate orange x Ruby sweet orange)
11. C35	C35 citrange (trifoliate orange x Ruby sweet orange)
12. C61	Sunki x macrophylla
13. FD	Flying Dragon
14. FF6	Sunki x MTO trifoliate orange
15. F80/3	F80/3 citrumelo
16. F80/9	F80/8 citrumelo
17. GT	Gou Tou
18. HRS 802	Siamese pummelo x trifoliate orange
19. HRS 809	Changsa x English large flowered trifoliate orange
20. HRS 812	Sunki mandarin x Beneke trifoliate orange
21. IRL	Indian rough lemon
22. JC	Japanese citron
23. JT	Jacobsen trifoliate
24. K	Konejime
25. KC	Koethen citrange
26. ML	Milan lemon
27. MXT	Minneola x trifoliate
28. N	Natsudaidai
29. O	Orlando tangelo

SYMBOL		ROOTSTOCK
30.	PT	Pomeroy trifoliate
31.	RC	Rusk citrange
32.	RL-C	Rough lemon Cairn
33.	RL-S	Rough lemon Schaub
34.	RL-W	Rough lemon Wallace
35.	RP	Rangpur lime
36.	RT	Rubidoux trifoliate
37.	RXT	Rangpur x Troyer
38.	SC	Swingle citrumelo
39.	SCS	Sun chu sha
40.	SFS	Smooth flat Seville
41.	SM	Shekwasha mandarin
42.	SO	Sour orange
43.	ST	Sampson tangelo
44.	Sunki 1112	Flying Dragon x Sunki (1112)
45.	Sunki 1113	Flying Dragon x Sunki (1113)
46.	Sunki 1116	Flying Dragon x Sunki (1116)
47.	TB	Terrabella
48.	TC	Troyer citrange
49.	Volk	Volkameriana
50.	X639	Cleopatra mandarin x trifoliate
51.	YC	Yuma citrange
52.	61 AA3	Cleopatra mandarin x *P. trifoliata*
53.	75 AB 12/13	McCarthy grapefruit x *P. trifoliata*
54.	79 AC 6/2	Cleo x Swingle citrumelo

6.4.2 **Project summary: Cape and Inland areas**

Genoa lemon in Citrusdal: The objective of the trial is to evaluate the performance of the Genoa lemon in the Citrusdal areas well as the performance of Genoa on different rootstocks. This was the fifth year of production, the first year of good yields with larger fruit size than last season, although the fruit were harvested later. Volckameriana, Rough lemon and Trifoliate X had the largest tree size, Rangpur lime and Benton citrange the smallest. Volckameriana had by far the highest yield followed by Japanse sitroen, Rough lemon and Rangpur, which were similar. Benton had the poorest yield, followed by Swingle citrumelo and Minneola x Trifoliate. Rough lemon and Volckameriana had the largest fruit size while Minneola x Trifoliate and Trifoliate x Sweet orange had the smallest but most desirable size. All rootstocks had good juice percentages, easily meeting the minimum export standards. Trifoliate x Sweet and Rough lemon had slightly earlier fruit colour, Japanse sitroen, Carrizo citrange and Minneola x Trifoliate slightly later. Swingle and Minneola x Trifoliate had the most high shoulders, Benton, Rough lemon and Carrizo the least. There was virtually no oleocellosis. Minneola x Trifoliate had the most non exportable fruit due to wind scarring. Swingle had the most severe bud union bench while Trifoliate x Sweet orange was slightly inverted. Rangpur and Japanse Sitroen had nodules and sucker growth on the rootstock and Benton occasional sucker growth. Evaluations to continue for one more season.

Delta Valencia in Marble Hall: To evaluate the performance of Delta Valencia on 42 different rootstocks in replant soils, over an eight-year production cycle in the Marble Hall area. There was sufficient water available for normal irrigation practices and the problem with the blocked irrigation line was fixed. The production on all the rootstocks increased promisingly compared to the previous seasons. The internal quality decreased slightly, with a lower Brix^{o} on all the combinations (below export minimum). This will be the final season for evaluations on the trial.

Midknight and Delta Valencias in Letaba: To evaluate the performance of different rootstocks on replant soils in an intermediate citrus production area. There was a decrease in production for the Delta Valencia trees. The internal quality of almost all the combinations did not comply with the minimum export standards. The average fruit size produced for this season increased by one count. RL-C produced the best yield for the 2006 season (66.8kg/tree). The Midknight trees produced a smaller fruit size this season. Most of the selections evaluated did not comply with the export standards, producing too low Brix°. Generally the trees produced a better yield in comparison with the 2005 season. Both the selections will be evaluated for the final time in the 2007 season.

Star Ruby grapefruit at Letaba: To evaluate the performance of different rootstocks on replant soils in an intermediate citrus production area. Please take the water problems in consideration when interpreting the following trial data. At one stage the trees were only irrigated with 100L per week (December). Fruit size was smaller for most of the combinations in comparison with the previous season. The average fruit size peaked at count 64. Star Ruby on Swingle citrumelo produced the best yield with 106.2 kg/tree.

Navels and Valencias in Vaalharts: The performance of Navel and Valencia selections on different rootstocks is being be investigated to determine the best rootstock for the Vaalharts area. Both Bahianinha and Royal Late trees recovered well after the cold damage of 2004. This season Bahianinha produced an average yield with medium fruit size. The internal quality of the fruit complied with the minimum packing specifications required. Royal Late produced a lighter crop with smaller fruit size. The internal quality looks promising with high Brixº and acceptable acid levels. The Valencia trees recovered well after the cold damage of 2004. The average fruit size was on the small side between count 144 and 88. Yield was low, but will increase for the next production season. There is potential for improvement in internal quality, because most of the combinations did not comply with the export standards. Evaluations will continue for 2007.

Valencias in Malelane: The performance of Midknight and Delta Valencias on new, imported rootstocks on replant soils must be investigated. The production, internal quality and rind colour must be improved and at the same time fruit size must be increased. The production on all the rootstock was lower in comparison with the 2005 season. There was a slight increase in fruit size, but none of the combinations complied with the export minimum standards (lower Brixº). This might be an off season and 2007 will bring possible explanations for the scenario.

Grapefruit in Swaziland: The performance of grapefruit cultivars on new, imported rootstocks on heavy, replant soils must be investigated. The production, fruit size, internal quality and rind colour must be improved. Star Ruby and Marsh produced a good crop after being transplanted from Tabankulu Estates. The average fruit size remained the same and peaked at count 48 for Star Ruby and count 40 for Marsh, in comparison with the 2005 season. Both selections complied with the export standards for juice content, but unfortunately not for the Brixº. The new plantings establish well and the trees produced enough fruit for evaluations. The internal quality looks very promising and all the combinations comply with the packing specs.

Valencias in Komaitpoort: Evaluate and assess the horticultural performance and capability of various new Valencia selections on different rootstocks. Determine the superior rootstock combinations for these new selections, and be able to make credible commercial recommendations. This rootstock trial was harvested for the second time this season and keep in mind the trees are still young. The difference in yield production increased compared with 2005 and valuable information is available. Capital investment to establish an orchard will bring in returns via early production. The highest increase in production from 2005 to 2006 was for Midknight, taking the young tree age in consideration. McClean SL produced the highest yield compared with the other cultivars.

Projekopsomming: Kaap- en Binnelandsestreke

Genoa suurlemoen te Citrusdal: Die doel van die proef is om die prestasie van Genoa suurlemoen in die Citrusdal area te beproef, asook die prestasie op tien verskillende onderstamme. Dit was die vyfde jaar van produksie (die eerste goeie oes) met groter vrugte as die vorige seisoen (vrugte was later geoes). Volckameriana, Growweskilsuurlemoen and Trifoliate X het die grootste bome gehad, Rangpur lemmetjie en Benton citrange die kleinste. Volckameriana het die meeste gedra, gevolg deur Japanse sitroen, Growweskilsuurlemoen en Rangpur. Benton het die swakste oes gehad, gevolg deur Swingle citrumelo en Minneola x Trifoliate. Growweskilsuurlemoen en Volckameriana het die grootste vruggrootte gehad, Minneola x Trifoliate en Trifoliate x Sweet orange die kleinste, maar meer gewenste vruggroote. Al die onderstamme het maklik die sap uitvoerstandaarde behaal. Trifoliate x Sweet orange en Growweskilsuurlemoen het effens vroeër vrugkleur gehad, Japanse sitroen, Carrizo citrange en Minneola x

Trifoliate effens later. Swingle en Minneola x Trifoliate het die meeste hoër skouers gehad, Benton, Growweskilsuurlemoen en Carrizo die minste. Daar was baie min oleo. Minneola x Trifoliate het die meeste nie uitvoerbare vrugte weens windletsels gehad. Swingle het 'n tipiese bankagtige entverbinding gehad terwyl Trifoliate x Sweet orange effens omgekeerd was. Rangpur en Japanse sitroen het knoppies en suiers op die onderstamme gehad en Benton enkele suiers. Evaluasies moet vir nog een seisoen voortgaan.

Delta Valencia te Marble Hall: Die prestasie van Delta Valencia op 42 verskillende onderstamme in herplant gronde, moet oor 'n agt jaar produksietyd in die Marble Hall omgewing geëvalueer word. Daar was voldoende water in die opgaardamme vir effektiewe besproeiing en 'n probleem met die pyplyn is opgeklaar. Die produksie by meeste van die onderstamme het baie toegeneem, maar die interne kwaliteit, veral Brixº het gedaal onder die minimum uitvoer standaard perke in. Hierdie proef sal vir nog een seisoen ge-evalueer word.

Midknight en Delta Valencias te Letaba: Die prestasie van verskillende onderstamme op herplant grond is in 'n intermediêre sitrusproduksie gebied geëvalueer. Hierdie seisoen het die ernstige water tekorte weer baie produksie probleme opgelewer. Die Delta valencias se gemiddelde oes wat geproduseer is, het gedaal. Die interne kwaliteit van meeste kombinasies het nie aan die minimum uitvoer vereistes voldoen nie. Volgens die verskillende vruggroottes wat geproduseer was, het die vrugtelling een vlak grootter opgeskuif. RL-C het die hoogste produksie per boom behaal vir hierdie seisoen (66.8%). Die Midknight bome se vruggroote het effens gedaal vir die 2006 seiseon. Meeste van die seleksies het ook nie aan die uitvoerstandaarde voldoen nie, met te lae Brixº. Die algemene produksie van die bome het gestyg in vergelyking met die 2005 seisoen. Albei hierdie seleksies sal die laaste keer vir 2007 ge-evalueer word.

Star Ruby pomelo te Letaba: Die prestasie van verskillende onderstamme op herplant grond is in 'n intermediêre sitrusproduksie gebied geëvalueer. Neem asseblief die water probleme in ag wanneer die inligting van die volgende proef geintepreteur word. Op een stadium was die bome slegs 100L per week toegedien (Desember). Die vruggrootte het kleiner geword by meeste van die kombinasies vir hierdie seisoen. Die algemene vruggrootte het 'n piek bereik by telling 64. Star Ruby op Swingle citrumelo het die beste oes geproduseer met 106.2 kg/boom.

Navels en Valencias te Vaalharts: Die prestasie van Nawel en Valencia variëteite op verskillende onderstamme ondersoek en die beste onderstam vir die betrokke seleksie te bepaal in die Vaalharts omgewing. Bahianinha en Royal Late bome het goed herstel na die koue skade. Hierdie seisoen het die Bahianinha bome gemiddelde oeste geproduseer, met medium vruggrootte. Die interne kwaliteit van die vrugte by meeste van die onderstamme het aan die uitvoer standaarde voldoen. Royal Late het het ligter oeste geproduseer, met kleiner vruggroottes. Die interne kwaliteit het belowend gelyk met hoë Brixº en aanvaarbare suur vlakke. Die proef sal weer in 2007 ge-evalueer word. Die Valencia bome het goed herstel na die koue skade van 2004. Die algemene vruggrootte wat op die bome geproduseer was, het gewissel tussen telling 144 en 88. Oor die algemeen was die produksie laag, maar sal verseker toeneem met die volgende seisoen se evaluasie. Intern is daar baie potensiaal vir verbetering, want meeste van die seleksies was nie uitvoerbaar nie. Evaluasies sal weer vir 2007 uitgevoer word.

Valencias te Malelane: Die prestasie van Midknight en Delta Valencias op nuwe, ingevoerde onderstamme op herplant grondtipes moet ondersoek word. Die produksie, interne gehalte en skilkleur moet verbeter word, terwyl vruggrootte moet toeneem. Die produksie het effens gedaal by albei seleksies met 'n geringe toename in vruggrootte. Die Brixº by al die kombinasies het nie aan die uitvoer standaarde voldoen nie (te laag). Hierdie kan 'n moontlike af jaar wees en die volgende evaluasies sal moontlike verduidelikings bied.

Pomelos te Swaziland: Die prestasie van pomelo variëteite op nuwe, ingevoerde onderstamme op swaar, herplant grondtipes moet ondersoek word. Die produksie, vruggrootte, interne gehalte en skilkleur moet verbeter word. Star Ruby en Marsh het 'n beter produksie gelewer (vestig goed) nadat die bome herplant is vanaf Tambankulu Estate. Die gemiddelde vruggrootte het dieselfde gebly op telling 48 vir Star Ruby, en telling 40 vir Marsh in vergelyking met 2005. Albei seleksies het aan die minimum uitvoer standaarde vir sap% voldoen, maak nie aan die Brixº nie. Die nuwe aanplantings het goed gevestig en is vir die eerste seisoen ge-evalueer. Intern het die vrugte baie goed gevaar en aan al die uitvoer spesifikasies voldoen.

Valencias te Komaitpoort: Evalueer en bepaal die tuinboukundige potensiaal en vermoë van verskillende Valencia variëteite op verskillende onderstamme. Bepaal die beste onderstam kombinasie vir hierdie nuwe variëteite. Maak betekenisvolle kommersïele aanbevelings vir die produsente. Hierdie onderstam proef is vir die tweede keer ge-oes en die bome is nog jonk. Die verskille in oes produksie het nou grootter geword en waardevolle inligting word beskikbaar. Die kapitaal wat uitgelê word vir vestiging kan gouer in winste omgesit word met vroeër produksie op die bome. Midknight het die grootste toename op so 'n jong ouderdom getoon, alhoewel McClean SL die hoogste produksie tussen die verskillende kultivars het vir die 2006 seisoen.

6.4.3 **Evaluation of Genoa Lemon on various rootstocks in Citrusdal**
Experiment 588 by C J Alexander (Private Contractor)

Opsomming

Die doel van die proef is om die prestasie van Genoa suurlemoen in die Citrusdal area te beproef, asook die prestasie op tien verskillende onderstamme. Dit was die vyfde jaar van produksie (die eerste goeie oes) met groter vrugte as die vorige seisoen (vrugte was later geoes). Volckameriana, Growweskilsuurlemoen and Trifoliate X het die grootste bome gehad, Rangpur lemmetjie en Benton citrange die kleinste. Volckameriana het die meeste gedra, gevolg deur Japanse sitroen, Growweskilsuurlemoen en Rangpur. Benton het die swakste oes gehad, gevolg deur Swingle citrumelo en Minneola x Trifoliate. Growweskilsuurlemoen en Volckameriana het die grootste vruggrootte gehad, Minneola x Trifoliate en Trifoliate x Sweet orange die kleinste, maar meer gewenste vruggroote. Al die onderstamme het maklik die sap uitvoerstandaarde behaal. Trifoliate x Sweet orange en Growweskilsuurlemoen het effens vroeër vrugkleur gehad, Japanse sitroen, Carrizo citrange en Minneola x Trifoliate effens later. Swingle en Minneola x Trifoliate het die meeste hoër skouers gehad, Benton, Growweskilsuurlemoen en Carrizo die minste. Daar was baie min oleo. Minneola x Trifoliate het die meeste nie uitvoerbare vrugte weens windletsels gehad. Swingle het 'n tipiese bankagtige entverbinding gehad terwyl Trifoliate x Sweet orange effens omgekeerd was. Rangpur en Japanse sitroen het knoppies en suiers op die onderstamme gehad en Benton enkele suiers. Evaluasies moet vir nog een seisoen voortgaan.

Introduction

Genoa is a newly acquired slightly earlier maturing lemon selection. The trial was established in Citrusdal to determine the performance of this selection in the area on ten different rootstocks and in so doing possibly provide an alternative to the currently planted lemon selections and different rootstock options.

Materials and methods

Genoa lemon was budded to ten different rootstocks and planted at Hexrivier, Citrusdal in January 2000 in adjacent rows. The trees were evaluated according to certain criteria, including production, sets, tree size, compatibility, fruit size, juice percentage, average seed, rind thickness, high shoulders, oleocellosis and wind scars. Commercial Genoa and Lisbon lemons on Rough lemon that form part of the orchard are included in the evaluations. The list of rootstocks and number of trees evaluated is presented in Table 6.4.3.1.

Results and discussion

The trees were evaluated on 24 April, harvested on 22 June and tree height and diameter measured on 3 July. There is some variation in the tree size and therefore only the better trees have been used for evaluation purposes – refer Table 6.4.3.1. Fruit from each rootstock were picked into bins, which were weighed in the packhouse and 50 fruit per bin measured. The commercial Genoa and Lisbon lemon trees are included in the tables but not in the discussion. The commercial Lisbon lemon results must be read with caution as it was since discovered that some of the rootstocks are mixed, Rough lemon and Rangpur lime.

Table 6.4.3.1. List of rootstocks, rootstock selection, number of trees evaluated and average tree height and diameter with Genoa lemon as scion and commercial Genoa and Lisbon lemons at Hexrivier, Citrusdal during 2006. Tree spacing 5.5 x 2.0m.

Rootstock	Selection	No of trees evaluated/ harvested	Average yield/tree (kg)	Ave. tree height (m)	Ave. tree * diameter (m)
Cairn Rough lemon	163	10	62.9	3.8	3.6
Volckameriana	575	10	80.0	3.8	3.8
Trifoliate X	1242	10	47.1	3.8	3.5
Benton citrange	980	10	32.0	3.3	3.0
Trifoliate x Sweet orange	1287	10	44.3	3.6	3.2
Japanse sitroen	184	10	64.8	3.5	3.3
Minneola x Trifoliate (M x T)	1238	10	39.2	3.4	3.5
Swingle citrumelo	715	10	37.2	3.4	3.2
Rangpur lime	225	5	62.2	3.1	3.2
Carrizo citrange	608	5	41.0	3.4	3.4
Commercial Genoa/Rough lemon		10	54.6	3.4	3.2

Commercial Lisbon/Rough lemon		6	40.3	3.6	3.3

* Trees are pruned between the rows to allow tractors to pass through.

Volckameriana had by far the highest yield and largest tree size. Rough lemon had the second largest tree size and third highest yield. Japanse sitroen had a medium tree size and the second highest yield, while Rangpur had high yields and a smaller tree size. Benton had the lowest yield with one of the smallest tree sizes.

Table 6.4.3.2. Visual evaluation of tree production and sets, tree growth and health, fruit colour in April and bud union on 24 April and 3 July, 2006. Rootstocks arranged in order of planting.

Rootstock	Production /sets	Tree growth and health	Fruit colour	Bud union and rootstock appearance
Rough lemon	Good. 1 set	Large vigorous, spreading trees, green leaves	6	Smooth, some rootstock regrowth, some fluting
Volckameriana	Good. 1 set	Large, vigorous, spreading trees, slightly paler leaves	6	Smooth, some rootstock regrowth, fluted
Trifoliate X	Good. 1 set	Paler trees, some yellow	5-6	Smooth to very slight bench, fluted
Benton citrange	Fair – good	Variable tree size, narrower shape. Some pale trees	5-6	Very slight bench, occasional shoot, fluting
Trifoliate x Sweet orange	Good. 1 set	Trees slightly more open, mostly green	6	Slightly inverted bench, slight fluting
Japanse Sitroen	Good	Dense trees, fairly green	6	Smooth, distinct paler rootstock, nodules, odd suckers, fluting
Minneola x Trifoliate	Fair – good. 1-2 sets?	Fairly green - green	6	Very slight bench, fluting
Swingle citrumelo	Good–excellent	Green to slightly yellow	6	Bench (most severe of all), slight fluting
Rangpur lime	Good–excellent 2 sets	Green	6 and 7	Smoothish, nodules, a lot of regrowth, slight fluting
Carrizo citrange	Fair – good	Slightly pale trees	6	Very slight bench, fluting
Commercial Genoa/RL	Good	Reasonably green	6	Smooth, occasional shoots, slight fluting
Commercial Lisbon/RL	Excellent?	Green	6	Smooth, occasional shoots, fluting

There were some slight differences in tree leaf colour. Rough lemon, Rangpur and Lisbon had the greenest leaves; Volckameriana, Japanse sitroen, M x T, commercial Genoa and Tri x Sweet not as green/slightly paler; Benton, Swingle and Carrizo pale and Tri X the palest to yellow. Lisbon fruit appear slightly rounder. Stem and scion diameters were not measured as the trees were allowed to branch just above the bud union in the nursery that also makes it difficult to always clearly see the bud union.

Table 6.4.3.3. Average fruit size and percentage fruit per count of Genoa lemon on various rootstocks. Rootstocks arranged according to descending fruit size.

Rootstock	Ave fruit size (mm)	Percentage fruit per count 2006										% in counts 216-113
		216	189	162	138	113	100	88	75	64	>64	
Rough lemon	67.2	1	1	2	10	9	15	17	13	24	8	23
Volckameriana	67.2	1	1		4	18	17	19	14	17	9	24
Japanse sitroen	65.5			4	7	19	22	13	18	14	3	30
Rangpur lime	65.3		4	4	4	16	14	34	12	8	4	28
Swingle citrumelo	64.4	2	1	2	14	12	20	29	11	9		31
Benton citrange	64.4	2	4	6	8	21	14	21	6	10	8	41
Trifoliate X	63.5	1	6	8	11	18	13	18	19	4	2	44
Carrizo citrange	63.3		2		12	26	28	18	10	4		40
Tri x Sweet orang	61.2	3	9	5	13	31	16	14	7	2		60
Minneola x Trifol	60.2	2	8	9	19	28	22	6	3	3		66

Comm Genoa/RL	65.3		3	3	6	19	19	12	17	14	7	31
Comm Lisbon/RL	62.7	2	8		12	23	29	14	4	2	6	45

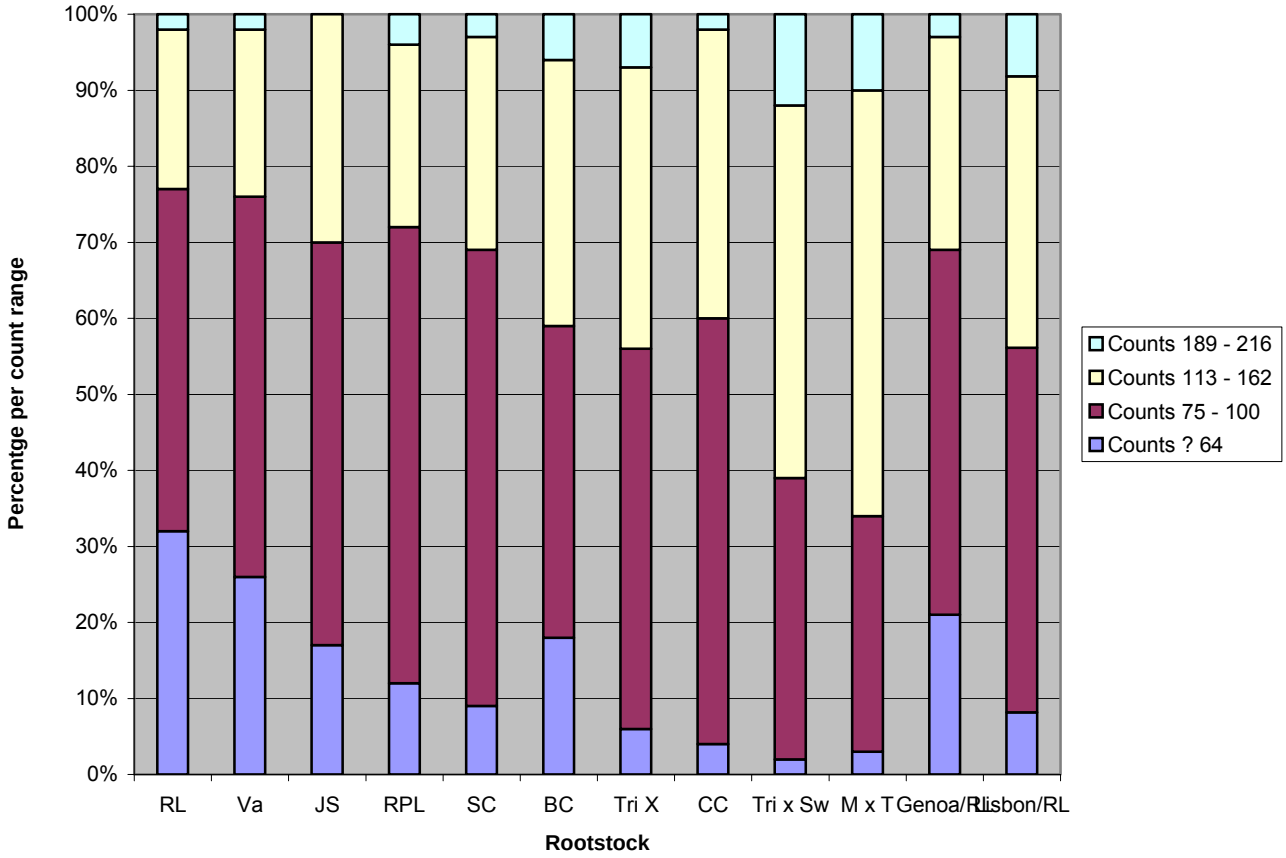


Figure 6.4.3.1. Fruit size distribution of Genoa lemon on various rootstocks per count range**.**

Last season 58 – 88 percent of the fruit fell between counts 113 – 216 whereas this season the percentage dropped to between 23 – 66. Tri x Sweet and M x T had more optimum fruit size, whereas Rough lemon and Volckameriana were too large. The trees were also harvested later than in 2005 which could also account for the larger fruit size.

Table 6.4.3.4. Average fruit size of Genoa lemon on various rootstocks over the past five seasons and colour transparency recorded on 24 June 2006. Rootstocks arranged according to descending fruit size.

Rootstock	**Average fruit size (mm)**					**Colour transparency (2006)**				
	2002	**2003**	**2004**	**2005**	**2006**	**T1**	**T2**	**T3**	**T4**	**T5**
Rough lemon	72.4	62.9	63.4	61.5	67.2	4	2	54	40	
Volckameriana	71.8	63.1	65.1	61.6	67.2	4	2	48	46	
Japanse sitroen	70.8	63.7	61.6	60.3	65.5	2	4	40	54	
Rangpur Lime	71.6	63.1	63.9	59.9	65.3	4	2	44	48	2
Swingle citrumelo	64.6	57.5	59.5	59.3	64.4		6	50	44	
Benton citrange	69.8	61.9	60.5	58.6	64.4		14	42	40	4
Trifoliate X	64.6	61.0	60.9	60.4	63.5		18	32	50	
Carrizo citrange	64.0		59.1	58.9	63.3		6	36	50	8
Tri x Sweet orange	67.6	58.8	59.1	58.4	61.2	4	14	54	20	8
Minneola x Trifoliate	63.2	57.1	57.8	58.8	60.2		8	28	58	6
Comm Genoa/RL			64.9	60.0	65.3	6	10	30	32	22
Comm Lisbon/RL			61.5	59.2	62.7	2	6	34	46	12

The average fruit size over all the rootstocks was slightly larger than 2005 by 4.4 mm. Rootstocks with smaller fruit had a lesser shift in fruit size between the two seasons (trees harvested later). Tri x Sweet had the earliest colour while Japanse sitroen, Carrizo and M x T were later colouring.

Table 6.4.3.5. Test sample size and colour, juice percentage (tested 24 June 2002, 13 May 2003, 12 May 2004, 26 April 2005 and 22 June, 2006), average seed counts and rind thickness of the Genoa lemon on various rootstocks. Rootstocks arranged according to descending juice percentage in 2006.

Rootstock	Sample Count	Sample colour	Juice percentage per year					Average seed per fruit	Rind thickness (mm)
	2006	2006	2002	2003	2004	2005	2006	2006	2006
Carrizo citrange	88	2-3	44.4		47.3	51.1	50.8	2.2	5.6
Rangpur lime	88	3	46.7	47.0	48.3	52.6	50.4	4.9	5.2
Benton citrange	88	2-3	47.4	45.4	49.0	48.6	50.2	4.8	5.6
Swingle citrumelo	88	3	45.0	43.8	46.5	41.8	49.7	1.6	5.8
Trifoliate X	88	3	42.9	44.3	47.9	50.4	49.5	3.0	5.7
Cairn Rough lemon	88	3	46.0	45.2	45.6	53.7	49.3	4.3	5.9
Volckameriana	88	3	45.2	43.0	47.6	49.0	48.9	3.1	6.0
Minneola x Trifoliate	88	3-4	45.0	43.0	47.3	49.3	48.7	2.5	5.0
Japanse sitroen	88	3	46.6	36.7	47.5	50.7	47.8	3.8	6.3
Tri x Sweet orange	88	3	46.3	46.6	46.0	51.4	47.3	3.8	5.8
Genoa/RL comm	88	3			44.9	47.9	48.7	4.1	6.0
Lisbon/RL comm	88	3			46.0	45.5	42.8	5.3	6.4

All rootstocks except for Lisbon (the lowest but acceptable), easily met the minimum juice export standards of 40% with little difference between them. Larger fruit were tested this season, which probably resulted in a slight average drop in juice percentages over last season (0.63%). Over the past seasons, Rangpur had most tests with higher juice percentages followed by Benton and M x T most of the lowest. Commercial Lisbon were also of the lowest. The rind thickness does not necessarily correspond with the juice percentages. Seed counts were much lower this season.

Table 6.4.3.6. Analyses of high shoulders and wind scars of Genoa lemons on various rootstocks. Rootstocks arranged from least to most high shoulders.

Rootstock	Percentage High shoulders*					Percentage Wind scars *						
	0	1	2	3	4	0	1	2	3	4	5	6
Rough lemon	78	16	2		4	62	24	8	2	2	2	
Carrizo citrange	78	14		4	4	24	32	20	18	2	4	
Benton citrange	82	8	2	4	4	20	30	34	16			
Trifoliate X	72	18	4	4	2	22	26	28	14	8	2	
Volckameriana	66	14	4	8	8	52	22	12	6	4	2	2
Rangpur lime	72	16	8	4		36	34	14	10	2	4	
Tri x Sweet orange	62	24	8	4	2	42	28	12	14	4		
Japanse sitroen	74	10	10	4	2	68	18	10	2		2	
Minneola x Trifoliate	52	24	10	4	10	38	24	14	12	4	4	4
Swingle citrumelo	50	16	4	2	28	58	16	8	14	4		
Genoa/RL comm	68	18	10	2	2	30	26	24	12	4	4	
Lisbon/RL comm	76	14	4	2	4	46	28	8	6	2	10	

There were some fruit with high shoulders, Swingle the worst and to a lesser extent M x T. All except Rangpur had some non exportable fruit. There was little oleocellosis; only two percent (T1) on each of Volckameriana, M x T and Rangpur lime and all the fruit were exportable. M x T had the most non exportable fruit with wind scars, while Benton, Swingle and Tri x Sweet had 100% exportable fruit.

* Evaluations are based on CRI Colour Prints for Blemish and Appearance standards. High Shoulders (Set 39), prints 0 – 3 exportable: Oleocellosis (Set 28), prints 0 - 3 exportable: Wind Scars (Set 8), prints 0 - 4 exportable.

Highlights of packhouse comments included the following:
Green fruit - Tri x Sweet and Swingle the most (low percentages)
Bollworm - Trifoliate X and Lisbon (low percentages)
Scale - Rough lemon. Lisbon double the amount
Sunburn - Benton
Thrips - Rangpur, Carrizo, Tri x Sweet, Lisbon and to a lesser extent Volckameriana

All rootstocks had mature flesh colour and mostly closed cores. Lisbon fruit overall appeared to be shorter (therefore rounder). Between the rootstocks, Trifoliate X and Carrizo also had slightly rounder fruit.

Summary of highlights per rootstock

Rough lemon: Large, vigorous trees with high yields and large fruit size. Average fruit colour to slightly earlier. Good juice percentage and of the thickest rinds. Smooth bud union. Red scale damage.

Volckameriana: Large, vigorous trees with highest yield and large fruit size. Average fruit colour. Good juice percentage and of the thickest rinds. Some high shoulders. Smooth bud union. Thrip damage.

Trifoliate X: Large trees, some pale/yellow, below average yield and a smaller, better fruit size. Average fruit colour. Good juice percentage and average rind thickness. Smooth bud union to very slight bench. Have the most wind damage and a little Bollworm damage.

Benton citrange: Variable, fairly large, narrow sometimes pale trees. Poorest yield with average fruit size and average rind colour. Good juice percentage with average rind thickness. Very slight bud union bench with occasional rootstock shoots. The most sunburnt fruit and fair amount of wind damage.

Trifoliate x Sweet orange: Medium large, more open trees, below average yield and second smallest (second best) fruit size. Earliest fruit colour. Lowest (but good) juice percentage with average rind thickness. Slightly inverted bud union. Most thrip damage.

Japanse sitroen: Dense, medium large trees. High yields of large fruit size. Of the latest coloured fruit. Second lowest but good juice percentage and thickest rind. Bud union is smooth with a paler rootstock, nodules and odd sucker growth. The least wind scars.

Minneola x Trifoliate: Medium tree size with low yields and the smallest, but best fruit size. Later fruit colour. Good juice percentage with the thinnest rind and high shoulders. A very slight bud union bench. Most non exportable wind damaged fruit.

Swingle citrumelo: Medium tree size with green to slightly yellow leaves. Second lowest yield with average to large fruit size and average fruit colour. Good juice percentage with average rind thickness, most high shoulders and the lowest seed count. Most benched bud union.

Rangpur lime: Smallest trees, high yield and large fruit size. Two sets and average colour. Second highest, good juice percentage and second thinnest rinds. Smoothish bud union, nodules and a lot of rootstock regrowth. A fair amount of wind damage and of the most thrip damage.

Carrizo citrange: Medium tree size with slightly pale leaves. Low yields with smaller, better fruit size and of the latest colour. Highest juice percentage and average rind thickness. Very slightly benched bud union. A fair amount of wind scarring and of the most thrip damage.

Lisbon lemon on Rough lemon: Large tree size with low yields and smaller, better fruit size and later colour. A much lower (acceptable) juice percentage than Genoa with the thickest rind and the most seed. Has the least high shoulders (rounder fruit). A smooth bud union with occasional shoots. The most non exportable wind scarred fruit, the most scale and most thrip damaged fruit.

Conclusions

This was the fifth year of production, the first good yield and fruit size larger than last season, although the fruit was harvested later. Volckameriana, Rough lemon and Trifoliate X had the largest tree size, Rangpur lime and Benton citrange the smallest. Volckameriana had by far the highest yield followed by Japanse sitroen, Rough lemon and Rangpur, which were similar. Benton had the poorest yield, followed by Swingle citrumelo and Minneola x Trifoliate. Rough lemon and Volckameriana had the largest fruit size while Minneola x Trifoliate and Trifoliate x Sweet orange had the smallest but most desirable size.

All rootstocks had good juice percentages, easily meeting the minimum export standards. Trifoliate x Sweet and Rough lemon had slightly earlier fruit colour with Japanse sitroen, Carrizo citrange and Minneola x Trifoliate slightly later.

Swingle and Minneola x Trifoliate had the most high shoulders, Benton, Rough lemon and Carrizo the least. There was virtually no oleocellosis. Minneola x Trifoliate had the most non exportable fruit due to wind scarring. Swingle had the most severe bud union bench while Trifoliate x Sweet orange was slightly inverted. Rangpur and Japanse sitroen had nodules and sucker growth on the rootstock and Benton occasional sucker growth.

Future evaluations

Continue evaluations for another season.

6.4.4 Evaluation of Delta Valencia rootstock trial at Moosrivier Estate

Experiment 94 by J. Joubert (CRI)

Opsomming

Die prestasie van Delta Valencia op 42 verskillende onderstamme in herplant gronde, moet oor ’n agt jaar produksietyd in die Marble Hall omgewing geëvalueer word. Daar was voldoende water in die opgaardamme vir effektiewe besproeiing en ’n probleem met die pyplyn is opgeklaar. Die produksie by meeste van die onderstamme het baie toegeneem, maar die interne kwaliteit, veral Brixº het gedaal onder die minimum uitvoer standaard perke in. Hierdie proef sal vir nog een seisoen ge-evalueer word.

Introduction

To evaluate the performance of Delta Valencia on 42 different rootstocks in replant soils, over an eight-year production cycle in the Marble Hall area.

Materials and Methods

A randomised block design comprising of 22 rootstocks of two replicates of five trees each, the other 20 rootstocks were planted in a non-randomised design comprising of 10 trees per rootstock. Delta Valencia on the following rootstocks are being evaluated: F80/8, F80/3, C32, C35, AT, X639, RL-C, RL-S, RL-W, PT, HRS812, K, ChM, N, RxT, CLM, Sunki 1113, CM, C, SCS, GT, CO, CC, TC, Volk, KC, TB, ML, OT, CA, RC, JT, RT, JC, BC, Sunki 1112, ST, SC, RP, SM, SFS, Sunki 1116. The trees were planted in 1998. Trees were evaluated at Moosrivier Estate (Marble Hall), in Mpumalanga during the 2006 season.

Results and discussion

Internal fruit quality analysis (Table 6.4.4.1)

- Juice%: F80/8 and C produced the highest Juice content (55.3%) followed by N (54.9%) and F80/3 (54.5%). Ten of the rootstocks evaluated (C32, CM, C35, ChM, CLM, TC, KC, TB, ST, RL-W) produced lower than 52% juice and did not comply with the packing specifications. The lowest juice content was produced by CM (49.9%).

- Brixº: The highest Brixº was produced by RT (10.4) followed by Sunki 1112 (10.3), RC (10.2) and PT (10.2). All of the rootstocks evaluated produced lower than 10.5 Brixº and did not comply with the minimum export standards. The lowest Brixº was produced by Volk (7.9), similar to the previous season.

- Acid%: CO rootstock provided the highest acid content (1.36%), followed by N (1.26% and SM (1.25%). The lowest acid content measured 0.83% (Volk) and did not comply with the minimum export specifications of 0.85%. The rest of the rootstocks did comply with the standards.

Fruit size distribution (Table 6.4.4.2)

- The fruit size evaluation shows the largest peak at count 72 on 22 of the 42 rootstocks. The next highest count in fruit size was count 56 with 15 rootstocks. The third highest count evaluated in fruit size was count 88 with 27 rootstocks. Considering that count 72, followed by count 56 and then count 88 was ranked from the highest percentage fruit per rootstock to the lowest percentage.

Production per tree (Table 6.4.4.3)

- CM rootstock produced the best yield per tree (112.9 kg) in comparison with the other 42 rootstocks. Sunki 1112 was the second highest producer with 101.8 kg per tree, followed by JT (96.5 kg) and BC (89.5 kg). OT produced the lowest yield for the 2006 season with 44.7 kg per tree.

- There is an increase in yield production between the 2005 and 2006 season. The trend shows the severe impact of the water problems that occurred in this area on the different rootstocks. The 2006 season had a better rainfall and the problems with the irrigation pipelines were solved.

- The table shows the variation in yield for 2003, 2004, 2005 and 2006 between the different rootstocks. The analysis points out that for the 2004 season there was a decrease in yield. It is very important to compare the recovery rate for the rootstocks from 2003 to 2006. Please bear in mind that during the 2006 season there was enough water for normal irrigation practices.

Conclusions and recommendations

The internal quality of the fruit decreased in comparison with the 2005 season, with none of the Brixº analysis complying with the export standards. There was a substantial increase in fruit size produced per tree. The average fruit size moved from count 105/125 to count 72, followed by count 56 and 88. The income will increase drastically with this scenario. The production per tree increased considerable compaired to 2005 (from 79.8 kg to 112.9 kg).

Table 6.4.4.1. Internal fruit quality of Delta Valencia on different rootstocks at Moosrivier Estate (Marble Hall) on 5 July 2006.

Root-stock	Count	Juice %	Brix º	Acid %	Ratio	Ave. seed	Colour
F80/8	72-48	55.3	9.8	1.14	8.60	0.0	T1
PT	72-48	54.1	10.2	1.15	8.87	0.0	T1
C32	64-48	51.9	9.3	1.16	8.02	0.0	T1-3
AT	72-56	52.6	10.1	0.97	10.41	0.0	T1
HRS 812	64-40	52.8	9.8	0.97	10.10	0.7	T1-2
K	88-48	53.9	9.2	1.00	9.20	0.0	T1-2
CM	64-56	49.9	8.5	1.09	7.80	0.0	T3-4
C35	72-48	51.7	10.0	1.08	9.26	0.0	T1-3
C	72-64	55.3	9.8	1.17	8.38	0.0	T1-3
SCS	88-48	54.0	9.5	1.09	8.72	0.0	T1-2
X639	72-56	52.7	9.9	1.13	8.76	0.0	T2-3
GT	72-48	53.4	10.1	1.12	9.02	0.0	T1
ML	72-40	54.0	9.2	0.97	9.48	0.0	T1-2
OT	72-56	53.8	9.1	0.95	9.58	0.0	T1
CA	72-48	53.4	9.9	0.99	10.00	0.0	T1
RC	64-40	52.7	10.2	1.24	8.23	0.0	T1-2
JT	88-56	53.3	9.7	1.01	9.60	0.0	T1
RL-S	88-56	52.8	9.3	1.12	8.30	0.0	T1-3
SC	72-64	52.6	9.7	1.12	8.66	0.0	T2-3
RP	88-64	53.0	8.3	1.06	7.83	0.0	T1-3
SM	72-56	53.4	9.6	1.25	7.68	0.0	T2-3
ChM	72-56	51.8	10.1	1.23	8.21	0.2	T1-2
N	72-64	54.9	9.2	1.26	7.30	0.0	T2-3
RxT	72-56	52.8	9.5	1.05	9.05	0.0	T2-3
RL-C	72-56	53.1	8.6	1.02	8.43	0.0	T1-3
CLM	88-56	51.7	9.7	1.11	8.74	0.0	T1-3
Sunki 1113	72-56	52.0	10.0	1.00	10.00	0.0	T2-3
CO	88-56	52.7	9.9	1.36	7.28	0.0	T1
CC	72-48	53.2	9.8	1.15	8.52	0.0	T1-3
TC	88-48	50.5	9.6	1.07	8.97	0.0	T1-2

	Count	Juice	Brix	Acid	Ratio	Ave.	Colour
Volk	88-48	52.0	7.9	0.83	9.52	0.0	T1-2
KC	72-48	51.9	9.9	1.22	8.11	0.0	T1-2
TB	88-48	51.4	9.4	1.18	7.97	0.0	T3-4
RT	88-56	53.9	10.4	1.29	8.06	0.0	T1-2
JC	72-48	53.0	9.2	1.08	8.52	0.0	T1
BC	88-56	54.4	9.4	1.11	8.47	0.0	T1-2
F80/3	72-56	54.5	9.5	1.23	7.72	0.3	T1
Sunki 1112	72-56	53.9	10.3	1.23	8.37	0.0	T1-2
ST	72-56	51.6	9.9	1.10	9.00	0.0	T1-2
SFS	72-56	52.2	9.8	1.02	9.61	0.0	T1
Sunki 1116	72-56	52.9	9.6	1.20	8.00	0.0	T1-3
RL-W	72-56	50.9	10.0	1.16	8.62	0.0	T3

Table 6.4.4.2. Fruit size distribution of Delta Valencia on different rootstocks at Moosrivier Estate (Marble Hall) during the 2006 season.

Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
F80/8	48	5.16	ChM	48	1.30	Sunki 1112	48	3.98
F80/8	56	31.52	ChM	56	19.57	Sunki 1112	56	20.56
F80/8	72	36.95	ChM	72	32.25	Sunki 1112	72	33.05
F80/8	88	17.63	ChM	88	23.77	Sunki 1112	88	25.03
F80/8	105/125	8.28	ChM	105/125	20.32	Sunki 1112	105/125	16.40
F80/8	144	0.45	ChM	144	2.80	Sunki 1112	144	0.98
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
PT	48	2.16	N	48	2.92	ST	48	5.54
PT	56	20.25	N	56	21.35	ST	56	27.44
PT	72	35.27	N	72	28.25	ST	72	31.65
PT	88	24.48	N	88	21.92	ST	88	18.26
PT	105/125	16.93	N	105/125	21.19	ST	105/125	15.45
PT	144	0.91	N	144	4.38	ST	144	1.65
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
C32	48	23.03	RxT	48	7.84	SFS	48	13.95
C32	56	44.56	RxT	56	37.01	SFS	56	38.05
C32	72	19.51	RxT	72	32.36	SFS	72	25.56
C32	88	7.46	RxT	88	14.04	SFS	88	14.93
C32	105/125	4.37	RxT	105/125	8.39	SFS	105/125	6.93
C32	144	1.07	RxT	144	0.36	SFS	144	0.59
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
AT	48	6.15	RL-C	48	3.32	Sunki 1116	48	7.43
AT	56	32.33	RL-C	56	22.26	Sunki 1116	56	35.72
AT	72	30.52	RL-C	72	30.49	Sunki 1116	72	29.37
AT	88	15.68	RL-C	88	23.77	Sunki 1116	88	16.95
AT	105/125	13.99	RL-C	105/125	18.72	Sunki 1116	105/125	9.52
AT	144	1.33	RL-C	144	1.43	Sunki 1116	144	1.00
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
HRS812	48	16.10	CLM	48	11.99	RL-W	48	17.24
HRS812	56	49.91	CLM	56	36.16	RL-W	56	43.49
HRS812	72	24.29	CLM	72	27.34	RL-W	72	25.47
HRS812	88	7.25	CLM	88	15.70	RL-W	88	9.50

HRS812	105/125	2.35	CLM	105/125	8.64	RL-W	105/125	3.62
HRS812	144	0.09	CLM	144	0.18	RL-W	144	0.69
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
K	48	10.22	Sunki 1113	48	16.01	JT	48	10.80
K	56	36.53	Sunki 1113	56	35.62	JT	56	26.83
K	72	29.68	Sunki 1113	72	28.42	JT	72	29.79
K	88	15.84	Sunki 1113	88	13.78	JT	88	17.42
K	105/125	7.11	Sunki 1113	105/125	5.39	JT	105/125	12.20
K	144	0.62	Sunki 1113	144	0.77	JT	144	2.96
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
CM	48	6.48	CO	48	2.67	RL-S	48	8.20
CM	56	27.23	CO	56	21.05	RL-S	56	33.85
CM	72	33.59	CO	72	28.95	RL-S	72	31.88
CM	88	19.32	CO	88	25.05	RL-S	88	16.41
CM	105/125	12.49	CO	105/125	20.53	RL-S	105/125	9.03
CM	144	0.89	CO	144	1.75	RL-S	144	0.62
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
C35	48	15.15	CC	48	7.81	SC	48	5.28
C35	56	42.76	CC	56	42.41	SC	56	30.51
C35	72	26.85	CC	72	32.32	SC	72	30.34
C35	88	10.74	CC	88	13.28	SC	88	20.28
C35	105/125	4.31	CC	105/125	4.02	SC	105/125	12.24
C35	144	0.19	CC	144	0.15	SC	144	1.34
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
C	48	5.99	TC	48	3.86	RP	48	3.36
C	56	25.45	TC	56	37.44	RP	56	40.15
C	72	34.38	TC	72	34.89	RP	72	32.24
C	88	19.87	TC	88	17.41	RP	88	16.17
C	105/125	13.25	TC	105/125	6.24	RP	105/125	7.27
C	144	1.05	TC	144	0.16	RP	144	0.82
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
SCS	48	4.78	Volk	48	5.63	SM	48	3.26
SCS	56	30.68	Volk	56	28.46	SM	56	28.19
SCS	72	32.93	Volk	72	31.91	SM	72	37.54
SCS	88	18.06	Volk	88	18.33	SM	88	20.33
SCS	105/125	12.62	Volk	105/125	14.87	SM	105/125	10.09
SCS	144	0.93	Volk	144	0.80	SM	144	0.59
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
X639	48	2.36	KC	48	13.97	BC	48	6.55
X639	56	23.99	KC	56	34.34	BC	56	29.82
X639	72	36.53	KC	72	28.65	BC	72	33.74
X639	88	22.98	KC	88	13.97	BC	88	20.03
X639	105/125	13.38	KC	105/125	8.36	BC	105/125	9.40
X639	144	0.76	KC	144	0.71	BC	144	0.46
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
GT	48	5.26	TB	48	1.99	F80/3	48	4.76
GT	56	29.03	TB	56	21.81	F80/3	56	28.46

GT	72	34.51	TB	72	34.57	F80/3	72	39.59
GT	88	20.69	TB	88	24.49	F80/3	88	18.10
GT	105/125	10.17	TB	105/125	15.98	F80/3	105/125	8.41
GT	144	0.34	TB	144	1.17	F80/3	144	0.68
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
ML	48	2.60	RT	48	3.31	CA	48	7.88
ML	56	17.05	RT	56	22.49	CA	56	31.52
ML	72	27.63	RT	72	29.44	CA	72	35.15
ML	88	21.45	RT	88	20.92	CA	88	15.45
ML	105/125	25.95	RT	105/125	21.23	CA	105/125	9.39
ML	144	5.32	RT	144	2.60	CA	144	0.61
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
OT	48	7.03	JC	48	18.45	RC	48	41.34
OT	56	37.81	JC	56	38.04	RC	56	29.33
OT	72	32.19	JC	72	24.30	RC	72	16.37
OT	88	14.38	JC	88	11.32	RC	88	7.01
OT	105/125	7.66	JC	105/125	6.74	RC	105/125	5.53
OT	144	0.94	JC	144	1.15	RC	144	0.43

Table 6.4.4.3. Production of Delta Valencia on different rootstocks at Moosrivier Estate (Marble Hall) during the 2006 season.

Rootstock Selection	Kg/tree 2003	Kg/tree 2004	Kg/tree 2005	Kg/tree 2006	Variation kg (03-04)	Variation kg (03-05)	Variation kg (03-06)	Variation kg (04-05)	Variation kg (04-06)
JT	26.3	30.8	43.0	96.5	4.50	16.70	70.20	12.20	65.70
RT	36.8	35.7	30.0	79.0	-1.10	-6.80	42.20	-5.70	43.30
CM	57.4	51.0	56.5	112.9	-6.40	-0.90	55.50	5.50	61.90
KC	39.0	32.7	50.8	75.1	-6.30	11.80	36.10	18.10	42.40
Sunki 1116	32.5	25.8	43.1	74.7	-6.70	10.60	42.20	17.30	48.90
SFS	51.4	40.1	37.8	73.2	-11.30	-13.60	21.80	-2.30	33.10
BC	61.7	47.7	66.5	89.5	-14.00	4.80	27.80	18.80	41.80
RP	59.4	45.7	79.8	85.0	-13.70	20.40	25.60	34.10	39.30
PT	43.1	32.5	47.6	75.6	-10.60	4.50	32.50	15.10	43.10
RC	50.1	37.4	40.1	78.2	-12.70	-10.00	28.10	2.70	40.80
F80/8	48.4	33.4	49.4	71.9	-15.00	1.00	23.50	16.00	38.50
RxT	36.2	22.5	41.6	75.6	-13.70	5.40	39.40	19.10	53.10
CC	37.0	22.0	45.2	95.6	-15.00	8.20	58.60	23.20	73.60
Sunki 1113	48.4	28.7	45.8	83.8	-19.70	-2.60	35.40	17.10	55.10
ML	60.5	35.4	42.3	93.9	-25.10	-18.20	33.40	6.90	58.50
ST	18.5	10.6	25.4	78.2	-7.90	6.90	59.70	14.80	67.60
X639	47.2	26.3	48.1	77.6	-20.90	0.90	30.40	21.80	51.30
F80/3	49.9	27.1	53.8	79.8	-22.80	3.90	29.90	26.70	52.70
CA	21.1	11.2	26.0	45.6	-9.90	4.90	24.50	14.80	34.40
Sunki 1112	43.9	22.9	37.4	101.8	-21.00	-6.50	57.90	14.50	78.90
JC	23.4	11.9	38.0	58.8	-11.50	14.60	35.40	26.10	46.90
Volk	56.7	28.1	42.8	82.4	-28.60	-13.90	25.70	14.70	54.30
RL-C	55.7	26.8	43.7	82.0	-28.90	-12.00	26.30	16.90	55.20
HRS 812	50.7	24.0	25.4	81.8	-26.70	-25.30	31.10	1.40	57.80
C35	58.4	27.4	39.6	80.9	-31.00	-18.80	22.50	12.20	53.50
AT	25.3	11.8	20.6	55.2	-13.50	-4.70	29.90	8.80	43.40
C32	54.9	25.4	38.6	73.5	-29.50	-16.30	18.60	13.20	48.10
RL-S	54.3	25.1	52.0	66.8	-29.20	-2.30	12.50	26.90	41.70
SM	28.8	13.3	23.9	45.4	-15.50	-4.90	16.60	10.60	32.10

Rootstock Selection	Kg/tree 2003	Kg/tree 2004	Kg/tree 2005	Kg/tree 2006	Variation kg (03-04)	Variation kg (03-05)	Variation kg (03-06)	Variation kg (04-05)	Variation kg (04-06)
RL-W	34.8	14.2	46.1	76.3	-20.60	11.30	41.50	31.90	62.10
CO	31.1	11.3	36.5	60.4	-19.80	5.40	29.30	25.20	49.10
TB	49.5	16.9	50.0	90.8	-32.60	0.50	41.30	33.10	73.90
CLM	31.7	9.6	12.7	40.1	-22.10	-19.00	8.40	3.10	30.50
OT	23.4	6.9	26.4	44.7	-16.50	3.00	21.30	19.50	37.80
SC	40.0	10.9	27.3	80.2	-29.10	-12.70	40.20	16.40	69.30
ChM	34.9	9.0	16.4	65.0	-25.90	-18.50	30.10	7.40	56.00
N	46.2	11.3	49.4	76.9	-34.90	3.20	30.70	38.10	65.60
GT	26.3	5.8	29.8	58.8	-20.50	3.50	32.50	24.00	53.00
TC	50.6	11.1	52.7	75.2	-39.50	2.10	24.60	41.60	64.10
C	18.7	3.7	26.8	62.5	-15.00	8.10	43.80	23.10	58.80
SCS	34.9	5.5	16.4	50.0	-29.40	-18.50	15.10	10.90	44.50
K	25.1	3.7	46.5	56.9	-21.40	21.40	31.80	42.80	53.20

6.4.5 **Evaluation of Midknight and Delta Valencia rootstock trial at Letaba Estates**

Experiment 137A by J. Joubert (CRI)

Opsomming

Die prestasie van verskillende onderstamme op herplant grond is in 'n intermediêre sitrusproduksie gebied geëvalueer. Hierdie seisoen het die ernstige water tekorte weer baie produksie probleme opgelewer. Die Delta valencias se gemiddelde oes wat geproduseer is, het gedaal. Die interne kwaliteit van meeste kombinasies het nie aan die minimum uitvoer vereistes voldoen nie. Volgens die verskillende vruggroottes wat geproduseer was, het die vrugtelling een vlak grootter opgeskuif. RL-C het die hoogste produksie per boom behaal vir hierdie seisoen (66.8%). Die Midknight bome se vruggroote het effens gedaal vir die 2006 seiseon. Meeste van die seleksies het ook nie aan die uitvoerstandaarde voldoen nie, met te lae Brixº. Die algemene produksie van die bome het gestyg in vergelyking met die 2005 seisoen. Albei hierdie seleksies sal die laaste keer vir 2007 ge-evalueer word.

Introduction

To evaluate the performance of different rootstocks on replant soils in an intermediate citrus production area. Commercially grown cultivars in the area were used as scion, *viz*. Midknight Valencia and Delta Valencia. The trial was established in February 1997. Soils are deep clay and irrigated with a micro irrigation system.

Materials and methods

Rootstock seeds were collected locally and abroad. Seeds were propagated by an accredited nursery using normal practices. Buds of Midknight Valencia and Delta Valencia trees were budded onto 30 different rootstocks. The trees were planted in February 1997 at Letaba Estates near Letsitele, Limpopo Province. The trial layout is a randomised block design comprising two replicates of 10 trees per replicate (total of 20 trees per rootstock).

Table 6.4.5.1. List of cultivar x rootstock combinations planted at Letaba Estates.

Selection	Rootstock	No. of trees
Delta Valencia	F80/9	10
Delta Valencia	SC	10
Delta Valencia	CC	10
Delta Valencia	F80/3	10
Delta Valencia	C35	10
Delta Valencia	KC	10
Delta Valencia	MxT	10
Delta Valencia	BC	10
Delta Valencia	X639	10
Delta Valencia	RL-C	8
Midknight Valencia	RL-C	10

Midknight Valencia	F80/9	6
Midknight Valencia	BC	9
Midknight Valencia	MxT	10
Midknight Valencia	SC	10
Midknight Valencia	F80/3	10
Midknight Valencia	CC	9
Midknight Valencia	C35	8
Midknight Valencia	KC	10
Midknight Valencia	X639	10
Midknight Valencia	61AA 3	10
Midknight Valencia	79AC 6/2	10
Midknight Valencia	75AB 12/3	7

Results and discussion

Delta Valencia

Internal fruit quality analysis (Table 6.4.5.2a)

- Juice %: CC produced the highest juice content (58.5%) followed by KC (57.8%) and BC (57.4%). All the rootstocks complied with the export standards, with F80/9, F80/3 and MxT producing the lowest juice content (54.7%).

- Brixº: The highest Brixº was produced by F80/9 (11.6) followed by MxT (11) and F80/3 (10.9). SC, CC, KC and X639 was below the export standard with RL-C the lowest (8.6).

- Acid%: RL-C produced the highest acid content (1.21%) followed by F80/9, F80/3 (1.09%) and C35 (1.08%). CC and BC was below the minimum standard with 0.82%.

Fruit size distribution (Table 6.4.5.2b)

- The fruit size evaluation shows the largest peak at count 72 on 4 of the 9 rootstocks. The next highest count in fruit size was again count 72 with 3 rootstocks. The third highest count evaluated in fruit size was between count 88 and 105/125 with 4 rootstocks. Considering that count 72, followed by count 88 and 105/125 was ranked from the highest percentage fruit per rootstock to the lowest percentage.

Production per tree (Table 6.4.5.2c)

- RL-C produced the highest yield per tree (66.8 kg), followed by KC with 64.6 kg/tree and BC with 55.5 kg/tree MxT produced the lowest yield on the trees of 15.8 kg/tree.

Midknight Valencia

Internal fruit quality analysis (Table 6.4.5.3a)

- Juice %: C35 and BC produced the highest juice content (60.3%) followed by F80/9 (59.6%) and KC (59.2%). The juice content on all the rootstocks complied with the minimum export standards with rootstock 61AA3 measuring 54.6% (lowest).

- Brixº: 61AA 3 produced the highest Brixº (10.8) followed by 75AB 12/3 (10.6) and C35, KC (10.5). All the other combination was below the minimum export standards (10.5). The lowest Brixº was produced by RL-C (9.1).

- Acid%: 61AA 3 produced the highest acid content (1.57%) by the time of harvest, followed by C35 (1.56%) and 75AB 12/3 (1.37%). The lowest acid content was produced by 79AC 6/2 (1.03%). All the selections comply with the minimum export standards (0.85%).

Fruit size distribution (Table 6.4.5.3b)

- The optimal fruit size for Valencias is between count 72 and 88. The fruit size evaluation shows the largest peak at count 105/125 on 9 of the 13 rootstocks. The next highest count in fruit size was

count 72 with 3 rootstocks. The third highest count evaluated in fruit size was between count 72 and 88 with 11 rootstocks. Considering that count 105/125, followed by count 72 was ranked from the highest percentage fruit per rootstock to the lowest percentage.

Production per tree (Table 6.4.5.3c)

- Midknight Valencia on X639 produced the highest yield (99.1 kg/tree). C35 produced 87.0 kg/tree, followed by F80/3 with 82.8 kg/tree and F80/9 with 68.0 kg/tree. MXT produced the lowest yield in this trial evaluated (30.8 kg/tree).

Conclusions and recommendations

To understand the production scenario better for the 2006 season, the following history was important. During the crop setting period there was only 250l of water available per tree per week. In November (20/11/2005) the area received 75mm of rain, forcing the trees to develop a second flower. This was the cause of out of season fruit production on most of the trees. December there was only enough water to irrigate 100l per tree per week. From January onwards up to April there were heavy rainfalls, influencing the internal quality of the fruit drastically.

Delta Valencia:
The internal quality scenario improved in comparison with the 2005 season. Most of the combinations comply with the export standards. The trees produced a larger fruit size in general with lower yield production per tree.

Midknight Valencia:
Internally the biggest problem was with the Brixº were most of the combinations (9) did not comply with the export standards. The juice and acid content was acceptable.

Table 6.4.5.2a. Internal fruit quality data of Delta Valencia on different rootstocks at Letaba Estates Letsitele) on 2 August 2006.

Root-Stock	Count	Juice %	Brix º	Acid %	Ratio	Ave. seed	Colour
F80/9	72-40	54.7	11.60	1.09	10.64	0.0	T1
SC	72-48	55.3	10.20	0.93	10.97	0.0	T1
CC	105-40	58.5	10.00	0.82	12.20	0.0	T1
F80/3	88-56	54.7	10.90	1.09	10.00	0.5	T1
C-35	88-40	55.0	10.50	1.08	9.72	0.0	T1
KC	105-48	57.8	10.40	0.87	11.95	0.0	T1
MxT	105-40	54.7	11.00	1.03	10.68	0.0	T1
BC	88-40	57.4	10.60	0.82	12.93	0.0	T1
X639	125-40	55.2	10.20	0.86	11.86	0.0	T1
RL-C	88-72	56.3	8.60	1.21	7.11	6.0	T1-2

Table 6.4.5.2b. Fruit size distribution of Delta Valencia on different rootstocks at Letaba Estates (Letsitele) during the 2006 season.

Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
F80/9	48	3.06	KC	48	1.10
F80/9	56	29.14	KC	56	17.21
F80/9	72	30.32	KC	72	29.43
F80/9	88	19.87	KC	88	24.42
F80/9	105/125	15.24	KC	105/125	23.96
F80/9	144	2.36	KC	144	3.89
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
SC	48	5.40	MxT	48	6.35
SC	56	31.72	MxT	56	24.21
SC	72	30.08	MxT	72	25.00
SC	88	20.04	MxT	88	18.65

SC	105/125	10.50	MxT	105/125	19.71
SC	144	2.26	MxT	144	6.08
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
CC	48	2.78	BC	48	5.90
CC	56	21.54	BC	56	23.42
CC	72	26.59	BC	72	18.23
CC	88	22.23	BC	88	12.62
CC	105/125	21.58	BC	105/125	22.49
CC	144	5.28	BC	144	17.34
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
F80/3	48	3.20	X639	48	1.05
F80/3	56	18.63	X639	56	11.78
F80/3	72	25.69	X639	72	24.19
F80/3	88	21.31	X639	88	24.08
F80/3	105/125	24.05	X639	105/125	28.94
F80/3	144	7.12	X639	144	9.96
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
C35	48	5.69	RL-C	48	0.00
C35	56	30.57	RL-C	56	0.65
C35	72	27.39	RL-C	72	9.38
C35	88	17.07	RL-C	88	27.10
C35	105/125	16.01	RL-C	105/125	51.00
C35	144	3.28	RL-C	144	11.88

Table 6.4.5.2c. Production per tree of Delta Valencia trees on different rootstocks at Letaba Estates (Letsitele) during the 2006 season.

Rootstock	Kg/tree
F80/9	27.4
SC	53.2
CC	43.6
F80/3	30.2
C35	22.6
KC	64.6
MxT	15.8
BC	55.5
X639	31.4
RL-C	66.8

Table 6.4.5.3a. Internal fruit quality data of Midknight Valencia on different rootstocks at Letaba Estates (Letsitele) on 17 July 2006.

Root-Stock	Count	Juice %	Brix º	Acid %	Ratio	Ave. seed	Colour
RL-C	125-56	55.9	9.10	1.44	6.32	0.4	T1-2
F80/9	105-56	59.6	9.50	1.16	8.19	0.7	T1
BC	72-40	60.3	9.70	1.11	8.74	0.3	T1
MxT	105-48	58.4	9.50	1.29	7.36	1.4	T1
SC	88-64	59.6	9.70	1.25	7.76	1.7	T1
F80/3	88-64	59.1	9.30	1.15	8.09	0.4	T1
CC	88-48	58.7	9.70	1.34	7.24	1.0	T1
C35	88-64	60.3	10.50	1.56	6.73	1.3	T1
KC	105-64	59.2	10.50	1.17	8.97	0.8	T1
X639	105-64	57.5	9.30	1.21	7.69	1.0	T1
61AA 3	125-72	54.6	10.80	1.57	6.88	0.0	T4

79AC 6/2	88-40	56.0	9.40	1.03	9.13	0.4	T1-3
75AB 12/3	125-72	57.9	10.60	1.37	7.74	0.0	T1

Table 6.4.5.3b. Fruit size distribution of Midknight Valencia on different rootstocks at Letaba Estates (Letsitele) during the 2006 season.

Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
RL-C	48	3.21	C35	48	1.21
RL-C	56	22.68	C35	56	12.30
RL-C	72	26.18	C35	72	25.38
RL-C	88	18.30	C35	88	23.43
RL-C	105/125	22.53	C35	105/125	31.78
RL-C	144	7.10	C35	144	5.90
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
F80/9	48	1.44	KC	48	0.18
F80/9	56	13.15	KC	56	10.36
F80/9	72	25.32	KC	72	25.06
F80/9	88	23.30	KC	88	27.38
F80/9	105/125	30.10	KC	105/125	31.11
F80/9	144	6.69	KC	144	5.90
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
BC	48	5.67	X639	48	0.40
BC	56	40.09	X639	56	8.79
BC	72	32.24	X639	72	28.00
BC	88	13.43	X639	88	30.06
BC	105/125	4.70	X639	105/125	28.56
BC	144	3.86	X639	144	4.19
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
MxT	48	1.12	61AA 3	48	0.26
MxT	56	7.61	61AA 3	56	5.13
MxT	72	18.65	61AA 3	72	12.08
MxT	88	23.60	61AA 3	88	16.25
MxT	105/125	39.66	61AA 3	105/125	39.10
MxT	144	9.36	61AA 3	144	27.19
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
SC	48	0.60	79AC 6/2	48	0.96
SC	56	9.21	79AC 6/2	56	8.98
SC	72	22.43	79AC 6/2	72	20.40
SC	88	26.49	79AC 6/2	88	21.81
SC	105/125	34.13	79AC 6/2	105/125	35.24
SC	144	7.15	79AC 6/2	144	12.61
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
F80/3	48	0.70	75AB 12/3	48	0.09
F80/3	56	10.92	75AB 12/3	56	1.28
F80/3	72	26.23	75AB 12/3	72	6.29
F80/3	88	28.51	75AB 12/3	88	13.77
F80/3	105/125	28.68	75AB 12/3	105/125	52.67
F80/3	144	4.95	75AB 12/3	144	25.90

Rootstock	Size	% Fruit
CC	48	3.13
CC	56	16.80
CC	72	22.58
CC	88	20.25
CC	105/125	27.73

CC	144	9.51

Table 6.4.5.3c. Production of Midknight Valencia on different rootstocks at Letaba Estates (Letsitele) during the 2006 season.

Rootstock	Kg/tree
RL-C	48.5
F80/9	68.0
BC	74.3
MxT	30.8
SC	66.9
F80/3	82.8
CC	66.5
C35	87.0
KC	74.3
X639	99.1
61AA 3	43.5
79AC 6/2	57.7
75AB 12/3	59.1

6.4.6 **Evaluation of Star Ruby rootstock trial at Letaba Estates**

Experiment 137B by J. Joubert (CRI)

Opsomming

Die prestasie van verskillende onderstamme op herplant grond is in 'n intermediêre sitrusproduksie gebied geëvalueer. Neem asseblief die water probleme in ag wanneer die inligting van die volgende proef geintepreteur word. Op een stadium was die bome slegs 100 L per week toegedien (Desember). Die vruggrootte het kleiner geword by meeste van die kombinasies vir hierdie seisoen. Die algemene vruggrootte het 'n piek bereik by telling 64. Star Ruby op Swingle citrumelo het die beste oes geproduseer met 106.2 kg/boom.

Materials and methods

Rootstock seeds were collected locally and abroad. Seeds were propagated by an accredited nursery using normal practices. Buds of Star Ruby grapefruit were budded onto 30 different rootstocks. The trees were planted in February 1997 at Letaba Estates near Letsitele, Limpopo Province. The trial layout is a randomised block design comprising two replicates of 10 trees per replicate (total of 20 trees per rootstock).

Results and discussion

Internal fruit quality analysis (Table 6.4.6.1)

- Juice %: RL-C produced the highest juice content (58.8%) followed by X639 (58.3%) and BC (56.8%). Internally CC and F80/3 produced the lowest juice content of 47.9%. CC, C35 and F80/3 did not comply with the minimum juice export requirements of 50%.

- Brixº: Internally CC and C35 produced the highest Brixº (9.1) for this Star Ruby trial. The second highest Brixº was produced by SC (9) followed by F80/3 with 8.9. RL-C produced the lowest Brixº of 7.3. SC, CC and C35 were the only three rootstocks to comply with the export standards of 9 Bixº minimum.

- Acid%: The highest acid content by the time of harvest was produced by F80/3 (2.09%) followed by CC (1.98%) and C35 (1.8%). The lowest acid content for this trial was on KC with 1.35%.

Fruit size distribution (Table 6.4.6.2)

- All the combinations peaked at count 64, except for MxT and F80/9 (peaked at count 48). The next highest count in fruit size was 48 with 8 of the 10 rootstocks. The third highest count evaluated in fruit size was 40 with all 10 of the rootstocks.

Production per tree (Table 6.4.6.3)

- Most of the rootstock combinations do have a decrease in production in this trial. In comparison to the 2005 season only SC, CC and MxT increased in fruit produced per tree. SC produced the highest yield (106.2kg/tree) followed by CC (105.6kg/tree) and MxT (97.3kg/tree). The lowest production for this season was on F80/3 with 63.1kg/tree.

Conclusions and recommendations

To understand the production scenario better for the 2006 season, the following history was important. During the crop setting period there was only 250 L of water available per tree per week. In November (20/11/2005) the area received 75mm of rain, forcing the trees to develop a second flower. This was the cause of out of season fruit production on most of the trees. December there was only enough water to irrigate 100 L per tree per week. From January onwards up to April there were heavy rainfalls, influencing the internal quality of the fruit drastically.

The average fruit size for this trial was on the small side. The lack of water does have a direct influence on the production, internal quality and size of the fruit produced. The increase in fruit production per tree of some rootstocks shows the ability of certain combinations to tolerate severe drought conditions.

Table 6.4.6.1. Internal fruit quality of Star Ruby grapefruit on different rootstocks at Letaba Estates (Letsitele) on 11 May 2006.

Root-Stock	Count	Juice %	Brix º	Acid %	Ratio	Ave. seed	Colour
RL-C	64-36	58.8	7.30	1.37	5.33	0.1	T1-2
CC	56-27	47.9	9.10	1.98	4.60	0.6	T1-2
SC	56-36	54.1	9.00	1.62	5.56	0.1	T1-2
F80/9	64-36	56.0	8.40	1.67	5.03	0.1	T1-2
MxT	64-32	51.3	8.10	1.79	4.53	0.0	T1-2
BC	64-27	56.8	8.30	1.43	5.80	0.0	T1-2
X 639	64-40	58.3	8.60	1.50	5.73	0.1	T1-2
KC	56-27	55.9	8.70	1.35	6.44	0.6	T1-2
C-35	64-23	49.7	9.10	1.80	5.06	0.7	T1-2
F80/3	64-36	47.9	8.90	2.09	4.26	0.2	T2

Table 6.4.6.2. Fruit size distribution of Star Ruby grapefruit on different rootstocks at Letaba Estates (Letsitele) during the 2006 season.

Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
RL-C	27	2.08	BC	27	1.31
RL-C	32	1.93	BC	32	1.08
RL-C	36	6.39	BC	36	3.47
RL-C	40	11.24	BC	40	7.98
RL-C	48	34.80	BC	48	26.56
RL-C	64	43.56	BC	64	59.59
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
SC	27	1.57	MxT	27	1.53
SC	32	1.96	MxT	32	2.01
SC	36	5.22	MxT	36	8.33
SC	40	12.37	MxT	40	16.06
SC	48	33.65	MxT	48	37.16

SC	64	45.23	MxT	64	34.91
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
F80/3	27	3.76	C35	27	5.72
F80/3	32	2.72	C35	32	2.09
F80/3	36	8.92	C35	36	6.97
F80/3	40	16.82	C35	40	12.50
F80/3	48	31.99	C35	48	31.61
F80/3	64	35.79	C35	64	41.11
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
CC	27	2.63	X639	27	2.10
CC	32	1.77	X639	32	1.89
CC	36	4.34	X639	36	6.86
CC	40	8.40	X639	40	12.19
CC	48	26.37	X639	48	33.20
CC	64	56.49	X639	64	43.79
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
KC	27	1.24	F80/9	27	3.78
KC	32	1.63	F80/9	32	3.78
KC	36	4.65	F80/9	36	10.42
KC	40	12.42	F80/9	40	16.41
KC	48	29.95	F80/9	48	34.05
KC	64	50.11	F80/9	64	31.58

Table 6.4.6.3. Production of Star Ruby Grapefruit on different rootstocks at Letaba Estates (Letsitele) during the 2006 season.

Rootstock	Kg/tree
RL-C	73.4
SC	106.2
F80/3	63.1
CC	105.6
KC	94.6
BC	87.7
MxT	97.3
C35	79.8
X639	84.7
F80/9	77.9

6.4.7 **Evaluation of Navel orange rootstock trial at Vaalharts**

Experiment 146A by J.Joubert (CRI)

Opsomming

Die prestasie van Navel variëteite op verskillende onderstamme ondersoek en die beste onderstam vir die betrokke seleksie te bepaal in die Vaalharts omgewing. Die beste produksie, vruggrootte, interne kwaliteit en eksterne kleur moet verkry word vir uitvoere na bv. die VSA markte. Die Bahianinha en Royal Late bome het goed herstel na die koue skade. Hierdie seisoen het die Bahianinha bome gemiddelde oeste geproduseer, met medium vruggrootte. Die interne kwaliteit van die vrugte by meeste van die onderstamme het aan die uitvoer standaarde voldoen. Royal Late het het ligter oeste geproduseer, met kleiner vruggroottes. Die interne kwaliteit het belowend gelyk met hoë Brix^{o} en aanvaarbare suur vlakke. Die proef sal weer in 2007 ge-evalueer word.

Introduction

The progress of Navel selections on different rootstocks must be investigated to determine the best rootstock for the Vaalharts area. To optimise the best production, fruit size, internal quality and external fruit colour for export to markets such as the USA.

Materials and methods

Field evaluations and laboratory analysis were conducted on Bahianinha navels on the following rootstocks: C32, C35, F80, IRL, KC, MxT, RL-W, RxT, SFS, TB, X639 and Royal Late on the following rootstocks: BC, C32, C35, CC, GT, RC. The trees were planted in 1998.

Table 6.4.7.1. Number of trees per rootstock in the Bahianinha and Royal Late navel trial at Vaalharts.

Selection	Rootstock	No. of trees
Bahianinha	C32	13
Bahianinha	C35	12
Bahianinha	F80	14
Bahianinha	IRL	9
Bahianinha	KC	12
Bahianinha	MxT	36
Bahianinha	RL-W	3
Bahianinha	RxT	13
Bahianinha	SFS	5
Bahianinha	TB	19
Bahianinha	X639	11
Royal Late	BC	16
Royal Late	Gou Tou	17
Royal Late	Rusk Citrange	17
Royal Late	C32	14
Royal Late	C35	15
Royal Late	CC	14

Results and discussion

Bahianinha

Internal fruit quality analysis (Table 6.4.7.2)

- Juice %: The highest juice content was produced by RxT (52.6%) followed by KC (52.5%) and SFS (52.1%). RL-W produced the lowest juice content with 49.1%. All the other selections comply with the export standards.

- Brixº: Bahianinha on C32 produced the highest Brix° (10.4) followed by Terrabella (10.3) and KC (10.2). IRL and RL-W did not comply with the export standards. The lowest Brix° was produced by IRL (8.1).

- Acid%: The lowest acid content was produced by IRL with 0.62%. IRL was the only rootstock that did not comply with the export standards. The highest acid content was produced on Terrabella (0.94%) followed by C35 (0.88%) and F80 (0.84%) by the time of harvest.

Fruit size distribution (Table 6.4.7.3)

- Count 72 and 105/125 peaked at 5 each of the 11 rootstocks evaluated in this trial. The second highest fruit size was count 88 with 5 of the 11 rootstocks. Count 88 was also the third highest fruit size count produced in this trial with 5 of the 11 rootstocks.

Production per tree (Table 6.4.7.4)

- The lowest crop produced in this trial was by RxT (22.4kg/tree). The best yield was produced by RL-W (94.7kg/tree) followed by IRL (66.7kg/tree) and X639 (59.3kg/tree).

Royal Late

Internal fruit quality analysis (Table 6.4.7.2)

- Juice %: Benton and C32 produced the best juice content in this trial with 52.3%. The second highest juice content was produced by C35 (52.2%) followed by CC (49.8%). RC had the lowest juice content measuring 49.3%. All the rootstocks comply with the minimum export standards.

- Brixº: The highest Brixº was measured with C35 (12.6) followed by C32 (11.2) and RC (10.8). CC and GT produced the lowest (10.2) Brixº. Fortunately all the combinations comply with the packing specifications.

- Acid%: C35 measured the highest acid content with 1.08%, followed by GT (0.95%) and C32 (0.90%). All the combinations were above the minimum acid content required for exports. CC produced the lowest content of 0.81%

Fruit size distribution (Table 6.4.7.3)

- There was a decrease in fruit size from the 2003 season when the trial was harvested before the cold damage. Count 105/125 peaked with 6 out of the 6 rootstocks. This was followed by count 88 with 4 out of the 6 rootstocks and a combined third place shared by count 88, 72 and 144 with 2 out of 6.

Production per tree (Table 6.4.7.4)

- C35 seems more susceptible to low temperatures (cold damage) in comparison to the other rootstocks, producing the lowest yield (22.4kg/tree). The best production was on Benton (57.9kg/tree) followed by RC (31.1kg/tree).

Conclusions and recommendations

The tree size seems small to medium in comparison with similar selections in the other regions. Please bear in mind the severe cold temperatures during winter time, influencing the growth patens of the trees. Bahianinha produced an average yield on the trees with medium fruit size, and acceptable internal quality after been harvested for the first time since 2003. The fruit size peaked between count 72 and 105/125 and the highest production was on RL-W (94.7 kg/tree).

Royal Late produced a similar internal quality with higher acid levels, but smaller fruit size and peaked at count 105/125. The production per tree was also lower in comparison with Bahianinha, and Benton produced the best yield of 57 kg/tree. C35 performed well with good internals, small fruit size and unfortunately low crop. Once again the cold temperatures in the winter play a major role.

Table 6.4.7.2. Internal fruit quality data of navels on different rootstocks at Vaalharts on 4 May 2006.

Selection	Root-stock	Count	Juice %	Brix º	Acid %	Ratio	Ave. seed	Colour
Bahianinha	C32	105-56	51.0	10.4	0.83	12.53	0.00	T1-2
Bahianinha	C35	88-48	51.3	10.0	0.88	11.36	0.00	T1-2
Bahianinha	F80	72-48	50.2	10.1	0.84	12.02	0.00	T3
Bahianinha	IRL	88-56	50.4	8.1	0.62	13.06	0.00	T3-4
Bahianinha	KC	72-56	52.5	10.2	0.80	12.75	0.00	T3-5
Bahianinha	MxT	88-48	50.6	9.7	0.83	11.69	0.00	T3-4
Bahianinha	RL-W	88-56	49.1	8.2	0.78	10.51	0.00	T4-5
Bahianinha	RxT	88-56	52.6	10.0	0.70	14.29	0.00	T2-3
Bahianinha	SFS	105-40	52.1	9.2	0.81	11.36	0.00	T4-6
Bahianinha	Terrabella	105-56	51.6	10.3	0.94	10.96	0.00	T3-4
Bahianinha	X639	72-48	51.3	9.5	0.75	12.67	0.00	T4-5
Royal Late	Benton	88-48	52.3	10.7	0.82	13.05	0.00	T4-6
Royal Late	C32	105-56	52.3	11.2	0.90	12.44	0.00	T3-4
Royal Late	C35	125-64	52.2	12.6	1.08	11.67	0.00	T3-4

Royal Late	CC	88-56	49.8	10.2	0.81	12.59	0.00	T4-6
Royal Late	GT	88-64	49.4	10.2	0.95	10.74	0.00	T4
Royal Late	RC	88-64	49.3	10.8	0.86	12.56	0.00	T4-6

Table 6.4.7.3. Fruit size distribution per rootstock at Vaalharts during the 2006 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Bahianinha	SFS	48	0.43	Royal Late	Benton	48	0.99
Bahianinha	SFS	56	8.34	Royal Late	Benton	56	8.49
Bahianinha	SFS	72	22.24	Royal Late	Benton	72	25.76
Bahianinha	SFS	88	26.50	Royal Late	Benton	88	24.49
Bahianinha	SFS	105/125	33.97	Royal Late	Benton	105/125	32.98
Bahianinha	SFS	144	8.51	Royal Late	Benton	144	7.29
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Bahianinha	RxT	48	0.22	Royal Late	C32	48	0.82
Bahianinha	RxT	56	8.85	Royal Late	C32	56	6.82
Bahianinha	RxT	72	23.70	Royal Late	C32	72	18.83
Bahianinha	RxT	88	28.60	Royal Late	C32	88	22.24
Bahianinha	RxT	105/125	33.21	Royal Late	C32	105/125	35.74
Bahianinha	RxT	144	5.41	Royal Late	C32	144	15.55
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Bahianinha	KC	48	1.28	Royal Late	C35	48	0.29
Bahianinha	KC	56	15.86	Royal Late	C35	56	4.03
Bahianinha	KC	72	26.49	Royal Late	C35	72	13.67
Bahianinha	KC	88	24.00	Royal Late	C35	88	20.72
Bahianinha	KC	105/125	24.54	Royal Late	C35	105/125	42.01
Bahianinha	KC	144	7.84	Royal Late	C35	144	19.28
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Bahianinha	MxT	48	1.63	Royal Late	CC	48	1.12
Bahianinha	MxT	56	21.35	Royal Late	CC	56	8.70
Bahianinha	MxT	72	35.88	Royal Late	CC	72	26.37
Bahianinha	MxT	88	21.35	Royal Late	CC	88	25.11
Bahianinha	MxT	105/125	16.53	Royal Late	CC	105/125	33.52
Bahianinha	MxT	144	3.26	Royal Late	CC	144	5.19
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Bahianinha	Terrabella	48	0.17	Royal Late	GT	48	0.39
Bahianinha	Terrabella	56	5.59	Royal Late	GT	56	4.57
Bahianinha	Terrabella	72	22.15	Royal Late	GT	72	15.56
Bahianinha	Terrabella	88	25.66	Royal Late	GT	88	23.37
Bahianinha	Terrabella	105/125	39.68	Royal Late	GT	105/125	37.54
Bahianinha	Terrabella	144	6.75	Royal Late	GT	144	18.58
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Bahianinha	IRL	48	3.14	Royal Late	RC	48	0.21
Bahianinha	IRL	56	20.28	Royal Late	RC	56	5.24
Bahianinha	IRL	72	28.87	Royal Late	RC	72	21.27
Bahianinha	IRL	88	21.00	Royal Late	RC	88	25.39
Bahianinha	IRL	105/125	21.15	Royal Late	RC	105/125	39.16
Bahianinha	IRL	144	5.55	Royal Late	RC	144	8.74
Cultivar	**Rootstock**	**Size**	**% Fruit**				
Bahianinha	RL-W	48	4.65				
Bahianinha	RL-W	56	29.71				
Bahianinha	RL-W	72	33.61				
Bahianinha	RL-W	88	16.35				
Bahianinha	RL-W	105/125	12.37				

Bahianinha	RL-W	144	3.32
Cultivar	**Rootstock**	**Size**	**% Fruit**
Bahianinha	F80	48	0.96
Bahianinha	F80	56	14.04
Bahianinha	F80	72	26.27
Bahianinha	F80	88	24.18
Bahianinha	F80	105/125	27.71
Bahianinha	F80	144	6.84
Cultivar	**Rootstock**	**Size**	**% Fruit**
Bahianinha	C32	48	0.27
Bahianinha	C32	56	5.86
Bahianinha	C32	72	12.42
Bahianinha	C32	88	15.91
Bahianinha	C32	105/125	41.08
Bahianinha	C32	144	24.46
Cultivar	**Rootstock**	**Size**	**% Fruit**
Bahianinha	C35	48	0.59
Bahianinha	C35	56	15.55
Bahianinha	C35	72	28.08
Bahianinha	C35	88	24.39
Bahianinha	C35	105/125	24.32
Bahianinha	C35	144	7.07
Cultivar	**Rootstock**	**Size**	**% Fruit**
Bahianinha	X639	48	0.10
Bahianinha	X639	56	5.15
Bahianinha	X639	72	18.04
Bahianinha	X639	88	24.84
Bahianinha	X639	105/125	38.41
Bahianinha	X639	144	13.47

Table 6.4.7.4. Production of Bahianinha and Royal Late on different rootstocks at Vaalharts during the 2006 season.

Cultivar	**Rootstock**	**Kg/tree**
Bahianinha	SFS	46.8
Bahianinha	RxT	41.6
Bahianinha	KC	51.2
Bahianinha	MxT	44.8
Bahianinha	Terrabella	51.3
Bahianinha	IRL	66.7
Bahianinha	RL-W	94.7
Bahianinha	F80	51.6
Bahianinha	C32	52.9
Bahianinha	C35	43.6
Bahianinha	X639	59.3
Royal Late	C35	22.4
Royal Late	C32	24.3
Royal Late	Benton	57.9
Royal Late	GT	40.8
Royal Late	RC	31.1

6.4.8 **Evaluation of Valencia orange rootstock trial at Vaalharts**
Experiment 146B by J.Joubert (CRI)

Opsomming

Die prestasie van Valencia variëteite op verskillende onderstamme word ondersoek en die beste onderstam vir die betrokke seleksie te bepaal in die Vaalharts omgewing. Die beste produksie, vruggrootte, interne kwaliteit en eksterne kleur moet verkry word vir uitvoere na bv. die VSA markte. Die Valencia bome het goed herstel na die koue skade van 2004. Die algemene vruggrootte wat op die bome geproduseer was, het gewissel tussen telling 144 en 88. Oor die algemeen was die produksie laag, maar sal verseker toeneem met die volgende seisoen se evaluasie. Intern is daar baie potensiaal vir verbetering, want meeste van die seleksies was nie uitvoerbaar nie. Evaluasies sal weer vir 2007 uitgevoer word.

Introduction

The performance of Valencia selections on different rootstocks must be investigated to determine the best rootstock combination for the Vaalharts area. To optimise the best production, fruit size, internal quality and external fruit colour for export to markets such as the USA.

Materials and methods

Field evaluations and laboratory analysis were conducted on Delta Valencia on the following rootstocks: BT, C35, CC, F80, SFS, X639 and Midknight Valencia on the following rootstocks: C35, CC, X639. The trees were planted in 1998.

Table 6.4.8.1. Number of trees per rootstock in the Delta and Midknight Valencia trial at Vaalharts.

Selection	Rootstock	No. of trees
Delta Valencia	BC	17
Delta Valencia	C35	12
Delta Valencia	CC	10
Delta Valencia	F80	13
Delta Valencia	SFS	8
Delta Valencia	X639	10
Midknight	C35	7
Midknight	CC	9
Midknight	X639	8

Results and discussion

Delta Valencia

Internal fruit quality analysis (Table 6.4.8.2)

- Juice %: CC (51.2%), F80 (48.5%) and SFS (50.2%) was below the minimum packing specifications. Benton produced the highest juice content (54.1%) followed by C35 (53.2%) and X639 (52.9%).
- Brixº: C35 was the only selection complying with the export standards (10.9º). The lowest production was on SFS with 9.3%.
- Acid%: F80 was above the maximum acid level allowed for export by the time of harvest (1.72%). All the other selections comply with the standards. Benton produced the lowest acid content of 1.22%.

Fruit size distribution (Table 6.4.8.3)

- The fruit size evaluation shows the largest peak at count 105 on 4 of the 4 rootstocks. The next highest count in fruit size was count 144 with 3 rootstocks. The third highest count evaluated in fruit size was count 88 with 3 rootstocks. Considering that count 105, followed by count 144 and 88 was ranked from the highest percentage fruit per rootstock to the lowest percentage.

Production per tree (Table 6.4.8.4)

- Benton produced the highest yield per tree (79.7 kg), followed by C35 with 59.4 kg/tree and X639 with 48 kg/tree. SFS produced the lowest yield on the trees of 40.3 kg/tree.

Midknight Valencia

Internal fruit quality analysis (Table 6.4.8.2)

- Juice %: All the selections comply with the export standards above 52% juice content. C35 produced the highest level (55.7%) followed by X639 (53.4%) and CC (52%).

- Brix°: There was a similar scenario in regards to the juice content production. C35 produced the highest level (10.1) followed by X639 (9.5) and CC (9.4). All the combinations were below the minimum levels for packing.

- Acid%: All three rootstocks produced too high acid levels above 1.5% and did not comply to export standards.

Fruit size distribution (Table 6.4.8.3)

- The fruit size evaluation shows the largest peak at count 105 on 3 of the 5 rootstocks. The next highest count in fruit size was again count 105 with 2 rootstocks. The third highest count evaluated in fruit size was count 88 with 3 rootstocks. Considering that count 105, followed by count 88 was ranked from the highest percentage fruit per rootstock to the lowest percentage.

Production per tree (Table 6.4.8.4)

- CC produced the highest yield per tree (49.9 kg), followed by X639 with 47.7 kg/tree and C35 with 41.9 kg/tree.

Conclusions and recommendations

Both cultivars produced a small to medium fruit size that will increase in future. Please remember the cold damage in the 2004 season, resulting in harvesting the trial for the first time in 2006. The internal quality must improve to export the fruit, as well as the production per tree. Evaluations will continue for 2007.

Table 6.4.8.2. Internal fruit quality data of valencias on different rootstocks at Vaalharts on 11 July 2006.

Selection	Root-stock	Count	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
Delta Valencia	Benton	125-64	54.1	10.1	1.22	8.28	0	T1
Delta Valencia	C35	125-72	53.2	10.9	1.45	7.52	0	T1
Delta Valencia	CC	125-72	51.2	9.8	1.39	7.05	0	T1
Delta Valencia	F80	125-64	48.5	10.3	1.72	5.99	0	T1
Delta Valencia	SFS	105-72	50.2	9.3	1.42	6.55	0	T1
Delta Valencia	X639	125-72	52.9	10.0	1.3	7.69	0	T1
Midknight	C35	105-64	55.7	10.1	1.69	5.98	0.3	T1
Midknight	CC	105-48	52.0	9.4	1.67	5.63	0.1	T1
Midknight	X639	105-72	53.4	9.5	1.68	5.65	0.4	T1

Table 6.4.8.3. Fruit size distribution per rootstock at Vaalharts during the 2006 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midknight	C35	48	0.00	Delta	CC	48	0.00
Midknight	C35	56	1.52	Delta	CC	56	0.07
Midknight	C35	72	15.65	Delta	CC	72	2.41
Midknight	C35	88	28.83	Delta	CC	88	8.18
Midknight	C35	105/125	46.88	Delta	CC	105/125	50.17
Midknight	C35	144	7.11	Delta	CC	144	39.17

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midknight	X639	48	0.00	Delta	F80	48	0.00
Midknight	X639	56	2.33	Delta	F80	56	0.06
Midknight	X639	72	11.11	Delta	F80	72	0.76
Midknight	X639	88	16.90	Delta	F80	88	4.05
Midknight	X639	105/125	47.57	Delta	F80	105/125	55.29
Midknight	X639	144	22.09	Delta	F80	144	39.84
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Midknight	CC	48	0.00	Delta	SFS	48	0.00
Midknight	CC	56	2.84	Delta	SFS	56	0.29
Midknight	CC	72	16.11	Delta	SFS	72	3.23
Midknight	CC	88	26.20	Delta	SFS	88	9.69
Midknight	CC	105/125	46.86	Delta	SFS	105/125	47.74
Midknight	CC	144	7.99	Delta	SFS	144	39.05
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Delta	C35	48	0.05	Delta	Benton	48	0.08
Delta	C35	56	0.05	Delta	Benton	56	4.73
Delta	C35	72	2.00	Delta	Benton	72	17.64
Delta	C35	88	8.25	Delta	Benton	88	20.17
Delta	C35	105/125	43.76	Delta	Benton	105/125	44.18
Delta	C35	144	45.90	Delta	Benton	144	13.21
Cultivar	**Rootstock**	**Size**	**% Fruit**				
Delta	X639	48	0.00				
Delta	X639	56	0.30				
Delta	X639	72	1.17				
Delta	X639	88	3.70				
Delta	X639	105/125	30.80				
Delta	X639	144	64.03				

Table 6.4.8.4. Production of Delta and Midknight Valencia trees on different rootstocks at Vaalharts during the 2006 season.

Cultivar	Rootstock	Kg/tree
Midknight	C35	41.9
Midknight	X639	47.7
Midknight	CC	49.9
Delta	C35	59.4
Delta	X639	48.0
Delta	CC	41.9
Delta	F80	41.8
Delta	SFS	40.3
Delta	Benton	79.7

6.4.9 **Evaluation of Valencias on new imported rootstocks in the Malelane area**

Experiment 416A by J.Joubert (CRI)

Opsomming

Die prestasie van Midknight en Delta Valencias op nuwe, ingevoerde onderstamme op herplant grondtipes moet ondersoek word. Die produksie, interne gehalte en skilkleur moet verbeter word, terwyl vruggrootte moet toeneem. Die produksie het effens gedaal by albei seleksies met 'n geringe toename in vruggrootte. Die Brix° by al die kombinasies het nie aan die uitvoer standaarde voldoen nie (te laag). Hierdie kan 'n moontlike af jaar wees en die volgende evaluasies sal moontlike verduidelikings bied.

Introduction

The performance of Midknight and Delta Valencias on new, imported rootstocks on replant soils must be investigated. The production, internal quality and rind colour must be improved and at the same time fruit size must be increased.

Materials and methods

Seed of HRS 802, HRS 812, HRS 809 and C61 was imported and propagated in 1996 by Esselen Nursery, an accredited nursery in Malelane.

Delta Valencia was budded onto the following three newly imported rootstock hybrids at Esselen nursery in 1997: Sunki x Beneke HRS 802, Sunki x Beneke HRS 812 and Sunki x MTO trifoliate orange (FF6). Midknight Valencia was budded onto Sunki x Beneke HRS 812. The trees were planted at Esselen Nursery in March 1999.

Table 6.4.9.1. Number of trees per rootstock in the Delta and Midknight Valencia trial at Malelane.

Selection	Rootstock	No.of trees
Midknight	Sunki 812	4
Delta	Sunki 812	5
Delta	Sunki 802	5
Delta	FF-6	5

Results and discussions

Midknight Valencia
Internal fruit quality complied with the export specifications, except for the Brixº (Table 6.4.9.2). Brixº of 10.4 was lower than the 2005 season and the juice content (60%) was impressive. Production decreased from 64.9 kg/tree (2005) to 46.8 kg/tree for the 2006 season (Table 6.4.9.4). Fruit size peaked at count 56, followed by count 48 and count 72 (Table 6.4.9.3).

Delta Valencia
Sunki 812 produced the best yield on the trees (65.4 kg/tree) followed by FF-6 (62.9 kg/tree) and Sunki 802 (58.5 kg/tree). All the combinations complied with the export standards for juice and acid content, but not for Brixº. The fruit size peaked at count 72 followed by count 56.

Conclusions and recommendations

The internal quality on Midknight and Delta decreased and did not comply with the packing specifications. There was a decrease in yield with a slight increase in fruit size this season. Theft might be one of the reasons explaining this scenario.

Table 6.4.9.2. Internal fruit quality of Midknight and Delta Valencias on different rootstocks at Esselen Nursery (Malelane) on 20 July 2006.

Selection	Root-stock	Count	Juice %	Brix º	Acid %	Ratio	Ave. seed	Colour
Midknight	Sunki 812	64-48	60.0	10.40	1.19	8.74	0.2	T1
Delta	Sunki 812	72-56	56.6	10.20	1.31	7.79	0.0	T1
Delta	Sunki 802	88-48	57.4	10.10	1.15	8.78	0.0	T1
Delta	FF-6	72-48	58.3	9.50	0.92	10.33	0.0	T1

Table 6.4.9.3. Fruit size distribution at Esselen nursery during the 2006 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midknight	Sunki 812	48	31.95	Delta	Sunki 802	48	2.68
Midknight	Sunki 812	56	43.70	Delta	Sunki 802	56	18.31
Midknight	Sunki 812	72	15.72	Delta	Sunki 802	72	29.43
Midknight	Sunki 812	88	4.66	Delta	Sunki 802	88	21.24
Midknight	Sunki 812	105/125	3.11	Delta	Sunki 802	105/125	23.16
Midknight	Sunki 812	144	0.86	Delta	Sunki 802	144	5.18
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Delta	Sunki 812	48	7.95	Delta	FF-6	48	3.59
Delta	Sunki 812	56	28.72	Delta	FF-6	56	26.41
Delta	Sunki 812	72	28.46	Delta	FF-6	72	31.21
Delta	Sunki 812	88	19.57	Delta	FF-6	88	21.43
Delta	Sunki 812	105/125	13.50	Delta	FF-6	105/125	15.73
Delta	Sunki 812	144	1.79	Delta	FF-6	144	1.63

Table 6.4.9.4. Production per tree of Midknight and Delta Valencia trees on different rootstocks at Esselen Nursery (Malelane) during the 2006 season.

Cultivar	Rootstock	Kg/tree
Midknight	Sunki 812	46.8
Delta	Sunki 812	65.4
Delta	Sunki 802	58.5
Delta	FF-6	62.9

6.4.10 **Evaluation of Grapefruit varieties on new imported rootstocks in the Swaziland area**
Experiment 416B by J.Joubert (CRI)

Opsomming

Die prestasie van pomelo variëteite op nuwe, ingevoerde onderstamme op swaar, herplant grondtipes moet ondersoek word. Die produksie, vruggrootte, interne gehalte en skilkleur moet verbeter word. Star Ruby en Marsh het 'n beter produksie gelewer (vestig goed) nadat die bome herplant is vanaf Tambankulu Estate. Die gemiddelde vruggrootte het dieselfde gebly op telling 48 vir Star Ruby, en telling 40 vir Marsh in vergelyking met 2005. Albei seleksies het aan die minimum uitvoer standaarde vir sap% voldoen, maak nie aan die Brix° nie. Die nuwe aanplantings het goed gevestig en is vir die eerste seisoen ge-evalueer. Intern het die vrugte baie goed gevaar en aan al die uitvoer spesifikasies voldoen.

Introduction

The performance of grapefruit cultivars on new, imported rootstocks on heavy, replant soils must be investigated. The production, fruit size, internal quality and rind colour must be improved.

Materials and methods

Seed of HRS 802, HRS 812, HRS 809 and C61 was imported and propagated in 1996 by Esselen Nursery, an accredited nursery in Malelane.

Star Ruby grapefruit was budded onto the four rootstock hybrids, Marsh grapefruit onto three rootstocks, and Oroblanco onto one rootstock in 1997. The newly imported rootstock hybrids include: Pummelo x trifoliate orange HRS 802, Changsa x English large flowered trifoliate orange HRS 809, Sunki x Beneke HRS 812 and Sunki x macrophylla C61. The trees were planted at Tambankulu Estates, Swaziland, in 1999. Experimental trees at Tambankulu Estates were transplanted at Tambuti Estates in Swaziland during November 2000 as certain orchards were to be removed from Tambankulu Estates. The trees were cut back and painted with white PVA. Making use of an excavator, the trees were uprooted and transplanted immediately at the new site. The trees were well watered and in good condition at the time of transplanting.

There was a second planting in 2003, 10 trees Marsh, NelRuby and Star Ruby all on C35, MxT, SC, and X639.

Table 6.4.10.1. Number of trees per rootstock in the grapefruit trial at Tambuti, Swaziland.

Planted 2000		
Selection	**Rootstock**	**No.of trees**
Marsh	812	1
Marsh	809	2
Marsh	C61	4
Star Ruby	C32	4
Star Ruby	802	2
Star Ruby	809	1
Star Ruby	812	2
Star Ruby	C61	5
Star Ruby	C35	4
Star Ruby	SC	8
Planted 2003		
Selection	**Rootstock**	**No.of trees**
Marsh	C35	10
Marsh	MxT	10
Marsh	SC	10
Marsh	X639	10
NelRuby	C35	10
NelRuby	MxT	10
NelRuby	SC	10
NelRuby	X639	10
Star Ruby	C35	10
Star Ruby	MxT	10
Star Ruby	SC	10
Star Ruby	X639	10

Results and discussions

Planted 2000:

Marsh
Marsh on Sunki 812 (Table 6.4.10.2) produced the highest juice content (57.6%) followed by C61 (56.3%) and Sunki 809 (56.2%). The Brixº values (Table 6.4.10.2) were below the export minimum of 9 for the Japan markets, ranging from 7.6 to 8.7º. The fruit size production (Table 6.4.10.3) peaked at count 40 followed by count 48 and 36. Marsh in combination with rootstock C61 (Table 6.4.10.4) produced the best yield (116 kg/tree) followed by Sunki 812 (111 kg/tree) and Sunki 809 (108.3 kg/tree).

Star Ruby
The highest juice content (Table 6.4.10.2) was produced on Sunki 812 (61.6%) followed by C32 (60.4%) and SC (59%). All the combinations complied with the juice content export standards, but unfortunately only C32 (9.4) and C35 (9) was above the minimum Brixº (Table 6.4.10.2). The highest fruit size production peaked at count 40/36, followed by count 36/40 and count 48/27 (Table 6.4.10.3). Star Ruby on C32 produced the best yield on the trees with 155 kg followed by Sunki 802 (131.3 kg/tree) and SC (130 kg/tree) (Table 6.4.10.4).

Planted 2003:

Marsh
All the combinations produced a Brixº above 9, complying with the export standards. MxT outperformed the rest of the rootstocks with 10.3 Brixº. C35 produced the best juice contend (56.1%) followed by X639 (55.1%) and SC (53.4%) (Table 6.4.10.5). Marsh produced the largest fruit size count at 40, followed by count 36 and count 32 (Table 6.4.10.6). Marsh on SC outperformed the rest of the combinations and produced 41.5 kg/tree. The other rootstocks varied between 7.6 and 19.1 kg/tree (Table 6.4.10.7).

NelRuby
Internally NelRuby performed very promising this season and comply with all the minimum export standards. The highest juice content was produced on X639 with 59.5%, and Brixº on MxT with 10.8 (Table 6.4.10.5).

Fruit size peaked at count 48 followed by count 40 and count 36. Considering that count 48, followed by count 40 and count 36 was ranked from the highest percentage fruit per rootstock to the lowest percentage (Table 6.4.10.6). NelRuby on SC produced 19.4 kg/tree by the time of harvest, followed by C35 (12.4%) and MxT (10.6%) (Table 6.4.10.7).

Star Ruby
Star Ruby on all the rootstocks complied with the export standards. The fruit produced on MxT resulted in the best juice content (58.4%) and Brixº (10.4) for this trial (Table 6.4.10.5). Al the fruit size counts peaked between 48/40 and 36/64 (Table 6.4.10.6). Production peaked at 19.9 kg/tree on SC, followed by MxT (12.6 kg/tree) and C35 (4.6 kg/tree) (Table 6.4.10.7).

Conclusions and recommendations

Planted 2000:
There was a slight decrease in Brixº for this season on the Marsh trees, but the juice content remained the same. The fruit size distribution stayed the same and this season C61 produced the best crop in comparison to Sunki 809 the previous season.

Star Ruby produced similar juice contents internally with the same scenario of lower Brixº. The fruit size increased by at least one count size compared to 2005. Yield production on the trees decreased, but C32 remained the best with 155kg/tree.

Planted 2003:
These combinations were evaluated for the first time this season, bearing enough fruit to sample. The internal quality on all three selections looks very promising and complied with the export standards. The trees are still too young to make conclusions on fruit size and production. Evaluations will continue.

Table 6.4.10.2. Internal fruit quality data of grapefruit on different rootstocks at Tambuti Estates on 25 May 2006 (Planted 2000).

Selection	Root-stock	Count	Juice %	Brix º	Acid %	Ratio	Ave. seed	Colour
Marsh	C61	56-32	56.3	7.60	1.07	7.10	3.9	T1-4
Marsh	Sunki 809	40-23	56.2	7.90	1.05	7.52	4.3	T2-5
Marsh	Sunki 812	48-23	57.6	8.70	1.13	7.70	4.0	T3-4
TSR	C32	40-32	60.4	9.40	1.19	7.90	0.2	T1
TSR	C35	40-23	58.3	9.00	1.11	8.11	0.3	T1
TSR	C61	40-23	56.7	7.20	0.99	7.27	0.8	T1
TSR	Sunki 802	40-23	57.9	7.30	1.10	6.64	0.4	T1-2
TSR	Sunki 809	40-23	56.5	7.30	1.08	6.76	0.3	T1
TSR	Sunki 812	40-27	61.6	8.80	1.15	7.65	0.8	T1-2
TSR	SC	40-23	59.0	8.20	1.14	7.19	0.2	T1

Table 6.4.10.3. Fruit size distribution per rootstock at Tambuti Estate during the 2006 season (Planted 2000).

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	C 61	27	23.94	Star Ruby	C 35	27	11.17
Star Ruby	C 61	32	11.67	Star Ruby	C 35	32	8.00
Star Ruby	C 61	36	21.93	Star Ruby	C 35	36	24.56
Star Ruby	C 61	40	26.06	Star Ruby	C 35	40	25.75
Star Ruby	C 61	48	14.98	Star Ruby	C 35	48	25.75
Star Ruby	C 61	64	1.42	Star Ruby	C 35	64	4.75
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Star Ruby	Sunki 812	27	8.79	Star Ruby	SC-C1	27	15.24
Star Ruby	Sunki 812	32	8.79	Star Ruby	SC-C1	32	12.64
Star Ruby	Sunki 812	36	19.48	Star Ruby	SC-C1	36	30.17
Star Ruby	Sunki 812	40	23.75	Star Ruby	SC-C1	40	26.78
Star Ruby	Sunki 812	48	33.49	Star Ruby	SC-C1	48	13.82

Star Ruby	Sunki 812	64	5.70	Star Ruby	SC-C1	64	1.34
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Star Ruby	Sunki 809	27	8.95	Marsh	C 61	27	7.02
Star Ruby	Sunki 809	32	11.28	Marsh	C 61	32	6.10
Star Ruby	Sunki 809	36	31.13	Marsh	C 61	36	20.98
Star Ruby	Sunki 809	40	29.57	Marsh	C 61	40	33.18
Star Ruby	Sunki 809	48	17.51	Marsh	C 61	48	29.14
Star Ruby	Sunki 809	64	1.56	Marsh	C 61	64	3.59
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Star Ruby	Sunki 802	27	10.94	Marsh	Sunki 812	27	2.34
Star Ruby	Sunki 802	32	10.02	Marsh	Sunki 812	32	7.03
Star Ruby	Sunki 802	36	33.44	Marsh	Sunki 812	36	20.31
Star Ruby	Sunki 802	40	29.89	Marsh	Sunki 812	40	34.77
Star Ruby	Sunki 802	48	13.87	Marsh	Sunki 812	48	32.81
Star Ruby	Sunki 802	64	1.85	Marsh	Sunki 812	64	2.73
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Star Ruby	C 32	27	19.03	Marsh	Sunki 809	27	8.54
Star Ruby	C 32	32	12.05	Marsh	Sunki 809	32	5.03
Star Ruby	C 32	36	21.83	Marsh	Sunki 809	36	24.54
Star Ruby	C 32	40	26.31	Marsh	Sunki 809	40	30.95
Star Ruby	C 32	48	17.51	Marsh	Sunki 809	48	27.44
Star Ruby	C 32	64	3.26	Marsh	Sunki 809	64	3.51

Table 6.4.10.4. Production per tree of grapefruit on different rootstocks at Tambuti Estates during 2006 (Planted 2000).

Cultivar	Rootstock	Kg/tree
Star Ruby	C 61	72.7
Star Ruby	Sunki 812	82.8
Star Ruby	Sunki 809	101.5
Star Ruby	Sunki 802	131.3
Star Ruby	SC-C1	130.0
Star Ruby	C 32	155.0
Star Ruby	C 35	128.0
Marsh	C 61	116.0
Marsh	Sunki 812	111.0
Marsh	Sunki 809	108.3

Table 6.4.10.5. Internal fruit quality data of grapefruit on different rootstocks at Tambuti Estates on 25 May 2006 (Planted 2003).

Selection	Root-stock	Count	Juice %	Brix º	Acid %	Ratio	Ave. seed	Colour
Marsh	C35	36-23	56.1	9.40	1.08	8.70	5.0	T1-2
Marsh	MxT	36-27	52.5	10.30	1.27	8.11	5.3	T1-2
Marsh	SC	32-23	53.4	9.90	1.16	8.53	4.9	T5
Marsh	X639	48-27	55.1	9.30	1.07	8.69	4.1	T3-5
NelRuby	C35	32-23	57.8	10.40	0.97	10.72	3.0	T1-2
NelRuby	MxT	56-27	56.7	10.80	1.07	10.09	3.2	T2-3
NelRuby	SC	48-27	55.1	10.40	1.00	10.40	1.9	T2-3
NelRuby	X639	48-23	59.5	10.30	1.00	10.30	2.4	T2
TSR	C35	36-23	55.7	10.10	1.22	8.28	0.9	T1-2
TSR	MxT	40-32	58.4	10.40	1.21	8.60	0.5	T1-2
TSR	SC	40-23	56.5	9.70	1.14	8.51	0.3	T1
TSR	X639	48-27	58.1	9.70	1.02	9.51	0.4	T1-2

Table 6.4.10.6. Fruit size distribution per rootstock at Tambuti Estate during the 2006 season (Planted 2003).

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Marsh	C 35	27	34.54	Nelruby	SC	27	2.33
Marsh	C 35	32	20.05	Nelruby	SC	32	4.29
Marsh	C 35	36	21.50	Nelruby	SC	36	15.92
Marsh	C 35	40	16.18	Nelruby	SC	40	30.05
Marsh	C 35	48	6.52	Nelruby	SC	48	40.43
Marsh	C 35	64	1.21	Nelruby	SC	64	6.98
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Marsh	MxT	27	4.34	Nelruby	X639	27	1.50
Marsh	MxT	32	7.14	Nelruby	X639	32	3.00
Marsh	MxT	36	21.68	Nelruby	X639	36	12.36
Marsh	MxT	40	35.97	Nelruby	X639	40	31.46
Marsh	MxT	48	26.79	Nelruby	X639	48	44.94
Marsh	MxT	64	4.08	Nelruby	X639	64	6.74
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Marsh	SC	27	20.94	Star Ruby	C 35	27	3.70
Marsh	SC	32	18.07	Star Ruby	C 35	32	2.22
Marsh	SC	36	36.04	Star Ruby	C 35	36	19.26
Marsh	SC	40	17.86	Star Ruby	C 35	40	29.63
Marsh	SC	48	4.41	Star Ruby	C 35	48	28.15
Marsh	SC	64	2.67	Star Ruby	C 35	64	17.04
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Marsh	X639	27	8.49	Star Ruby	MxT	27	1.92
Marsh	X639	32	10.38	Star Ruby	MxT	32	2.56
Marsh	X639	36	23.58	Star Ruby	MxT	36	12.18
Marsh	X639	40	38.68	Star Ruby	MxT	40	27.88
Marsh	X639	48	12.74	Star Ruby	MxT	48	39.42
Marsh	X639	64	6.13	Star Ruby	MxT	64	16.03
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Nelruby	C 35	27	9.37	Star Ruby	SC	27	4.12
Nelruby	C 35	32	5.44	Star Ruby	SC	32	8.04
Nelruby	C 35	36	22.05	Star Ruby	SC	36	25.77
Nelruby	C 35	40	26.59	Star Ruby	SC	40	29.90
Nelruby	C 35	48	26.28	Star Ruby	SC	48	27.42
Nelruby	C 35	64	10.27	Star Ruby	SC	64	4.74
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Nelruby	MxT	27	1.53	Star Ruby	X639	27	0.46
Nelruby	MxT	32	1.53	Star Ruby	X639	32	1.37
Nelruby	MxT	36	11.66	Star Ruby	X639	36	7.31
Nelruby	MxT	40	28.53	Star Ruby	X639	40	19.63
Nelruby	MxT	48	44.17	Star Ruby	X639	48	55.25
Nelruby	MxT	64	12.58	Star Ruby	X639	64	15.98

Table 6.4.10.7. Production per tree of grapefruit on different rootstocks at Tambuti Estates during 2006 (Planted 2000).

Cultivar	Rootstock	Kg/tree
Marsh	C35	19.1
Marsh	MxT	16.1
Marsh	SC	41.5
Marsh	X639	7.6
Nelruby	C35	12.4
Nelruby	MxT	10.6
Nelruby	SC	19.4
Nelruby	X639	8.6
Star Ruby	C35	4.6
Star Ruby	MxT	12.9
Star Ruby	SC	19.9
Star Ruby	X639	5.9

6.4.11 **Evaluation of various Valencia selections on different rootstocks in the Komatipoort area**

Experiment 590B by J.Joubert (CRI)

Opsomming

Evalueer en bepaal die tuinboukundige potensiaal en vermoë van verskillende Valencia variëteite op verskillende onderstamme. Bepaal die beste onderstam kombinasie vir hierdie nuwe variëteite. Maak betekenisvolle kommersïele aanbevelings vir die produsente. Hierdie onderstam proef is vir die tweede keer ge-oes en die bome is nog jonk. Die verskille in oes produksie het nou grootter geword en waardevolle inligting word beskikbaar. Die kapitaal wat uitgelê word vir vestiging kan gouer in winste omgesit word met vroeër produksie op die bome. Midknight het die grootste toename op so 'n jong ouderdom getoon, alhoewel McClean SL die hoogste produksie tussen die verskillende kultivars het vir die 2006 seisoen.

Introduction

Evaluate and assess the horticultural performance and capability of various new Valencia selections on different rootstocks. Determine the superior rootstock combinations for these new selections. Be able to make credible commercial recommendations.

Materials and methods

Five trees of each cultivar x rootstock combination were planted in 1998.
Evaluate visually to determine production per tree, trueness to type and compatibility with scion and harvest each tree with the sizer to determine production per tree as well as fruit size distribution per tree. Samples will be taken and internal quality tested and analysed. Fruit colour will be evaluated and analysed.

Table 6.4.11.1. List of cultivar x rootstock combinations in the Valencia trial at TSB Hectorspruit in the Komatipoort area.

Selection	Rootstock
Delta (Control)	C35
Delta (Control)	CC
Delta (Control)	KC
Delta (Control)	MxT
Delta (Control)	SC
Delta (Control)	Terrabella
Delta (Control)	X639
McClean SL	C35
McClean SL	CC
McClean SL	KC
McClean SL	MxT
McClean SL	SC
McClean SL	Terrabella

McClean SL	X639
Midknight	C35
Midknight	CC
Midknight	KC
Midknight	MxT
Midknight	SC
Midknight	Terrabella
Midknight	X639
Portsgate	C35
Portsgate	CC
Portsgate	KC
Portsgate	MxT
Portsgate	SC
Portsgate	Terrabella
Portsgate	X639

Results and discussion

Delta Valencia

Internal fruit quality analysis (Table 6.4.11.2)

- Juice %: All the selections comply with the export standards above 52% juice content. MxT produced the highest level (59.1%) followed by X639 (58.3%) and KC (57.9%).
- Brixº: CC produced the highest Brixº (10.8) followed by C35 (10.5) and KC (10.4). All the other combinations were below the minimum levels for packing.
- Acid%: All the rootstocks produced a too low acid level below 0.85% and did not comply with export standards.

Fruit size distribution (Table 6.4.11.3)

- The fruit size evaluation shows the largest peak at count 72 on 4 of the 7 rootstocks. The next highest count in fruit size was count 72 with 3 rootstocks. The third highest count evaluated in fruit size was count 88 with 3 rootstocks. Considering that count 72, followed by count 72 again and count 88 was ranked from the highest percentage fruit per rootstock to the lowest percentage.

Production per tree (Table 6.4.11.4)

- SC produced the highest yield per tree (57.2 kg), followed by TB with 32.2 kg/tree and C35 with 31.1 kg/tree

McClean SL

Internal fruit quality analysis (Table 6.4.11.2)

- Juice %: X639 produced the highest juice content (61.8%) followed by SC (59.5%) and CC (59.4%). All the selections comply with the export standards above 48% juice content.
- Brixº: SC and C35 did not comply with the minimum export standards and produced the lowest Brixº. KC, MxT, TB and X639 produced a 10.3 Brixº and CC 9.6.
- Acid%: All the selections comply with the minimum standards. MxT produced the highest level of 0.97% followed by SC (0.82%) and KC (0.76%).

Fruit size distribution (Table 6.4.11.3)

- The fruit size evaluation shows the largest peak at count 56 on 7 of the 7 rootstocks. The next highest count in fruit size was count 72 with 6 rootstocks. The third highest count evaluated in fruit size was count 88/48 with 3 of the 7 rootstocks.

Production per tree (Table 6.4.11.4)

SC produced the highest yield per tree (77kg), followed by C35 with 51.2 kg/tree and X639 with 42.7 kg/tree

Midknight

Internal fruit quality analysis (Table 6.4.11.2)

- Juice %: All the selections comply with the export standards above 52% juice content. CC produced the highest level (62.4%) followed by MxT (61.6%) and X639 (61.4%).
- Brixº: C35 (10.7º) and X639 (11.2º) were the only two rootstock complying with the export standards. The rest of the rootstocks was below the minimum level.
- Acid%: MxT produced the highest acid content of 0.97% followed by C35 with 0.95%. All the other rootstocks did not comply with the export standards.

Fruit size distribution (Table 6.4.11.3)

- The fruit size evaluation shows the largest peak at count 56 on 6 of the 7 rootstocks. The next highest count in fruit size was count 48/72 with 3 rootstocks. The third highest count evaluated in fruit size was once again count 72 with 4 rootstocks.

Production per tree (Table 6.4.11.4)

SC produced the highest yield per tree (57.2 kg), followed by TB with 32.2 kg/tree and C35 with 31.1 kg/tree

Portsgate

Internal fruit quality analysis (Table 6.4.11.2)

- Juice %: All the selections comply with the export standards above 48% juice content. SC produced the highest juice level with 60.4%.
- Brixº: C35, MxT, SC and X639 was below the minimum Brixº level. CC produced the highest level with 10.6º.
- Acid%: Portsgate produced the lowest acid contents in comparison to the other three cultivars, but still above the minimum export level. SC was the highest level with 0.86%.

Fruit size distribution (Table 6.4.11.3)

- The fruit size evaluation shows the largest peak at count 56 on 7 of the 7 rootstocks. The next highest count in fruit size was count 72 with 7 rootstocks. The third highest count evaluated in fruit size was count 88 with 6 rootstocks. Considering that count 56, followed by count 72 and 88 was ranked from the highest percentage fruit per rootstock to the lowest percentage.

Production per tree (Table 6.4.11.4)

- SC produced the highest yield per tree (64.4 kg), followed by TB with 44.5 kg/tree and C35 with 43.7 kg/tree

Conclusions and recommendations

The fruit set and crop production on the trees increased considerably with better internal qualities and fruit size. Compare the 2005 and 2006 season's yield production per tree and concentrate on the immaculate increase for Midknight. Delta produced 24.9 kg/tree on MxT and increased to 57.2 kg/tree on SC. McClean SL increased from 36.5 kg/tree to 77 kg/tree on SC. Midknight increased with the best crop production on the trees from 2.8 kg/tree to an amazing 57.2 kg/tree. Portsgate increased production from 32.7 kg/tree to 64.4 kg/tree.

The trial looks promising at this stage. It will be very valuable to evaluate the production increase on the young trees. Over the long term this will give an indication of the precocity of the combinations.

Table 6.4.11.2. Internal fruit quality data for Valencias on different rootstocks at TSB Hectorspruit on 24 July 2006.

Selection	Root-stock	Count	Juice %	Brix º	Acid %	Ratio	Ave. seed	Colour
Delta	C35	72-48	54.5	10.50	0.74	14.19	0.0	T1
Delta	CC	88-56	56.0	10.80	0.65	16.62	0.0	T1
Delta	KC	88-56	57.9	10.40	0.76	13.68	0.0	T1
Delta	MxT	72-48	59.1	9.60	0.77	12.47	0.0	T1
Delta	SC	88-56	57.6	9.60	0.84	11.43	0.0	T1
Delta	TB	125-48	57.1	9.80	0.79	12.41	0.0	T1
Delta	X639	72-48	58.3	9.90	0.76	13.03	0.0	T1
McClean SL	C35	64-36	58.4	9.20	0.70	13.14	0.0	T1
McClean SL	CC	64-40	59.4	9.60	0.72	13.33	0.0	T1-2
McClean SL	KC	72-48	58.4	10.30	0.76	13.55	0.0	T1
McClean SL	MxT	72-40	58.6	10.30	0.97	10.62	0.0	T1
McClean SL	SC	72-40	59.5	9.20	0.82	11.22	0.0	T1-2
McClean SL	TB	88-48	59.3	10.30	0.74	13.92	0.0	T1
McClean SL	X639	72-40	61.8	10.30	0.75	13.73	0.0	T1
Midknight	C35	64-40	61.3	10.70	0.95	11.26	0.2	T1
Midknight	CC	72-56	62.4	10.10	0.80	12.63	0.0	T1
Midknight	KC	88-48	59.9	10.10	0.84	12.02	0.6	T1
Midknight	MxT	56-48	61.6	10.00	0.97	10.31	0.2	T1
Midknight	SC	72-48	58.6	10.30	0.90	11.44	0.3	T1
Midknight	TB	56-36	59.7	10.00	0.90	11.11	0.0	T1
Midknight	X639	72-40	61.4	11.20	0.73	15.34	0.0	T1
Portsgate	C35	72-48	57.6	9.60	0.71	13.52	0.0	T1
Portsgate	CC	72-48	57.8	10.60	0.69	15.36	0.0	T1
Portsgate	KC	72-56	57.9	10.40	0.72	14.44	0.0	T1
Portsgate	MxT	64-48	58.4	9.70	0.82	11.83	0.0	T1
Portsgate	SC	72-48	60.4	9.30	0.86	10.81	0.0	T1
Portsgate	TB	72-40	57.8	10.00	0.77	12.99	0.0	T1
Portsgate	X639	64-48	55.8	9.50	0.76	12.50	0.0	T1

Table 6.4.11.3. Fruit size distribution per rootstock at TSB Hectorspruit during the 2006 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midknight	C35	48	34.46	Delta	C35	48	6.19
Midknight	C35	56	43.69	Delta	C35	56	26.83
Midknight	C35	72	14.86	Delta	C35	72	30.96
Midknight	C35	88	3.15	Delta	C35	88	16.14
Midknight	C35	105/125	3.15	Delta	C35	105/125	16.89
Midknight	C35	144	0.68	Delta	C35	144	3.00
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Midknight	CC	48	14.01	Delta	CC	48	0.35
Midknight	CC	56	40.06	Delta	CC	56	14.01
Midknight	CC	72	27.17	Delta	CC	72	34.60
Midknight	CC	88	7.56	Delta	CC	88	23.53
Midknight	CC	105/125	9.80	Delta	CC	105/125	20.93
Midknight	CC	144	1.40	Delta	CC	144	6.57
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Midknight	KC	48	11.05	Delta	KC	48	4.17

Midknight	KC	56	29.07	Delta	KC	56	18.48
Midknight	KC	72	21.51	Delta	KC	72	25.00
Midknight	KC	88	11.63	Delta	KC	88	17.57
Midknight	KC	105/125	14.53	Delta	KC	105/125	26.99
Midknight	KC	144	12.21	Delta	KC	144	7.79
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Midknight	MxT	48	22.04	Delta	MxT	48	5.29
Midknight	MxT	56	55.92	Delta	MxT	56	32.19
Midknight	MxT	72	15.46	Delta	MxT	72	32.05
Midknight	MxT	88	4.28	Delta	MxT	88	17.60
Midknight	MxT	105/125	1.97	Delta	MxT	105/125	11.87
Midknight	MxT	144	0.33	Delta	MxT	144	1.00
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Midknight	SC	48	29.59	Delta	SC	48	5.87
Midknight	SC	56	51.12	Delta	SC	56	22.97
Midknight	SC	72	15.38	Delta	SC	72	31.38
Midknight	SC	88	2.84	Delta	SC	88	20.63
Midknight	SC	105/125	0.95	Delta	SC	105/125	17.79
Midknight	SC	144	0.12	Delta	SC	144	1.37
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Midknight	TB	48	43.91	Delta	TB	48	1.13
Midknight	TB	56	42.12	Delta	TB	56	7.07
Midknight	TB	72	9.38	Delta	TB	72	22.35
Midknight	TB	88	3.39	Delta	TB	88	23.47
Midknight	TB	105/125	1.00	Delta	TB	105/125	33.76
Midknight	TB	144	0.20	Delta	TB	144	12.22
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Midknight	X639	48	12.05	Delta	X639	48	1.82
Midknight	X639	56	31.92	Delta	X639	56	20.88
Midknight	X639	72	26.38	Delta	X639	72	27.08
Midknight	X639	88	12.38	Delta	X639	88	18.00
Midknight	X639	105/125	13.68	Delta	X639	105/125	25.11
Midknight	X639	144	3.58	Delta	X639	144	7.11
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Portsgate	C35	48	13.94	McClean SL	C35	48	12.08
Portsgate	C35	56	33.76	McClean SL	C35	56	36.34
Portsgate	C35	72	28.77	McClean SL	C35	72	23.42
Portsgate	C35	88	13.17	McClean SL	C35	88	15.13
Portsgate	C35	105/125	8.95	McClean SL	C35	105/125	11.55
Portsgate	C35	144	1.41	McClean SL	C35	144	1.47
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Portsgate	CC	48	9.88	McClean SL	CC	48	18.29
Portsgate	CC	56	25.68	McClean SL	CC	56	37.17
Portsgate	CC	72	24.69	McClean SL	CC	72	26.99
Portsgate	CC	88	20.49	McClean SL	CC	88	9.29
Portsgate	CC	105/125	16.30	McClean SL	CC	105/125	6.05
Portsgate	CC	144	2.96	McClean SL	CC	144	2.21
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Portsgate	KC	48	6.20	McClean SL	KC	48	17.47
Portsgate	KC	56	36.97	McClean SL	KC	56	41.84
Portsgate	KC	72	26.28	McClean SL	KC	72	23.22
Portsgate	KC	88	14.53	McClean SL	KC	88	8.25
Portsgate	KC	105/125	10.68	McClean SL	KC	105/125	8.06
Portsgate	KC	144	5.34	McClean SL	KC	144	1.15

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Portsgate	MxT	48	11.62	McClean SL	MxT	48	10.04
Portsgate	MxT	56	39.14	McClean SL	MxT	56	24.07
Portsgate	MxT	72	29.29	McClean SL	MxT	72	24.07
Portsgate	MxT	88	13.64	McClean SL	MxT	88	18.43
Portsgate	MxT	105/125	6.06	McClean SL	MxT	105/125	19.81
Portsgate	MxT	144	0.25	McClean SL	MxT	144	3.58
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Portsgate	SC	48	8.42	McClean SL	SC	48	14.23
Portsgate	SC	56	30.82	McClean SL	SC	56	38.03
Portsgate	SC	72	27.24	McClean SL	SC	72	27.26
Portsgate	SC	88	18.19	McClean SL	SC	88	11.82
Portsgate	SC	105/125	14.14	McClean SL	SC	105/125	7.61
Portsgate	SC	144	1.19	McClean SL	SC	144	1.05
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Portsgate	TB	48	3.31	McClean SL	TB	48	6.05
Portsgate	TB	56	30.63	McClean SL	TB	56	28.78
Portsgate	TB	72	28.91	McClean SL	TB	72	24.67
Portsgate	TB	88	18.17	McClean SL	TB	88	16.32
Portsgate	TB	105/125	15.66	McClean SL	TB	105/125	18.38
Portsgate	TB	144	3.31	McClean SL	TB	144	5.80
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Portsgate	X639	48	6.84	McClean SL	X639	48	8.67
Portsgate	X639	56	28.48	McClean SL	X639	56	33.21
Portsgate	X639	72	26.93	McClean SL	X639	72	25.40
Portsgate	X639	88	23.84	McClean SL	X639	88	16.60
Portsgate	X639	105/125	10.82	McClean SL	X639	105/125	13.14
Portsgate	X639	144	3.09	McClean SL	X639	144	2.97

Table 6.4.11.3. Production per tree of Valencia selections on different rootstocks at TSB Hectorspruit during the 2006 season.

Cultivar	Rootstock	Kg/tree (05)	Kg/tree (06)
Midknight	C35	2.0	31.1
Midknight	CC	1.9	20.5
Midknight	KC	0.6	11.3
Midknight	MxT	1.3	19.8
Midknight	SC	2.8	57.2
Midknight	TB	2.1	32.2
Midknight	X639	0.8	17.1
Portsgate	C35	13.4	43.7
Portsgate	CC	5.4	21.5
Portsgate	KC	14.0	24.5
Portsgate	MxT	14.2	21.8
Portsgate	SC	32.7	64.4
Portsgate	TB	18.7	44.5
Portsgate	X639	17.9	22.5
Delta	C35	5.3	27.0
Delta	CC	14.8	32.6
Delta	KC	3.4	20.1
Delta	MxT	24.9	37.2
Delta	SC	21.3	52.2
Delta	TB	7.3	26.5
Delta	X639	10.1	31.1
McClean SL	C35	26.7	51.2

McClean SL	CC	18.2	39.4
McClean SL	KC	17.2	30.6
McClean SL	MxT	6.3	36.5
McClean SL	SC	36.5	77.0
McClean SL	TB	15.1	37.9
McClean SL	X639	20.9	42.7

7 CITRUS IMPROVEMENT PROGRAMME 2006

By Thys du Toit and Louise Jackson (CRI)

7.1 PROGRAMME SUMMARY

Citrus Foundation Block: A total of 2 076 851 buds were supplied by the Citrus Foundation Block during 2006, which is 550 677 less than the quantity of buds supplied in 2005. The two most popular cultivars in 2006 were Star Ruby (17.9% of total supplied) and Midknight (13.0%). The drastic reduction in the demand for citrus seed – 1537 litres in 2006 compared to 2354 litres in 2005 - can be attributed to a shrink in demand from export markets in addition to loss of seed fruit during the August floods. Nine new cultivars were received from the ITSC for establishment, evaluation and increase, compared to seven cultivars in 2005. The second phase of the second insect-proof house, which can accommodate 45 700 increase trees was completed. Increase trees established from category 1 and 2 cultivars total 36 560 trees. A policy document relating to visits to the Citrus Foundation Block was implemented.

Tree Certification: During 2006, 1 613 914 trees were certified, compared to 1 866 176 trees in 2005.

Nursery Accreditation: 20 nurseries were audited in May and November 2006. All of the nurseries were accredited. In general the standard in nurseries is high. Growers must visit nurseries and specify in writing which category tree they wish to purchase.

Statutory Improvement Programme: The document in support of the application to have the Citrus Improvement Scheme registered has been submitted to the Registrar of Plant Improvement for provisional approval before it will be submitted to the Minister of Agriculture.

Protected zone around the CFB: The final document to establish a citrus-free area around the Citrus Foundation Block has been submitted to the Department of Agriculture, and we await their response.

Shoot-tip grafting and Gene bank: Shoot tip grafting is currently being performed on 32 cultivars at the Institute for Tropical and Subtropical Crops in Nelspruit. The CRI's Virological Department in Nelspruit has established 235 cultivars to date in a gene bank in its glasshouse. A further 42 cultivars are undergoing shoot tip grafting.

Import Control: A task team of experts has been formed on the recommendation of the Department of Agriculture in order to assist the Department with ensuring that no harmful organisms are brought into the country with imported citrus propagation material.

PROGRAMOPSOMMING

Sitrus Grondvesblok: 'n Totaal van 2 076 851 okuleerhout is deur die Sitrus Grondvesblok verskaf in 2006 wat 550 677 minder is as in 2005. Star Ruby met 17.9% en Midknight met 13.0% van die totaal is die twee mees populêre kultivars. 'n Drastiese afname in die verkope van saad van 1537 liter in 2006 in vergelyking met 2354 liter in 2005 wat toegeskryf word aan die krimpende uitvoer aanvraag asook die verlies aan saad vrugte as gevolg van die vloed in Augustus . Vanaf die ITSG is 9 nuwe kultivars ontvang vir vestiging, evaluering en vermeerdering in vergelyking met 7 in 2005. Die tweede insekbeheerde kweekhuis se tweede fase is votooi waarin 'n totaal van 45 700 vermeerderingsblokbome gevestig kan word, 36 560 is reeds geokuleer met kategorie 1 en 2 kultivars. 'n Beleidsdokument vir besoeke aan die Sitrus Grondvesblok is geimplimenteer.

Boomsertifisering: 1 613 914 Bome is in 2006 gesertifiseer in vergelyking met 1 866 176 in 2005.

Kwekery Akkreditasie: 20 Kwekerye is in Mei en November besoek en geakkrediteer. Oor die algemeen is die standaard goed. Produsente moet die kwekerye besoek en skriftelik spesifiseer oor die kategorie boom wat verlang word.

Statutêre Verbeteringsprogram: Die dokument ter voorlegging aan die Departement van Landbou om die Suid-Afrikaanse Sitrusverbeteringskema te registreer is tans by die Registrateur van Plantverbetering vir goedkeuring.

Beskermde sone rondom die SGB: Die finale dokument vir 'n sitrusvrye area rondom die SGB is aan die Departement van Landbou voorgelê en ons wag vir terugvoering.

Groeipuntenting en Genebron: By die LNR-ITSG word daar tans op 32 kultivars groeipuntenting gedoen. Die CRI se Virologie Departement in Nelspruit het tot op datum 235 kultivars gevestig as ’n genebron in ’n glashuis by die department en 42 kultivars ondergaan tans groeipuntenting.

Beheer oor invoer: Op aanbeveling van die Departement van Landbou is ’n taakspan kundiges voorgestel om met die departement saam te werk om te verhoed dat skadelike organismes die land binne kom deur middel van sitrus voorplantings materiaal.

An overview of operations in the CIP follows.

Citrus Foundation Block

Budwood supply during 2006 compared to 2 preceding years, 10 most popular cultivar selections.

2006			2005			2004		
Cultivar	**Buds**	**%**	**Cultivar**	**Buds**	**%**	**Cultivar**	**Buds**	**%**
TOTAL	**2076851**		**TOTAL**	**2627528**		**TOTAL**	**2314730**	
Star Ruby	371140	17.9%	Star Ruby	533913	20.3%	Midknight	284815	12.3%
Midknight	268973	13.0%	Midknight	337970	12.9%	Star Ruby	275550	11.9%
Palmer	121493	5.8%	Bahianinha	229710	8.7%	Bahianinha	183180	7.9%
Bahianinha	104820	5.0%	Delta	156165	5.9%	Du Roi	122870	5.3%
Late	95550	4.6%	Turkey	138150	5.3%	Eureka	116350	5.0%
Turkey	81700	3.9%	Palmer	126696	4.8%	Palmer	113680	4.9%
Du Roi	78170	3.8%	Du Roi	84610	3.2%	Delta	109140	4.7%
Washington	75692	3.6%	Autumn Gold	83200	3.2%	Turkey	102440	4.4%
Nadorcott 1	69500	3.3%	Cal.Lane Late	77500	2.9%	Nadorcott 1	67070	2.9%
Delta	60030	2.9%	Washington	73410	2.8%	Eureka SL	59432	2.6%

Budwood supply per area during 2006 compared to 2 preceding years.

Area	2006	%	2005	%	2004	%
Eastern Cape	469452	22.6%	478079	18.2%	427080	18.5%
Western Cape	311494	15.0%	383089	14.6%	368255	15.9%
Northern Cape	34940	1.7%	46850	1.8%	68720	3.0%
KwaZulu-Natal	31550	1.5%	15500	0.6%	16500	0.7%
Limpopo	777085	37.4%	1252503	47.7%	1090435	47.1%
Mpumalanga	312240	15.0%	312552	11.9%	228750	9.9%
North-West	135090	6.5%	82780	3.2%	86990	3.8%
Mozambique	3000	0.1%	12250	0.5%	0	0.0%
Swaziland	2000	0.1%	42225	1.6%	0	0.0%
Zimbabwe			1700	0.1%	28000	1.2%
TOTAL	**2076851**		**2627528**		**2314730**	

Budwood supply per area and variety during 2006 compared to 2 preceding years.

Variety	Year	Eastern Cape	KwaZulu-Natal	Limpopo	Mozambique	Mpumalanga	North-West Province	Northern Cape	Swaziland	Western Cape	Zimbabwe	Total 2006	Total 2005	Total 2004
Clementine	2006	900		1000	100	1800	1400	1700		25050		**31950**		
Clementine	2005	7700			100		1000	10100		19200			**38100**	
Clementine	2004	4610				1500	4820	20500		64425				**95855**
Ellendale	2006				100		200			200		**500**		
Ellendale	2005				100		1600			1000			**2700**	
Ellendale	2004						10	510		750				**1270**
Grapefruit	2006	23600	4000	153620	200	157500	3030	17500		15680		**375130**		
Grapefruit	2005	21000		396863	1050	111550	1050	8850		1690			**542053**	
Grapefruit	2004	22450		228500		60000		6700		1050				**318700**
Grapefruit Hybrid	2006	300		1000	100	3900						**5300**		
Grapefruit Hybrid	2005			5100	100	1800	100						**7100**	
Grapefruit Hybrid	2004			1500				200						**1700**
Kumquat	2006	600	1000	4000		3200	2230			1100		**12130**		
Kumquat	2005		500			3100	3050			2800			**9450**	
Kumquat	2004	50				4850	3000	500		1000				**9400**
Lemon	2006	10000	4000	4900	200	15900	10030	1000		11850		**57880**		
Lemon	2005	30120	1000	28400	500	37900	14400	500		8160			**120980**	
Lemon	2004	94072	1000	71600		26000	18500	500		32650				**244322**
Lime	2006	300	6500	6800	100	8300	4760			2910		**29670**		
Lime	2005	300		1800	100	800	30	400		3200			**6630**	
Lime	2004	500				2000	4000	200		2300				**9000**
Mandarin Hybrid	2006	89900		26070	400	8100	23400	200		53581		**201651**		
Mandarin Hybrid	2005	24398	2000	37950	400	2200	9550	3000		59914			**139412**	
Mandarin Hybrid	2004	21130	3500	11800			20570	760		81120				**138880**

Variety	Year	Eastern Cape	KwaZulu-Natal	Limpopo	Mozambique	Mpumalanga	North-West Province	Northern Cape	Swaziland	Western Cape	Zimbabwe	Total 2006	Total 2005	Total 2004
Midseason	2006									9970		**9970**		
Midseason	2005									1830			**1830**	
Midseason	2004	2900								240				**3140**
Navel	2006	160402	10250	190430	300	46400	51160	6540		115895		**581377**		
Navel	2005	223017	7000	328090	5000	69218	23900	15700		212795			**884720**	
Navel	2004	175320	11000	271280		59050	18250	28350		140400	10000			**713650**
Satsuma	2006	42050	2000	2000	300	5100	1000			385		**52835**		
Satsuma	2005	3344		40300	400	3800	4600			8250			**60694**	
Satsuma	2004	18588	1000	5000		20000	3040	500		24670				**72798**
Valencia	2006	141400	3800	387265	1200	62040	37880	8000	2000	74873		**718458**		
Valencia	2005	168200	5000	414000	4500	82184	23500	8300	42225	64250	1700		**813859**	
Valencia	2004	87460		500755		55350	14800	10000		19650	18000			**706015**
TOTALS PER ANNUM												**2076851**	**2627528**	**2314730**

Summary

The increase in budwood demand anticipated after the good fruit season in 2006 has not yet materialized. This can be attributed to a possible conservative approach on the part of growers in respect of developing new plantings. Star Ruby grapefruit and Midknight Valencia have been the most popular cultivars for the past 3 years. The other cultivars featuring in the ten most popular cultivars vary from year to year.

Seed supplied per rootstock cultivar, in South Africa during 2006 compared to 2 preceding years.

Area Name	Year	C35 Citrange	Carrizo Citrange	Minneola X Trifoliate	Rough Lemon	Rough Lemon (Schaub)	Swingle Citrumelo	Troyer Citrange	Volckameriana	X639	Yuma Citrange	Total 2006	% 2006	Total 2005	% 2005	Total 2004	% 2004
Eastern Cape	2004	20	97		41		20	1	3	23						205	10.7%
	2005	43	101		24		16	40	9	13				246	15.5%		
	2006	27	125		12	2	10	2	5	7		190	20.0%				
KwaZulu-Natal	2004						2			2						5	0.3%
	2005			2			8	4	2	2				18	1.1%		
	2006			2			5	3	3			13	1.4%				
Limpopo	2004	60	400	73	121		437	60	14	52						1217	63.5%
	2005	47	265	25	84		311	110	2	38	4			886	55.7%		
	2006	40	170	20	40	20	142	10	1	15		458	48.2%				
Mpumalanga	2004	5	24		14		22	3		4						72	3.8%
	2005	3	20		8		16	9		2				58	3.6%		
	2006		5		6							11	1.2%				
North-West Province	2004	14	45		7		7			2						75	3.9%
	2005	6	6		12		4		6	4				38	2.4%		
	2006	5	6		6		5		6	4		32	3.4%				
Northern Cape	2004				5					17						22	1.1%
	2005									16				16	1.0%		
	2006					4						4	0.4%				
Western Cape	2004	40	118		30	20	45	63		5						321	16.7%
	2005	50	155		75		20	10		20				329.5	20.7%		
	2006	21	33		45	7	32	59	36	10		243	25.6%				
TOTAL PER ANNUM												**951**		**1591.5**		**1917**	

Seed exported per rootstock cultivar during 2006 compared to 2 preceding years.

Area Name	Year	Australian Trifoliate	C35 Citrange	Carrizo Citrange	Cleopatra Mandarin	Flying Dragon	Minneola X Trifoliate	Rough Lemon	Rough Lemon (Schaub)	Swingle Citrumelo	Troyer Citrange	Volckameriana	X639	Total 2006	% 2006	Total 2005	% 2005	Total 2004	% 2004
Australia/NZ	2004			60				20	11		105							196	9.9%
	2005									8	30					38	5.0%		
	2006					12				12	40			64	10.9%				
Carribbean	2006	2	30	4								4		40	6.8%				
China	2004			1500														1500	75.9%
	2005		20	65			20			70			150			325	42.5%		
	2006			235						22				257	43.9%				
Europe	2005			100												100	13.1%		
	2006		30											30	5.1%				
Far East	2005				30											30	3.9%		
Mozambique	2004			1						7	2		1					11	0.6%
	2006			1			1			2	2	1	1	8	1.4%				
Other African States	2004		2							34	3	118						157	7.9%
	2005			2	2					50	7	1				62	8.1%		
	2006							12		4	11			27	4.6%				
USA	2004			110														110	5.6%
	2005			200												200	26.1%		
	2006		160											160	27.3%				
Vietnam	2005									7						7	0.9%		
Zimbabwe	2004							1			2					3	0.4%	3	0.2%
TOTAL PER ANNUM														**586**		**765**		**1977**	

Seed supplied per rootstock cultivar 2004-2006

Cultivar	2006	%	2005	%	2004	%
Australian Trifoliate	2	0.1%				
C35 Citrange	313	20.4%	169	7.2%	141	3.6%
Carrizo Citrange	579	37.7%	914	38.7%	2355	60.5%
Cleopatra Mandarin			32	1.4%		
Flying Dragon	12	0.8%				
Minneola X Trifoliate	23	1.5%	47	2.0%	73	1.9%
Rough Lemon	121	7.9%	202.5	8.6%	239	6.1%
Rough Lemon (Schaub)	33	2.1%			31	0.8%
Swingle Citrumelo	234	15.2%	517	21.9%	574	14.7%
Troyer Citrange	127	8.3%	210	8.9%	239	6.1%
Volckameriana	56	3.6%	20	0.8%	135	3.5%
X639	37	2.4%	245	10.4%	106	2.7%
Yuma Citrange			4	0.2%		
TOTAL	**1537**		**2360.5**		**3894**	

Seed supplied, local and export 2004-2006

	2006	%	2005	%	2004	%
South Africa	951	61.9%	1591.5	67.5%	1917	49.2%
Export	586	38.1%	765	32.5%	1977	50.8%
TOTAL	**1537**		**2356.5**		**3894**	

Summary

The reduced demand for seed in South Africa matches the reduction in demand for budwood. At the beginning of the seed harvest season in May 2006, 1800 litres of seed from the 2005 crop was dumped due to reduced germination viability because of the seed's age. The demand for seed in 2006, both locally and for the export market was much lower than in 2005. In order to reduce costs not all the seed fruit was harvested and processed. It was felt if demand picked up later in the year this seed could then be harvested and processed on demand. However, during August a storm and flood was experienced which blew the seed fruit off the trees, and mixed them up on the ground, rendering the crop useless. After that export orders were received which could not be fully supplied due to lack of stock.

New Cultivars

During 2006, 9 new cultivars were received from the ITSC, compared to 7 during 2005. Seven of these cultivars are under the control of private cultivar agents and information pertaining to these cultivars is treated as confidential. The 2 open cultivars are Sweet Spring Mandarin Hybrid and Genoa Lemon, which have been reintroduced into the scheme.

New Developments

The final phase of 4 bays of the second insect-controlled greenhouse, which consists of 11 bays of 6,5 x 80 m, was completed. This greenhouse can accommodate a total of 45 700 increase trees in 10 litre plant bags, of which 36 560 have been budded and 28 300 are already in full production. The expired increase trees in Shade-house One have been scrapped and plant bags are currently being prepared for replacement increase trees. This shade-house structure will be replaced in the future by the next phase of insect-controlled greenhouse, which will be erected over the already established increase trees.

Guidelines for visits to the Citrus Foundation Block

Policy Document

Objective

To ensure that the risk of infection by harmful pathogens at the Citrus Foundation Block be kept as low as possible, by restricting access to the facility.

International Visitors

- Application for all proposed visits must be submitted and approved at least one month in advance to the CEO of CRI and the Manager, Citrus Improvement Programme.
- Visits should preferably take place at the end of the visitor's tour to South Africa.
- Group size should be limited to not more than two people, if possible.
- Visitors must be met in person by the Manager, at the controlled entrance.
- No knives or secateurs may be brought into the controlled area.
- No citrus propagation material, leaves or fruit may be brought into the controlled area.
- All visitors will be provided with an overall and their shoe soles will be sterilized at the entrance.
- All visitors will be permitted only as far as the office.
- No visitors will be permitted into the greenhouses.
- If the fruit is in season, a display of fruit will be prepared at the office.
- A slide show depicting activities at the Citrus Foundation Block will be shown to visitors at the office, followed by a question and answer session.
- Visitors will be accompanied by the Manager to outside the controlled area, where the disposable clothing will be placed in a container to be burnt. Overalls will be washed and sterilized after each visit.

South African Visitors

- Application for all proposed visits must be submitted and approved at least one week in advance to the Manager, Citrus Improvement Programme.
- Information must be supplied as to whether the visitors have returned from recent international visits. Where proposed visitors have returned from international visits less than 3 weeks prior to the proposed CFB visit, these applications will automatically be declined.
- Group size should be limited to not more than two people, if possible.
- Visitors must be met in person by the Manager, at the controlled entrance.
- No knives or secateurs may be brought into the controlled area.
- No citrus propagation material, leaves or fruit may be brought into the controlled area.
- All visitors shoe soles will be sterilized at the entrance.
- Generally no visitors will be permitted into the greenhouses. However, the Manager may allow exceptions, provided that visitors wear protective clothing inside the greenhouses.
- If the fruit is in season, a display of fruit will be prepared at the office.
- A slide show depicting activities at the Citrus Foundation Block will be shown to visitors at the office, followed by a question and answer session.
- Visitors will be accompanied by the Manager to outside the controlled area, where any disposable clothing will be placed in a container to be burnt. Overalls will be washed and sterilized after each visit.

Tree Certification

Tree certification per area compared to 2 preceding years.

Area	2006		2005		2004	
	Trees	%	Trees	%	Trees	%
Botswana	2400	0.1%	270	0.0%	-	
Eastern Cape	306167	19.0%	401207	21.5%	530587	44.3%
Gauteng	19000	1.2%	23955	1.3%	-	
KwaZulu-Natal	47382	2.9%	54601	2.9%	-	
Limpopo	237932	14.7%	432375	23.2%	310968	25.9%
Mozambique		0.0%	600	0.0%	-	
Mpumalanga	477365	29.6%	666730	35.7%	181501	15.1%
Namibia	6895	0.4%				
North-West Province	20097	1.2%	30616	1.6%	5250	0.4%
Northern Cape	112966	7.0%	-		-	
Orange Free State	2000	0.1%				
Swaziland	32120	2.0%	39329	2.1%	-	
Western Cape	315727	19.6%	197493	10.6%	170067	14.2%
Zimbabwe	33863	2.1%	19000	1.0%	-	
TOTAL	**1613914**		**1866176**		**1198373**	

Trees registered per area and variety during 2006 compared to 2 preceding years.

Variety	Year	Botswana	Eastern Cape	Gauteng	KwaZulu-Natal	Limpopo	Mozambique	Mpumalanga	Namibia	North-West	Northern Cape	Orange Free State	Swaziland	Western Cape	Zimbabwe	2006 Total	2005 Total	2004 Total
Clementine	2006		343								10000			63549		73892		
	2005		8972					1200		3000				50547			63719	
	2004		15049											55790				70839
Ellendale	2006										500					500		
	2005							250									250	
Grapefruit	2006		19369		33700	102089		125831	20	260	14152		18000			313421		
	2005		5358	3535	32843	99279	200	171806		6000			27269	330			346620	
	2004		450			55169		23055										78674
Grapefruit Hybrid	2006					59		1610								1669		
	2005					1010		250									1260	
	2004					1717		1422										3139
Kumquat	2006													100		100		
	2005													450			450	
Lemon	2006		29172	1600		1526		33429						4285		70012		
	2005		92643	2700	16448	8625		72842		2000				3470			198728	
	2004		91429			835		18657						2198				113119
Lime	2006		250			450								560		1260		
	2004					50												50
Mandarin Hybrid	2006		21059		512	7786		15104	300	2130	500			62623		110014		
	2005		14619			9549		40770		9995				1334			76267	
	2004		36524			36611		42		250				35341				108768
Midseason	2006		900											81		981		
	2005		383											1101			1484	
Navel	2006	2400	141928	8000	3850	16380		176074	425	12832	65672	2000		146258		575819		
	2005	70	197711	20	4800	23952	400	216216		5690				103988			552847	
	2004		313536			90452		68838						29556				502382
Satsuma	2006		25015			5		23515	35	700	3750			4017		57037		
	2005		21075					15270						13411			49756	

Variety	Year	Botswana	Eastern Cape	Gauteng	KwaZulu-Natal	Limpopo	Mozambique	Mpumalanga	Namibia	North-West	Northern Cape	Orange Free State	Swaziland	Western Cape	Zimbabwe	2006 Total	2005 Total	2004 Total
	2004		31814					10528						27707				70049
Valencia	2006		68131	9400	9320	109637		101802	6115	4175	18392		14120	34254	33863	409209		
	2005	200	60446	17700	510	289960		148126		3931			12060	22862	19000		574795	
	2004		41785			134289		58959		5000				19475				259508
TOTALS																1613914	1866176	1206528

Tree Certificate percentage per variety per annum during 2006 compared to 2 preceding years.

Variety	Year	2006 Total	2006 % per Variety	2005 Total	2005 % per Variety	2004 Total	2004 % per Variety
Clementine	2006	73892	4.6%	63719	3.4%	70839	5.9%
Ellendale	2006	500	0.0%	250	0.0%		
Grapefruit	2006	313421	19.4%	346620	18.6%	78674	6.5%
Grapefruit Hybrid	2006	1669	0.1%	1260	0.1%	3139	0.3%
Kumquat	2006	100	0.0%	450	0.0%		
Lemon	2006	70012	4.3%	198728	10.6%	113119	9.4%
Lime	2006	1260	0.1%			50	0.0%
Mandarin Hybrid	2006	110014	6.8%	76267	4.1%	108768	9.0%
Midseason	2006	981	0.1%	1484	0.1%		
Navel	2006	575819	35.7%	552847	29.6%	502382	41.6%
Satsuma	2006	57037	3.5%	49756	2.7%	70049	5.8%
Valencia	2006	409209	25.4%	574795	30.8%	259508	21.5%
GRAND TOTAL		1613914		1866176		1206528	

The demand for trees certification reduced to 1 613 914 trees in 2006 compared to 1 866 176 in 2005. The quantity of trees certified is still significantly lower than the quantity of buds supplied to nurseries each year. Tree certification is still viewed by EurepGAP as a “minor must” for accreditation, and this requirement’s priority cannot easily be changed due to the international scope of EurepGAP. Tree certification is nevertheless important in the EurepGAP context, as a grower must score 95% in total on all the “minor must” categories.

Nursery Accreditation

In total 20 citrus nurseries applied for accreditation and were audited and accredited in May and November 2006. In general the nurseries are maintaining a good standard. Growers should visit nurseries they wish to buy from and ensure that they are satisfied with the standard in the nursery. They should also specify in writing which category tree they wish to be supplied with.

Currently 3 categories of trees are specified in the scheme, as follows:

Category	Name	Description
1	Mini tree	A tree showing bud (scion) growth of at least 15 cm.
2	Whip tree	A tree without any scaffold-branches, but with the stem already hardened off at the intended topping height. Trees may therefore be topped at any height to suit the grower's requirements.
3	Scaffold tree	A tree which has formed scaffold branches at a height required by the grower, with a stem thick enough to support the scaffold.

Any problems with nursery trees should first be discussed with the nursery in an effort to resolve the conflict. If no satisfaction is obtained through this route then contact the Manager, Citrus Improvement Programme, Thys du Toit at: Tel: 041-9925366 Fax: 041-9227416 Cell: 0828892363 E-mail: tdt@cri.co.za.

The following nurseries were accredited during 2006.

Nursery	Address	Telephone
Apapanzi	P O Box 147, Kirkwood, 6120	042 2301483
B F Joubert	P O Box 193, Kirkwood, 6120	042 2300309
Casmar	P O Box 3 Mooinooi, 0325	0145 743152
Du Roi	P O Box 66, Letsitele, 0885	015 3451650
Esselen	P O Box 100, Malelane, 1320	013 7900160
H J Joubert	P O Box 207, Montagu, 6720	023 6142237
Kleinbegin	P O Box 45 , Sunland, 6115	042 2340087
La Rhyn	P O Box 111, Citrusdal, 7340	022 9213541

Nursery	Address	Telephone
Letsitele	P O Box 1, Letsitele, 0885	015 3451600
Mistkraal	P O Box 16, Kirkwood, 6120	042 2301461
Ngwenya	P O Box 36, Malelane, 1320	013 7903004
Nucellar	P O Box 69, Simondium, 7670	021 8741033
Paksaam	P O Box 16, Patensie, 6335	042 2830201
Sondagsrivier	P O Box 304, Kirkwood, 6120	042 2300349
Stargrow	P O Box 12536. Die Boord, 7613	021 9212232
Tweeling	P O Box 190, Kirkwood, 6120	042 2301408
Vaalharts	P O Box 317, Hartswater, 8570	053 4740565
Waterfall	P O Box 339, Adelaide, 5760	046 6840738
Westfalia	P O Box 14, Duiwelskloof, 0835	015 3090011
Witkrans	P O Box 17, Boshoek, 0301	014 5733036

Statutory Improvement Programme

A concept document, which describes the South African Citrus Improvement Scheme, was drafted by an advocate, and circulated to all stakeholders in the industry for their comments and contributions. The necessary amendments were made to the document, which is now being translated into Afrikaans. The English document has already been submitted to the Registrar of Plant Improvement in the National Department of Agriculture for provisional approval before it will be submitted to the Minister of Agriculture.

Protected zone around the CFB

The final document to declare a 5 km radius citrus-free zone around the Citrus Foundation Block has been submitted to the Department of Agriculture for approval.

Shoot Tip Grafting and Gene bank

There are currently 32 cultivars in the STG pipeline at the ARC-ITSC. The CRI's Virological Department in Nelspruit have to date received 42 cultivars from clients for shoot tip grafting and indexing, of which 17 were submitted by the CRI's Cultivar Development Department. The virus-free gene bank at the ARC-ITSC has been partially duplicated by CRI and a total of 235 cultivars have been established in their glasshouse. This department's full report is discussed under 4.2.4.

Control of Citrus propagation material imports

A task team of experts has been formed on recommendation of the Department of Agriculture in order to assist the Department with ensuring that no harmful organisms are brought into the country with imported citrus material. It was proposed that the team include: Mr M Holtzhausen, Prof V Hattingh, Prof G Pietersen, Dr H Le Roux and Dr F van Vuuren.

8 INTERNATIONAL VISITS

8.1 S.D. MOORE

8.1.1 Report on a visit to Lucern, Switzerland for River Bioscience

Introduction

This visit took place from 21-26 October 2006 and was sponsored by River Bioscience (RB). The inaugural meeting of the International Biocontrol Manufacturers Association (IBMA) took place from 23-24 October in Lucerne.

Itinerary

Date/s	Destination	Institution/venue	Activity	Mode of travel
21-22 October	Lucerne, Switzerland	-	-	Air
23-24 October	Lucerne, Switzerland	KKL conference centre	IBMA meeting	-
25-26 October	Port Elizabeth	-	-	Air

Purpose of trip

Through participation in the inaugural meeting of the IBMA, the purpose of the trip was:

1. To identify products, manufactured elsewhere in the world, with potential for use in southern African agriculture (particularly citrus), that could be commercialised in the region by RB.
2. To identify markets for RB's existing products, elsewhere in the world, and potential partners who could commercialise RB's products in these other regions.
3. To build beneficial and potentially beneficial relationships with other role players in the biocontrol industry.
4. To gleam any information on the commercial development of biocontrol in the world, which might have any benefit for RB.

Programme: Sessions, Papers and Workshops

23 October

Poster session

Welcome (Chair: Lucius Tamm)

- Michel Guillon (President IBMA): Objectives of IBMA and ABIM
- Lucius Tamm (FiBL): Welcome on behalf of the organizing committee
- Fabio Cerutti (BLW): New evidence on the contribution of Swiss agriculture to the viability of rural areas
- Heinrich Hebeisen (Canton Lucerne): Biocontrol in a Swiss canton: the case of Lucerne

Plenary session 1 (Chair: Michel Guillon)
New developments in regulation and policy development

- Fabio Cerutti (BLW): Regulation of beneficial Insects and biocontrol agents in Switzerland
- Wolfgang Reinert (DG SANCO). The new regulation on plant protection products in the EU
- Ulf Heilig (IBMA): Public relations in regulatory affairs for IBMA
- Michael Braverman (Rudgers Univ.): IR-4 and international biopesticide registrations for speciality crop agriculture

Plenary session 2 (Chair: Bernard Blum)
New market developments

- Johann Züblin (Migros): EurepGAP and biocontrol

- Simeon Chenev (DG Enterprise): The European Union Support programmes for SMEs
- Thomas Jaekel (GTZ): The GTZ project “Commercialisation of Biopesticides”
- Reinhard Behrens (SOFI): Instruments for facilitating investments
- Stephane Delautre-Drouillon: Contribution and proposals for the promotion of the distribution of biopesticides

Plenary session 3 (chair: Richard Great Rex)
Macrobials

- Arne Peters (e-nema): The position of entomopathogenic nematodes in the biological control market
- Tiziana Irdani (CRA): Cryopreservation of nematode specimens avoiding liquid nitrogen
- Karel Bolckmans (Koppert): *Amblyseius swirskii*
- Firouz Kabiri (Biotop): Commercial development of beneficials for outdoor utilization in France: examples with *Trichogramma*, ladybirds
- Milan Hluchy (Biocont): Effect of Trichoplus on the cotton bollworm (*H. armigera*)

Annual General Assembly Meeting of IBMA

24 October

Programme

Plenary session 4 (Chair: Guido Sterk)
Microbials

- Daniel Zingg (Andermatt BIOCONTROL): Baculovirus products of Andermatt BIOCONTROL AG
- Roberto Kron-Morelli (Agrifutur): MELOCONT and GRANMET, two new fungal pest control products produced by Agrifutur
- Peter Lüth (Prophyta): The new biological nematicide BioAct, its production, application and efficacy
- Stefan Kunz (Bio-Protect): *Aureobasidium pullulans* – an effective yeast for biocontrol
- Don Edgecomb (AgraQuest): Serenade (*Bacillus subtilis*, strain QST 713): Use in integrated pest management

Plenary session 5 (Chair: Hubertus Kleeberg)
Botanicals, natural substances and semiochemicals

- Georges Magnier (AgriPlantes): Pyrethrum production in France: experience, status and perspectives
- Richard Meadow (BIOFORSK): ECOguard: a garlic-based insecticide for the control of root flies in brassica
- Olivier Klarzynski (Goëmar): Stimulation of natural defenses: a growing technique for plant protection against diseases
- Hubertus Kleeberg (Trifolio-M): Successful marketing of NeemAzal- T/S for the biological control of insect pests
  Quassia-extract-MD- a new botanical with interesting pest control properties
- Willem Ravensberg (Koppert): A novel, natural anti-microbial product for use as an agricultural bactericide and fungicide
- Mac McCreless (ACM-Texas): Effectiveness of unique nonhazardous desiccant powder
- Vittorio Veronelli (CBC): Pheromones present and future
- Eric Doye (PheromonTest): A reliable field test for development and registration of mating disruption techniques

Parallel sessions:
Meetings of national IBMA-organisations

Sustaining the long term efficacy of Cydia pomonella granulovirus (CpGV) products: European research to overcome CpGV resistance (Chair: Annegret Schmitt)

- Jürg Huber (BBA): History and new developments of CpGV
- Johannes Jehle (DLR): Research on CpGV resistance in Germany and in the European project "SustainCpGV"
- Antoine Bonhomme (NPP SAS; Arysta Life Science): Research on CpGV resistance in France
- Daniel Zingg (Andermatt BIOCONTROL): CpGV resistance research at Andermatt BIOCONTROL AG; Development and results of MADEX Plus
- Jutta Kienzle: Field results from Germany
- Edith Ladurner (Intrachem Bio Italia): Field results from Italy

Workshop REBECA (Chair: Ulf Heilig)

- Ralf-Udo Ehlers: The policy support action REBECA

KEY POINTS ON KEY PAPERS:

Objectives of IBMA and ABIM (Michel Guillon (President IBMA))
IBMA is 10 years old and has 100 members in 21 countries. The objectives of the association are to: promote biological control and alternative control methods; to lobby and support members in various organisations (e.g. OECD, FAO, EPA and EC authorities); to facilitate relationship with scientific partners (e.g. IOBC, GTZ, FIBL and National Research Organisations).

Biocontrol in a Swiss canton: the case of Lucerne (Heinrich Hebeisen)
Reported the use of Spinosad against Colarado Potato Beetle (*Leptiontarsa decemlineata*). Reported the use of a biological fungicide, called Myco-San.

IR-4 and international biopesticide registrations for speciality crop agriculture (Michael Braverman (Rudgers Univ.))
A USA funding programme for the development of biopesticides, all the way to registration and commercialization, was presented. The website for application to this initiative is: http://ir4.rutgers.edu/Biopesticides/EarlyAdvDemoGuidelinesForms-2007.doc
Two specific products were touched on: thymol for Varroa mite control in honey bee hives; and *Chondrostereum purpareum*, as a natural herbicide. These were recently commercialized through support from the IR-4 programme.

The GTZ project "Commercialisation of Biopesticides" (Thomas Jaekel (GTZ))
In 2004, the Thai biopesticide industry was valuded at US$3.24 million. In 2005, this went up to US$5.26 million. Bt products make up 96% of this market. The predicted growth per year is 30-40%.
Report was made of the commercial production and use of the protozoan parasite, *Sarcocystis singaporensis* for biological control of rodents.

Instruments for facilitating investments (Reinhard Behrens (SOFI))
The Swiss Organisation for Facilitating Investments (SOFI) supports developing and transition economies by investing in them. This they do by transferring capital, technical expertise and managerial know how. They also forge partnerships between enterprises in these countries and those in Switzerland. They are active in South Africa. SOFI provides start up funding for the first three years of a project. Up to 50% of project costs can be loaned, up to a maximum of CHF 500000. The borrower must be a Swiss company, who is in partnership with a company in a developing country. The loan should be repaid with 3-5 years at an interest rate of 4-7% (or it could be turned into a grant). The website is www.swissinvestforum.ch. Calls will be put out for June 2007.

Cryopreservation of nematode specimens avoiding liquid nitrogen (Tiziana Irdani (CRA))
Cryopreservation is conducted at -196°C, usually in liquid nitrogen. A technique, without the use of liquid nitrogen was presented. The objective is cell vitrification, through a rapid drop in temperature – in order to avoid intracellular ice formation. An amorphous glassy state is therefore maintained. This is done with the J2 EPN stage. EPNs can then be "dry stored" for up to 6 months, without any loss in motility or reproductive potential. This work has been published in Cryobiology 52 (2006): 319- . This work will be of interest in CRI's FCM EPN research project, which has commercial potential for RB.

Amblyseius swirskii (Karel Bolckmans (Koppert))
Typhlodromips swirskii predacious mites were very effective against *Frankliniella occidentalis* thrips – more so than were *Amblyseius* mites. Inoculative releases of *T. swirskii* were very effective, as it demonstrated a very rapid numerical response. *T. swirskii* is reared on a factitious host i.e. *Carpoglyphus lactis*. There is a patent pending on this rearing method. More information is available at www.allaboutswirskii.com.

Baculovirus products of Andermatt BIOCONTROL AG (Daniel Zingg (Andermatt BIOCONTROL))
Andermatt has 7 baculovirus products:
Madex, the codling moth GV, is available at 3 x 10^{13} OBs/L and is registered to be applied at 100 ml/ha.
Cryptex, the FCM GV, is available at 2 x 10^{13} OBs/L and was recommended to be applied at 200-330 ml/ha. Even at the highest rate, this would extrapolate to a 7.6 times lower rate of application per hectare than with Cryptogran (RB's product).
Helicovex, the bollworm NPV, is available at 7.5 x 10^{12} OBs/L and was recommended to be applied at 50-200 ml/ha. The highest rate would extrapolate to half the rate for which registration is being sought for Helicovir (RB's product) on citrus.

The new biological nematicide BioAct, its production, application and efficacy (Peter Lüth (Prophyta))
Melocon WG (produced by Prophyta) has *Paecilomyces lilacinus* as its active ingredient. This is the same fungus as that registered for use by BCP in South Africa i.e. PL-Plus. However, it is certain to be a different isolate. Melocon is registered in the USA, Bulgaria, the Philippines and other countries. An application for registration has been submitted in Morocco. In trials on vegetables (4 applications/season) and bananas (2 applications/season – 1 g/plant), Melocon gave better control of nematodes than did the chemical standard (Namathorin), as long as the soil was drenched with a large volume of water. Melocon also has a root promoting effect.

Aureobasidium pullulans – an effective yeast for biocontrol (Stefan Kunz (Bio-Protect))
A. pullulans is used against apple decay and fire blight in orchards. It has also been tested against *Botrytis*, *Penecillium*, *Monilia* and *Azicula* pathogens. This work is published in Leibinger et al. 1997. Phytophathology 97: 1103-1110. For post-harvest decay control, three applications are applied before harvest – 1, 3 and 5 weeks before harvesting begins (see Mogel & Kunz. 2006. Obstbau 31(9): 468-470). For fire blight, the product is applied on the blossoms. Results were better than those with *Bacillus subtilis* and similar to those with Streptomycin. See www.bio-protect.de.

Serenade (*Bacillus subtilis*, strain QST 713): Use in integrated pest management (Don Edgecomb (AgraQuest))
Formulated at 10^{9} cfu/g, as a combination of spores and metabolites. Used against *Alternaria* on tomatoes and other crops. If applied with Mancozeb, for control of banana leaf disease, reduces the amount of Mancozeb necessary by 50%.

Pyrethrum production in France: experience, status and perspectives (Georges Magnier (AgriPlantes))
Kenya produces 70% of the world's pyrethrum. Australia produces the other 30%.

ECOguard: a garlic-based insecticide for the control of root flies in brassica (Richard Meadow (BIOFORSK))
Ecoguard is produced by the Norwegian company, Ecospray. It is 99.9% food grade garlic. There are liquid and granular formulations. Control results against flies on cabbages were better than with either Perfekthion or Birlane. The product has no repellant effect, but is ovicidal (mainly) and larvicidal. Both digestive and respiratory enzymes in the eggs are affected.

Stimulation of natural defenses: agrowing technique for plant protection against diseases (Olivier Klarzynski (Goëmar))
The molecule, laminarin, is extracted from seaweed (*Laminaria digitata*) and used as a plant vaccine. It enables the plant to develop resistance against diseases (fungal and bacterial). It is formulated into a product called Iodus. On cereal crops, one application is effective for 40 days.

Successful marketing of NeemAzal- T/S for the biological control of insect pests (Hubertus Kleeberg (Trifolio-M))
100 kg of neem fruit is required to produce 0.1 kg Azadirachtin of a 34% a.i. This includes Azadirachtin A-K, but predominantly A. The insects hormonal system is affected through feeding inhibition, molting inhibition and fertility reduction. Azadirachtin has been shown to be effective against around 150 species of insect. See www.NeemAzal.de.

Quassia-extract-MD – a new botanical with interesting pestcontrol properties (Hubertus Kleeberg (Trifolio-M))
The sap from the bitterwood tree, *Quassia amara*, is used as an insecticide for control of fleas, mosquitoes, sawflies, whiteflies and aphids. It can also be applied systemically i.e. through drip irrigation.

A novel, natural anti-microbial product for use as an agricultural bactericide and fungicide (Willem Ravensberg (Koppert)) wravensberg@koppert.nl
Lactoperoxidase system: $SCN^{-} + H_2O_2$. It is present in milk, and used by the FAO, as a milk preservative. It is also used in toothpaste and chewing gum. Koppert have formulated it into a product for control of powdery mildew and *Fusarium*. A patent exists in certain countries.

Effectiveness of unique nonhazardous desiccant powder (Mac McCreless (ACM-Texas))
Proposed the use of brucitic marble – a naturally occurring combination of magnesium hydroxide and calcium carbonate (limestone) – as an insecticide. It has a physical effect on the insect, by creating an inhospitable environment, probably similar to kaolin. It is known to work against stable flies, cockroaches and bed bugs, within 4 h after application.

Pheromones present and future (Vittorio Veronelli (CBC))
Mating disruption products are currently available for more than 40 species of insects. MD is currently being developed for the argentine ant and the grape vine mealybug (*Planococcus ficus*).

FORMAL CONTRIBUTION BY SEAN MOORE TO THE PROGRAMME:

I presented a poster on River Bioscience, including details on CRYPTOGRAN and HELICOVIR. The poster was aimed at soliciting interest in the two products from companies elsewhere in the world.

SEMINAR: SUSTAINING THE LONG TERM EFFICACY OF *CYDIA POMONELLA* GRANULOVIRUS (CpGV) PRODUCTS: EUROPEAN RESEARCH TO OVERCOME CpGV RESISTANCE:

History and new developments of CpGV (Jürg Huber (BBA))
In a trial, eight chemical treatments were applied to an orchard (for codling moth control) and eight CpGV treatments were applied to another orchard. At the end of the season, mean numbers of overwintering larvae recorded per tree were 2.43 for the chemical orchard and 0.14 for the virus orchard.

Research on CpGV resistance in Germany and in the European project "SustainCpGV" (Johannes Jehle (DLR))
2003 – the first observation of "resistance" (or reduced susceptibility – not clear whether induced or always present – probably the latter) was recorded (Switzerland/Germany?).
2005 – resistance was measured at 1000 x.
2006 – resistance was recorded in France for the first time; a further 10 cases were confirmed in Germany.
The SustainGV project will look at the following:

1. CpGV susceptibilities of codling moth in the EU
2. Immune response of codling moth to CpGV
3. More CpGV isolates will be identified and tested
4. Production, formulation and field testing of new isolates

The Iranian isolate of CpGV, CpGV-I, has been found to overcome resistance.
Within 4-8 weeks, the project website (www.SustainCpGV.eu) will be up and running.

Research on CpGV resistance in France (Antoine Bonhomme (NPP SAS; Arysta Life Science))
CpGV used in France at 1013 OBs/ha every 10-12 days. In surface dose bioassays conducted with the LD_{95} for neonate codling moth larvae (2500 OBs/ul), resistance was found to be dominant, polygenic and at up to 13000 x lower susceptibility.

REBECA MEETING – The policy support action

Ralf-Udo Ehlers and Ulf Heilig reported on the initiative, details and progress with the programme to establish new registration protocols for biocontrol organisms in the EU. All of the information can be found at www.rebeca-net.de.

VALUABLE DISCUSSIONS HELD:

Louise Labuschagne
Louise Labuschagne is the CEO of the company Real IPM, in Kenya. She was previously the CEO of Dudutech, owned by Flamingo Holdings, UK. Real IPM manufactures and supplies biological control products in Kenya – primarily to the ornamental market. Louise expressed interest in RB's Helicovir product.

Milton Lore
Milton Lore is CEO of Bridgeworks, also a Kenyan company. Bridgeworks facilitates the commercialisation of biocontrol products and also commercialises products themselves. They have strong links with Swiss companies, such as Andermatt. However, Milton expressed his desire to collaborate with other African companies. We agreed that we would pursue opportunities for cooperation.

Emiru Seyoum
Emiru Seyoum is with the Department of Biology at Addis Ababa University. He expressed interest in Helicovir.

Patrick Buerger
Patrick Buerger is MD of Ag-Biotech Australia. We agreed that we would pursue the possibility for the production of a South African isolate of HearNPV (the bollworm virus) by Ag Biotech in Australia, if such an isolate can be shown to be more effective than the Australian isolate.

Thomas Jaekel
Thomas Jaekel works for GTZ (German Technical Cooperation) in SE Asia, particularly Indonesia and Thailand. He cautioned against the use of Chinese produced insect viruses, as the SE Asian experience was that these products were often not of acceptable quality.

PRODUCTS NOTED WHICH MAY BE OF INTEREST IN THE SOUTHERN AFRICAN CITRUS INDUSTRY

1. EasySlugStop for snail control (Andermatt, Switzerland)
2. Sirene (*Bacillus subtilis* spores and metabolites) for control of phytopathogenic fungi (AgraQuest, USA)
3. Melocon WG (*Paecilomyces lilacinus*) for control of nematodes (Prophyta, Germany)
4. *Aureobasidium pullulans* (yeast) for control of phytopathogenic fungi (Bio-Protect, Germany)
5. Iodus (*Laminaria digitata*) plant vaccine for resistance against diseases (Goëmar, France)
6. Quassia-extract-MD (*Quassia amara*, bitterwood sap) as a systemic insecticide (Trifolio-M, Netherlands)
7. Lactoperoxidase for control of phytopathogenic fungi (Koppert, Netherlands)
8. Prorodent (*Sarcocystis singaporensis*) for rodent control (Dept Agric Thailand, GTZ & Uniseeds Co, Thailand)
9. *Leptomastix dactylopii* for mealybug control – (Biological Agriculture Consulting & Engineering Co.)

Value of visit

The visit to Lucerne and participation in the meeting was of significant value. The opportunity was taken to establish and build relationships with researchers and commercial people (in particular), with whom valuable collaboration can be fostered. The opportunity was also taken to identify products (and ideas for products), which have potential for RB to commercialise in South Africa. It was also an opportunity to evaluate the potential benefit for RB in becoming a member of the IBMA. As this was the inaugural IBMA Congress, it is likely that this meeting will grow, both in size and value. It is planned that it be an annual event in Lucerne. I recommend that RB attend this meeting again next year and that a paper be presented.

Acknowledgements

River Bioscience is thanked for funding and facilitating the trip. River Bioscience's Directors are thanked for authorising the *ad hoc* proposal for this trip. CRI is thanked for supporting the application.

8.1.2 **Report on a visit to China and Australia for River Bioscience**

Introduction

This visit took place from 26 August to 10 September 2006 and was sponsored by River Bioscience. From 26 August to 4 September I was in China for the Society for Invertebrate Pathology 9th International Colloquium (held in Wuhan).

From 5-10 September I was in Australia, for the main reason of visiting Ag Biotech Australia in Queensland and New South Wales.

Itinerary

Date/s	Destination	Institution/venue	Activity	Mode of travel
27-31 August	Wuhan, China	Zhangnon Hotel	Society for Invertebrate Pathology meeting	Air
1 September	Wuhan outskirts	Trend Technologies	Visit virus production facility	Road
1-3 September	Yangtze River 3 Gorges	-	Excursion	Road and boat
4-5 September	Brisbane, Qld, Australia (via Sydney)	-	-	Air
5-7 September	Toowoomba, Qld	Ag Biotech	Visit virus production facility and hold discussions	Road
7 September	Brisbane	QDPI Insect Pathology Unit	Discussions with Principal Researchers	Road
8 September	Richmond, NSW	Ag Biotech	Visit virus production facility and hold discussions	Air and road
10 September	Joburg & PE			Air

Purpose of trip

China

The main purpose of my visit to China was to participate in the Society for Invertebrate Pathology 9th International Colloquium on Invertebrate Pathology and Microbial Control and 39th Annual Meeting. Researchers from all over the world, working on various aspects of insect pathology and microbial control, convene annually to present and discuss their work. Every four years, an international colloquium, an even larger event, is held. This was an opportunity for me to present and discuss RB's products and any CRI research which has the potential to culminate in microbial control products. It was also an opportunity to familiarise myself with and hold discussions on the latest research in insect pathology and microbial control. While in Wuhan, I also took the opportunity to visit a commercial virus production facility.

Australia

The main purpose of my visit to Australia was to meet with Ag Biotech Australia. This company produces the bollworm virus, which CRI is in the process of testing and RB is in the process of commercialising. Meetings were held with Ag Biotech's management to discuss all aspects pertaining to the importation and use of the virus in South Africa. I also visited both of Ag Biotech's virus production facilities. While in Australia, I also took the opportunity to meet with two of the insect pathology/entomology principal researchers from the Queensland Department of Primary Industries (QDPI).

Programme

China

The congress, which focused on research conducted on various aspects of all pathogens infecting a range of invertebrate hosts, was attended by researchers from all over the world. In total there were approximately

250 participants. The society consists of six specialist divisions: viruses, bacteria, fungi, microsporidia, nematodes and microbial control. The meetings were also structured around these areas of interest. The congress consisted of plenary addresses, symposia, workshops, contributed oral presentations and contributed poster presentations. There were five contributed paper sessions for viruses, more than for any other topic.

SYMPOSIA WHICH I ATTENDED, INCLUDING KEY POINTS:

Pathogen diversity and the efficacy of virus insecticides. JS Cory, DJ Hodgson, EM Redman. Ecology and Biocontrol Group, NERC Centre for Ecology and Hydrology, Oxford, UK.

Wild-type mixtures of baculovirus genotypes are more virulent than any genotypes, which are isolated on their own. The Pine Beauty moth wild-type NPV has a lower LD_{50} than any of its genotypes on their own.

Microbial control and biotechnology research on *Bacillus thuringiensis* (Bt) in China. D-F. Hunag, Chinese Academy Agric Sciences, Beijing, China.

Biocontrol products make up 12% of the agricultural plant protection market in China.

Microbial control in Japan. Y. Kunimi, Tokyo Univ of Agric & Tech, Tokyo, Japan.

World crop production is valued at US$950 billion. An estimated US$370 billion worth of production is lost annually due to pests. The annual expenditure on pesticides in Japan comes to US$1.03 billion. Microbials make up about 10% of this and Bt makes up about 80% of the microbial market. In SE Asia, microbials make up 1% of the total pesticide market. The FAO drives an IPM programme in the region.

Fungal biocontrol agents for arthropod pest control in India & Pakistan. T.M. Butt & L. Copping, University of Wales, Swansea, UK

India lose an estimated US$10.6 billion to agricultural pests annually. Annually, more than 86000 tons of pesticides are used, at a cost of US$630 million. Biocontrol agents make up less than 1% of the total pesticide market (in value).

Monitoring and management strategy of *Helicoverpa armigera* resistance to Bt cotton in China. K. Wu, Chinese Academy of Agric Sciences, Beijing, China.

Cry1A GM cotton was commercialized in 1997. Cry1A + CpTI cotton was commercialized in 1999. Bollworm egg densities on Bt cotton and non-Bt cotton did not differ. However, larval densities were very different. Resistance has been monitored since 1998. No change in susceptibility has been recorded to date.

What is the current situation in Australia for resistance to Bt cotton by *Helicoverpa armigera*? S.J. Downes & R. Mahon, CSIRO, Canberra, Australia.

Bollgard II (GM cotton) is a Cry1Ac + Cry2Ab modification. As a result of this product, insecticide usage on cotton has been reduced by 86%. A resistance management strategy has been put in place, consisting of: pupae busting, maintenance of mandatory refuges, and no use of Bt sprays in refuges.

Monsanto's global approach to resistance monitoring. G.P. Head & S. Sivasupramaniam, Monsanto, Chesterfield, UK.

In the USA, 3.6 million ha are planted with Bt cotton. In China, 3.2 million ha; in India 1.3 million ha; in Australia 0.3 million ha; in South Africa less than 0.05 million ha; and even less in Argentina, Mexico and Colombia.

New developments in the use of codling moth granulovirus. J. Huber, Institute for Biological Control, Darmstadt, Germany.

Codling moth granulovirus is produced as Madex in Switzerland, Granupom in Germany, Carpovirusine in France, Decyde in the USA (no longer on the market), Cyd-X (ThermoTrilogy/Certis) in the USA and Virosoft in Canada. Use of CpGV in Germany was minimal in 1993; <1000 ha in 1996/7; about 13000 ha in 2006. "Resistance" was detected in bioassays with larvae from orchards where growers complained about virus efficacy. It has been discovered that about 15% of individuals in these populations are nearly completely resistant. Resistance was detected in nearly all orchards where growers complained about efficacy. Resistance was detected in 16 our of 500-600 organic orchards. Consequently, the CpGV isolate has been

changed from CpGV-M (Mexican isolate) to CpGV-I12 (another Mexican genotype). This has been commercialized by Andermatt as Madex-Plus.

Abietiv: Field efficacy and registration of the balsam fir sawfly NPV in Canada. C.J. Lucarotti, Canadian Forest Service, Fredericton, Canada.

Sprays are applied aerially with 20% molasses, as an anti-evaporant. In 2000 – 150 ha; in 2005 – 5000 ha; in 2006 – 15000 ha (first commercial year).

New strategies for using viruses to control agricultural and forest pests in China. X. Sun, Z. Hu & H. Peng, Chinese Academy of Sciences, Wuhan, China.

There are 15 insect viruses registered in China. One of these is a recombinant (GM) HearSNPV with an egt-gene deletion and an aaIT gene insertion. From 1990 to 2002, about 400 tons of this virus was produced and sold per annum. This declined dramatically in 2004, due to the introduction of Bt cotton. The recombinant virus is as persistent in the soil as the wild-type virus but its ecological fitness is lower. It is also totally safe for use. Trichogramma egg parasitoids are also used to vector the virus. This is because <1% of applied virus is actually ingested by the target pests. On cotton, 33% of bollworm eggs are parasitized and 60% of eggs are infected with virus.

Analysis, interpretation, and avoidance of difficult data in bioassay. S.P. Wraight. USDA-ARS, New York, USA.

Three to four doses in a bioassay are considered to be enough. There should be a total of 60 test animals per dose. Control mortality of higher than 20% is considered to be acceptable. For calculating LD_{50}s, an ANOVA/non-parametric approach is recommended.

Top reasons why papers have been rejected for publication. M.S. Goettel, Q. Migheli & C.H. Pickett. Agriculture & Agri-Food, Canada.

Thirty to 50% of papers submitted are rejected. The main reasons for this are:

1. Clarity
2. Experimental design and statistics
3. Repetition of experiments
4. Focus! (Avoid extraneous material)
5. Inappropriate for the journal (international, regional or local interest? Think of target audience.)
6. Plagiarism
7. Double submission

Nucleopolyhedrosis virus introduction in Australia. P. Buerger, C. Hauxwell & D. Murray. Ag Biotech Australia, Richmond, Australia.

Barriers to the introduction of HearNPV into the market place are:

1. Regulatory:	Market geared to synthetics
	Data requirements
	Importation
2. Competition:	Low cost of synthetic pesticides
3. Perception:	Effectiveness of BVs
	Grower education and extension
4. Production:	*In vivo*
	Labour cost
	Low output
	Poor quality
	Risk
Opportunities for introduction:	Resistance to chemicals
	Community pressure
	Move to sustainable agriculture

Field resistance of codling moth to Cydia pomonella granulovirus: occurrence, genetics and breaking. J.A. Jehle, K.E. Eberle, S. Asser, S.M. Sayed & M.R. Rezapanah. Agricultural Service Centre Palantinate, Neustad, Germany.

80% of CpGV products are used in conventional farming; 20% is used in organic farming. In 2003, the first observations of resistance were observed – see Fritsch *et al*., 2005. In bioassays there was ± 100 x

difference in LC50s between susceptible and resistant strains of codling moth. In bioassays, a concentration of $2x10^5$ OBs/ml was used against all instars. 100% mortality of susceptible strains was achieved. For resistant strains, some mortality for L1 and L2 were achieved, but none for L3 to L5. See Stone (1968) for dominance factors for resistance: -1 = completely recessive, +1 = completely dominant, 0 = neither. Resistance is either autosomal, incompletely dominant (bad news) or polygenic. Now the Iranian strain of CpGV (i.e.CpGV-I12) is being used. This overcomes the resistance problem, but does work slower.

Ecological mechanism of sustainable pest control in pine plantation ecosystem. Z. Li, M. Fan, D. Ding, B. Wang & B. Han. Anhui Agricultural University, China.

Sublethal doses of virus reduce puptal size. Smaller pupal size leads to fewer eggs. Sublethal doses also reduce feeding – measured by faecal load. Highest virus production was recorde in L3 (not L5!) – $2.18x10^{10}$ OBs/larva.

Microbial insecticides: some thoughts on history, commercialization and the future. W. Gelernter. PACE Consulting. San Diego, USA.

Microbial control has been changed by genetic engineering and discovery and patent laws (since about 1986), food safety scares, pesticide resistance, organic agriculture. The biggest competition posed to microbial control comes from Bt crops, which are a derivative of insect pathology research; and microbial derivatives e.g. strobilurins, harpins etc. Estimated microbial control sales worldwide: Europe $101 m; USA $90 m; Canada & Mexico $20 m; rest of world $49 m. This is probably an underestimation, due to unregistered sales and incomplete data from developing countries. All successful commercial projects were at some time heavily subsidized with public funding.

Lessons learned from LUBILOSA. R. Bateman. Imperial College London, UK.

The LUBILOSA programme is 15 years old and has cost £10.2 m. Green Muscle is produced in South Africa by BCP and in Benin by the IITA. A ULV application is <1 L/ha. Results are only seen within a week. The concentration in Green Muscle is $5x10^{12}$ conidia/100 g – sufficient for 1 ha. Production cost is $20/ha. 25 g/ha might work as well, if in a good formulation. Chemicals cost about $10/ha. The project has produced >200 publications. See www.lubilosa.org and www.dropdata.org.

Laboratory bioassays of entomopathogenic fungi for control of western flower thrips *Franklinella occidentalis* in horticultural growing media. M.A. Ansari, M. Brownbridge, F.A. Shah, M. Whittaker, M. Prasad & T.M. Butt. AgResearch, Lincoln, New Zealand.

Fungi are cultured on SDA. Conidia are added to media (potting mix) at 10^{10} conidia/L. Water was added to field capacity. Fungi were compared with fipronil (Regent) at 1 g/L medium. Media was placed into cups and larvae were added. Temp = 24°C. Adult emergence was monitored at 4 and 7 days. All fungi were more effective than fipronil. *Metarhizium anisopliae* V275 and ERL700 were the best with 75-95% mortality. About 10% of the adults emerging, also became infected.

Study the infectivity of budded viruses of wild type and recombinant HearNPVs by quantitative PCR. H. Wang, M. Wang, W. Dai, F. Deng, Z. Hu & H. Wang. Chinese Academyh of Sciences, Wuhan, China.

The first registration of a GM HearNPV was in 1993. This was of HearSNPV, which was sequenced in 2001. Currently, there are two GM HearNPVs in China: Hear+egfp and Ha+gp64+egfp. Viral DNA copies are produced by real time PCR. Entry efficiency into larvae is quantified by Real time PCR at various intervals post infection. Results are given in “DNA copies/cell”.

FORMAL CONTRIBUTION BY SEAN MOORE TO THE PROGRAMME:

I presented a paper in one of the Microbial Control sessions. The abstract for that paper, which appears in the formal congress proceedings is as follows:

A phenologically based programme for season-long control of false codling moth on citrus, with particular use of a granulovirus and entomopathogenic nematodes

S.D. Moore[1,2], A. Malan[3] and W. Kirkman[2]

[1]River Bioscience, PO Box 20388, Humewood 6013, South Africa
[2]Citrus Research International, PO Box 20285, Humewood 6013, South Africa
[3]University of Stellenbosch, P/Bag X1, Matieland 7602, South Africa

False codling moth, *Cryptophlebia leucotreta* (Meyrick) (Lepidoptera: Olethreutidae), is a fruit pest of citrus, macadamias, stone fruits, avocadoes, grapes, peppers and litchis, in southern Africa. It can be a pest throughout the year, remaining active even in winter. It is capable of attacking newly set pea-sized fruit in spring, to ripe fruit in autumn and unharvested fruit in winter. It is therefore necessary to employ control measures throughout the year. The *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) has been used successfully for two years now for the commercial control of *C. leucotreta* on citrus. Studies are currently being conducted to test the potential of entomopathogenic nematodes (EPNs), in the families Heterorhabditidae and Steinernematidae, as biological control agents for *C. leucotreta*. A control programme incorporating a granulovirus and EPNs is proposed. Cryptogran has been found to reduce *C. leucotreta* infestation more effectively, when applied early in the season (early summer) and when timed against a flight peak. In a trial where the efficacy of sprays applied at different phenological times were compared, a treatment on 7 December, applied against a flight peak, reduced infestation by an average of 62% over a 14 week period. A treatment applied on 10 January, between two flight peaks, only reduced infestation by 42%, over a 7 week period. Sprays applied during the last six weeks before harvest induce control for an even shorter period. It is proposed that during autumn and winter, EPNs could be applied to control pre-pupae and pupae in the soil. A survey has been undertaken in citrus orchards to obtain endemic, locally adapted EPNs. Twelve isolates of EPNs have been recovered and maintained for further study. All isolates were identified as *Heterorhabditis*. Laboratory bioassays conducted with EPNs and sentinel larvae and pupae of *C. leucotreta*, demonstrated that mortality and penetration of pupae was much lower than for larvae. Highest mortality and penetration rate of both larvae and pupae was obtained for *Heterorhabditis zealandica*. We therefore propose a phenologically discerning control programme for *C. leucotreta* on citrus, based around the use of Cryptogran and EPNs.

NEW PRODUCTS WORKSHOP:

In this workshop, six companies made presentations of new microbial control products, which were on the market. I took the opportunity to present CRYPTOGRAN and HELICOVIR on behalf of River Bioscience. The other companies and products were:

1. Andermatt (baculoviruses – including Cryptex and Helicovex): Cryptex has $2x10^{13}$ OBs/L (Cryptogran has $5x10^{13}$ OBs/L).
2. Certis (Kleestek – a plant tissue culture for ethanol production)
3. Wits University (GM Cyanobacteria – Bti – for malaria control; and HearNPV – 8 different field isolates found, 3 of which are more pathogenic than any previously tested.)
4. Roy Bateman – a fungal spore harvester, used for solid state fermentation (Mycotech technique). Sylvan has produced another one. Silica gel is used in the drying cabinet.
5. CABI (Belinda Luke).

MICROBIAL CONTROL DIVISION MEETING:

The annual general meeting of the microbial control division was held. I was elected onto the committee for the next two years.

VALUABLE DISCUSSIONS HELD:

Martin Andermatt:

Martin Andermatt is the founder and CEO of Andermatt AG, a Swiss company which manufactures and markets a range of biological and biorational products for use in agriculture, veterinary science and home gardens. Andermatt is possibly the largest producer of microbial control agents in Europe. Amongst other products, they are the manufacturers of Cryptex and Helicovex – the same viruses as CRYPTOGRAN and HELICOVIR, although different isolates. Very little was discussed on the release of these two products on the South African market and their competition with RB's products. Andermatt will distribute these products through BCP. Andermatt also produces some entomopathogenic nematodes (EPNs). Andermatt's EPN production methodology was discussed, with relevance to CRI's exp 793 i.e. the collaborative project between University of Stellenbosch and CRI. It is a published methodology (Benning, Australia). However, Amdermatt claimed that there are certain details to making the system work, which he has perfected.

Nikolai van Beek:

Nikolai van Beek led Du Pont's GM baculovirus programme a few years ago. He is now looking for opportunities to get involved in the development of baculoviruses in the developing world. He is particularly enthusiastic to enter into a collaboration with RB and CRI. He believes that he may have discovered the reason for UV light debilitating the efficacy of baculoviruses, and that this is contradictory to the popularly held and documented belief. He confidentially shared his findings with me and offered to investigate them further, in collaboration with RB and CRI, if we could acquire funding for such work. The potential outcome is that a unique method for UV protection of viruses could be developed.

Juerg Huber:

Juerg Huber is head of the Insect Pathology Division of the German Institute for Biological Control. He has worked on applied aspects of the codling moth granulovirus (CpGV) since the 1970s. He reported that the addition of CpGV, at lower than registered concentrations, to all sprays applied to apples, produces very good control of codling moth in Europe. He suggested that we try a similar approach with CRYPTOGRAN.

Johannes Jehle:

Johannes Jehle is a German researcher on molecular aspects of insect viruses. He has also worked on the FCM granulovirus and was the first to publish a DNA profile of this virus (from Cape Verde). He confirmed the appearance of codling moth "resistance" to CpGV in Europe – approximately 5% of orchards. However, he stated that he believes that it was not CpGV usage induced resistance but that there had always been populations of codling moth with lower susceptibility. We discussed the relevance of pro-actively investigating the possible occurrence of a similar phenomenon in FCM in South Africa.

Michael Brownbridge:

Michael Brownbridge is a UK citizen, who is a researcher with AgResearch Limited in New Zealand. He works on the use and development of entomopathogenic fungi (EPFs) as microbial control agents, and has also worked on Bt. He was involved in a very exciting project where a range of EPFs were used successfully for control of thrips larvae and pupae in soil. Michael agreed to become involved in pursuing similar work for control of citrus thrips in South Africa.

Tariq Butt:

Tariq Butt is Professor of Insect Pathology at the University of Wales Swansea. He was involved with Michael Brownbridge in the thrips EPF study. He introduced me to a grant funding system, geared at identifying and sponsoring promising young academics in developing countries, and invited me to identify an individual and a project in South Africa which could qualify for funding.

Dudley Pinock:

Dudley Pinnock is an Australian researcher, working for a private company, who had discovered and was testing a new Bt strain, for control of veterinary ectoparasites, including fly larvae.

VISIT TO TREND TECHNOLOGIES:

I visited Trend Technologies with Patrick Buerger, Managing Director of Ag Biotech Australia. Trend Technologies is a Hong Kong owned company, on the outskirts of the city of Wuhan. They are one of approximately 10 bollworm virus (HearNPV) mass production facilities in China. Many of these facilities are no longer operational or have dramatically scaled downed activities, due to the introduction of and increase in commercially grown genetically modified cotton. This GM cotton has one or two genes incorporated for control of bollworm and other lepidopteran pests.

When we visited Trend Technologies, they were also not in operation at the time. Their production facilities were enormous – an estimated 10000 square metres, employing up to 84 people. They reported that they can make 2 tons of HearNPV per day, of a concentration of 2×10^{11} OBs/g (as an air-dried wettable powder). In my opinion, this would enable them alone to supply the world demand for this virus! They are able to inoculate and harvest 400000 trays of larvae per day i.e. 4 million larvae per day. However, they reported that due to a drop in market demand, they last did this in 2003. This reported production potential means

that 10^{11} OBs are harvested per larva. Patrick Buerger, who produces the same virus in Australia, claimed that they are only able to produce 10^{10} OBs per larva and was therefore sceptical.

Trend Technologies claimed a 3 year shelf-life for their product, if kept cooled; but only 1 year, without refrigeration. They sell their product to small users, at RMB5/50g for a concentration of 10^{10}OBs/g.

Trend Technologies also manufacture a granulovirus for diamond back moth control. This is sold at a higher price that is HearNPV, as a result of the greater input required to produce adequate quantities of virus from smaller larvae.

Australia

VISIT TO AG BIOTECH AUSTRALIA VIRUS PRODUCTION FACILITIES

Ag Biotech has two production facilities: one on the Bidstrup's farm, 1 hour's drive from Toowoomba, NSW; the other in Richmond, Sydney. Both production facilities were small but impressive. They were highly automated, enabling them each to be run by as few as two people. A number of ideas were obtained for improving sanitation and automation of RB's production facility. Despite the difference in automation, the production and formulation methods employed by Ag Biotech were very similar to those used by RB. Neither of the facilities was operational at the time of my visit, as large volumes of product had already been stockpiled. Some examples of technology which RB may want to use were noted. However, many of the appliances and designs used were specific to the *in vivo* production of HearNPV and were not directly applicable to *in vivo* production of CRYPTOGRAN.

Ag Biotech have a second product, which they manufacture, called Magnet. This is a plant-volatile attract and kill product for bollworm control. Currently, the formulation of Magnet is being improved. Once this has been completed, Ag Biotech will be able to supply RB or CRI with a sample for testing. The product can also be tested against other lepidopteran pests.

Ag Biotech have submitted a tender for the commercial rights to a fruit piercing moth bait, developed by Harry Fay and patented by DPI. This is another product, which CRI and RB can investigate in South Africa.

The time spent with Patrick Buerger (Managing Director) and Anthony Hawes (Marketing Director) of Ag Biotech, was very beneficial in building and reinforcing the business relationship already in place between RB and Ag Biotech.

VISIT WITH DR CAROLINE HAUXWELL, INSECT PATHOLOGY UNIT, DEPARTMENT OF PRIMARY INDUSTRIES (DPI), BRISBANE

Caroline Hauxwell, who heads up the Insect Pathology Unit, has been instrumental in the commercialisation of HearNPV in Australia, including the execution of extensive research trials. The unit still serves as a strong impartial support structure for Ag Biotech Australia – the main commercial HearNPV company in Australia – including providing a regular quality control service. Caroline invited me to spend a sabbatical at DPI.

VISIT WITH DR DAVE MURRAY, ENTOMOLOGY DIVISION, DPI

Dave Murray, along with Bob Teakle, was one of the pioneers of applied baculovirus research in Australia. Most of his work has been with HearNPV against bollworm. Unfortunately, he is no longer conducting baculovirus research, but is still involved in biocontrol and IPM research.

Value of visit

China

This was the fifth Society for Invertebrate Pathology Meeting which I have participated in, and by far the most beneficial. The greater benefit derived at this meeting was probably because I approached this meeting, not only from a scientific perspective, but also from a business perspective. This breathed new life into the programme for me. I also had a number of invaluable one-on-one discussions with other scientists and business people, some of which are already bearing fruit. The fact that I was elected to the Microbial Control Division Committee of the Society is also evidence that the work which CRI and RB are conducting in South Africa is being well regarded.

In addition to this, the visit to the enormous commercial baculovirus production facility in Wuhan was eye-opening and educational.

Australia

The visit to Australia was also extremely beneficial in the building of business and scientific relationships and in acquiring highly specific information which can be used to improve the commercial mass production of Cryptogran (and possibly any other viruses).

Acknowledgements

River Bioscience is thanked for funding and facilitating the trip. CRI is thanked for supporting the application.

8.2 G. PIETERSEN & H.F. LE ROUX

8.2.1 Report on a visit to Brazil to attend the Huanglongbing Workshop, July, 2006

General

Hectares to citrus has decreased in Brazil from 850 000ha to 650 000 ha in the past 5 years. The main reasons have been poor juice prices and diseases. However, total production has been stable as a result of increases in planting densities from 250 to 450 trees per ha, and an increase in the utilization of irrigation.

The price of orange juice concentrate increased from less than US$1000.00 per ton in 2004 to more than US$ 2000.00 per ton in 2006. The expectation is that the prices will not drop below US$1500.00 during the next five years. The reason for the industry not expanding area to citrus has been the competition for farmlands induced by the good price of sugar and the threat of HLB, citrus variegated chlorosis (CVC), leprosis and other diseases.

The number of citrus exporters has declined, from each of the large exporters (Cutrale, Fischer, Citrovita and Cambuhy), as well as Seven lakes farms, to only Cutrale. The main reason for this has been CBS, which has most probably entered Brazil on illegally imported Midnight Valencia budwood from the Karino area, South Africa.

Threats for South Africa

Brazillian citrus grower/s have been in discussions with the Angolan government to establish citrus plantings in Angola with the aim of setting up a processing plant. If the propagation material is imported from Brazil there is a clear risk that citrus canker, CVC, sudden death, Leprosis, Rubellosis, Asian greening and pests not currently in Southern Africa could be introduced. It is of the utmost importance that SAAFQHIS will engage in more serious negotiations with both the Angolan and Mozambican Governments to prevent the importation of ANY propagation material from the continent of South America. CRI should ensure that it is aware of all citrus developments in both these countries.

A Second threat relates to market access: Fundecitrus (Marcell Sposito) has done research on the spread of CBS from pycnidia from a fruit placed in a tree above small orange fruitlets. His results show that there is a much faster development of CBS on the infected fruit below than when infection is from dead leaves releasing ascospores. If misinterpreted, these results can do a lot of damage within the EU, by suggesting a the low possibility of spread of CBS through the fruit pathway. Somehow Fundecitrus should be approached to ensure that CRI gain an input into the article to ensure that statements are made correctly.

Leprosis continues to be the most expensive disease to control in Brazil because of the cost of controlling the *Brevipalpus* mite. Continued vigilance is required to ensure that this pathogen is not introduced into South Africa by either visitors from Brazil or by returning South Africans. South African growers must also be made aware of this danger and discouraged to visit Brazil.

The area in which Sudden death occurs has not increased, however the incidence of the disease in the area has increased to a stage where 60% of trees in the area is affected. To date 3 million trees have been removed as a result of this disease. Unlike huanglongbing, the fruit is still suitable for processing albeit considerably smaller than healthy ones. The etiological agent remains unclear with both CTV and a tymovirus being closely associated with all infected trees when under stress conditions. The current hypothesis is that the tymovirus reduces the resistance of the Rangpur lime rootstock allowing a unique strain of CTV to induce a typical rapid decline disease. This association appears to only occur within the

marginal citrus-producing areas and not where Citrus grows optimally (northern part of Sao Paulo province and Southern part of Mina Gerais) .The use of Swingle inarching has significantly alleviated the problem. In order to maintain drought tolerance young trees are planted on Rangpur lime and inarched with Swingle, Cleo or Sunki mandarin, one year after planting when the trees are still healthy. This devastating disease is one that must be kept out of South Africa at all costs.

The research division of Fundecitrus (Araraquarra), established in 1993 has developed into one of the foremost Citrus research facilities in the World. Combined with the expertise of the Instituto Agronomico, Centro Avancado de Pesquisa Tecnologica do Agronegocio de Citros Sylvio Moreira institute in Cordeiropolis they have succeeded in preventing the collapse of the industry due to citrus canker, CVC, sudden death and Huanglongbing. The South African research fraternity must take note of this and it is strongly recommended that closer ties should be forged between CRI and these organizations. If this had happened earlier articles such as those in the pipeline from Marcell Sposito would not threaten South African markets. In the past two years such close ties with Brazilan counterparts have made possible the implementation and local adaptation of PCR techniques to detect Xyllela fastidiosa (causual organism of CVC), leprosis and *Libiribacter americanus* in South Africa, and the authors wish to express gratefulness to the fine collaboration established in 2004.. Dr, Marco Mashado has recently produced transgenic citrus plants resistant to CTV and to CVC. South Africa may not have the funds for this type of research but must retain contact with this technology through collaboration and contact with Brazilian researchers in this field.

Huanglongbing/Greening

The Brazilian industry identified and had sensitive detection methods (PCR) in place within months of the first report of the disease. In less than a year the Brazilian Federal Government has enacted legislation to enforce eradication strategies (in South Africa this has not been possible in 90 years with the disease, including recent introduction to previously unaffected areas). 170 Million citrus trees have been inspected over the past two years for greening resulting in the removal of 250 000 infected trees. As a result of the eradication the incidence of greening within all the major citrus growing estates has progressively declined. Fischer/Citrosuco have removed 40 000 trees out of 8 million, Cutrale 23 000 out of 20 million and Cambuhy 13000. Infected trees, confirmed positive by PCR or official visual inspection, must be removed by law within two weeks of notification. If not these are removed at the cost of the grower by Federal authorities.

The rapid response to the HLB situation was made possible partly due to the excellent citrus nursery industry and inspection systems necessitated by the earlier introduction and control of CVC. In contrast, during the first sixty years since the discovery of African greening the citrus nurseries were responsible for the distribution of the disease to uninfected citrus-producing areas. Today Brazil has arguably the best nurseries in the world having visited South Africa in the 1990 when the South African citrus nursery industry was at its peak.

Though most of the larger estates do their own monthly inspections, Fundicitrus has 1900 inspectors who perform the inspections of both citrus canker and HLB. In order to ensure that symptoms in the upper reaches of the tree are not missed the growers have developed a tractor driven tree inspection platform Trees with greening are immediately removed by chain saw after which the stump is treated with either 2,4 D or glyphosate. The remaining branches, twigs and leaves are mechanically shredded and left as mulch.

Vector control is done by contact and systemic insecticides applied as foliar applications on old trees. Up to 24 applications per year is applied. On young trees systemic insecticides are applied as stem applications or as soil-drenches during the rainy season whereas foliar contact insecticides are applied during the dry season. The most common systemic insecticides used on young trees is Confidor and Actara. Confidor is also sprayed on older trees only yielding 14 days of control fo Psyllla. This method of application is not recommended for South Africa as this increases the chances of development of resistance. Phosdrin and malathion is used as contact insecticides whereas the use of endosulfan , commonly used in South Africa is prohibited in Brazil

The fact that such a low percentage of orchards is under irrigation makes the control of the psylla difficult. The seasonal dynamics of Diaphorina citri is still not known in Brazil. Once the population peaks are effectively controlled there should be certain months of the year when treatment may not be necessary.

Although *Liberibacter asiaticus* and *L. americanus* are thought to be organisms that can tolerate high temperatures there is still a maximum that can be tolerated. Both these organisms induce the clearest symptoms on trees in the cooler months as in the case of *L. africanus* (June to August).

The largest threat to control of HLB in Brazil is due to the presence of small growers who lack the resources to inspect and eradicate HLB infected trees effectively. Because of the high sugar prices it is expected that many of these small citrus growers will abandon citrus production in favour of sugar in the near future. Important is the fact that no neglected orchards with HLB is tolerated. The probability of success of eradication/control of HLB is directly related to the extent with which small grower control is performed.

Unlike the situation in South South Africa where alternate hosts of African greening are not known to not play a significant role in the epidemiology of the disease, orange Jassemine (Murraya paniculata) poses a serious threat as an alternative host for HLB in Brazil. Infected trees are eradicated in Brazil.
Symposium:

Brazilan scientist Silvio Lopes has shown that pruning for HLB in Brazil has proven completely ineffective in control the spread of HLB.

The symposium was attended by 250 delegates of which approximately 40 delegates were from overseas countries. This included HLB specialists such as Prof. Bove (France) and Prof. Zhaung (China). American scientists that attended Tim Gottwald, Ron Brlansky, Mary-Lou Polek, Richard Lee, Susan Halbert, Various citrus growers from California (Tom Mulholland), Texas and Florida attended along with large contingent of Brazilian growers and nurserymen..

The meeting presented an opportunity to share data with Tim Gottwald and to obtain advise and collaboration in analysing spatio-temporal data on disease spread.

A number of delegates from the USA and other countries requested samples of *Liberibacter africanus* DNA or *Diaphorina* samples. These will be sent to these on return to South Africa. Collaboration has also been concluded in the sequencing of various other parts of the genome of L. africanus in an attempt to define the variability of the bacteria in South Africa. Prof. Bove has requested that the authors collaborate in doing a survey of South African citrus greening affected areas for the presence of *L. americanus*. This will be combined with the collection of isolates for variability studies, as well as coinciding with the visit of two Brazilian scientists from Fundecitrus, both involved in HLB research.

Visits to Fundecitrus and Instituto Agronomico, Centro Avancado de Pesquisa Tecnologica do Agronegocio de Citros Sylvio Moreira institute in Cordeiropolis were specifically requested and utilised to obtain first hand knowledge of methods utilised by these two agencies to perform large-scale DNA extractions prior to greening PCR. While some handy tips were obtained neither unit appears to be able to do more than 40 samples per day, more or less the same in the same order as that done in South Africa at CRI@UP.

HLB in Florida

The Brazilian attitude differs significantly from Floridian researchers who do not appear to believe they can successfully control the disease. This is partly due to the Amercian failure to control citrus canker due to hurricanes over the past two seasons and various legal challenges to the eradication problem. Furthermore the cost of employing thousands of inspectors for HLB eradication is considered prohibitive by the Florida legislature. The situation in Florida is worse than initial reports emanating from Florida suggested. Some researchers privately expressed the opinion that the disease has been there for a number of years already. HLB infection was found to extent as far North as St. Lucie county, some 240km from the initial point of discovery by Dr. Susan Halbert (FDACS/DPI) in Miami. A panel of experts in Florida expressed the view that the disease has become endemic in Florida and delimitating surveys were requested. The disease has been shown to have spread over 12 counties in Florida. Some excellent spatial analyses have been performed by Dr. Tim Gottwald in this period and this information has significance to the control of the disease worldwide. Research of this nature should be performed on African greening.

HLB in China

HLB in China has been controlled in the past to a certain extent, however as is the case in South Africa the growers at a stage started to ignore the problem. As a result of this they now have what is referred to a the “second wave of HLB”. The incidence of HLB in infected provinces has increased dramatically and chances of successful control significantly reduced.

Delegates of the Huanglongbing Workshop during a field-trip to Fundecitrus. Thursday, 20th July, 2006. Photo courtesy of Clara Serena Garcia Darderes, Argentina.

8.3 P.J.R. CRONJÉ

8.3.1 Besoek aan die Gordons Research Konferensie, VSA – 9-14 Julie 2006

Gedurende 9 tot 14 Julie het ek saam met Mariana Jooste (**Sagtevrugte Produsent Trust**) en mede PhD-student van die Departement Hortologie, US, die voorreg gehad om die "Gordons Research" konferensie gefokus op naoes fisiologie in New London, Connecticut, VSA by te woon. Die doel van die konferensie was om 'n atmosfeer te skep waarbinne die nuutste naoes inligting gedeel kon word, basiese en toegepaste navorsingsmetodiek verduidelik kon word en waar nuwe kontakte opgebou kon word. Die konferensie se voorsittersfonds het $600.00 vir beide studente geskenk om te registreer en die res van die besoek was befonds deur die Sagtevrugte Produsente Trust, Citrus Growers Association en die Departement van Handel en Nywerheid se TRIP-program

Lesings wat deur vooraanstaande naoes fisioloë gelewer is, het gekonsentreer op die genetiese beheer van rypwording, etileen en rypwording, die fisiologie van varsgesnyde produkte, selwande, geur en aroma, koueskade, die beheer van veroudering en kommersiële naoes hanteringsisteme. Dit het duidelik uit hul navorsing gebleik dat die meeste naoes-laboratoriums spesialis-spanne het (bv. molekulêre bioloë, antioksidantspesialiste, selwand spesialiste, etileenspesialiste, ens.) wat saamwerk op 'n groter projek (meestal internasionale samewerking asook veelvoudige instellings) en so 'n baie meer gedetailleerde en deeglike oplossing kan bied aan hul industrieë. In teenstelling met ons situasie, waar die vrugte industrie grootliks verantwoordelik is vir befondsing, word die meeste van hierdie spanne deur hulle onderskeie regerings ondersteun. Daar is op die kongres van die geleentheid gebruik gemaak om met al die bewonende sitrus na-oes navorsers kontak te maak. Dr Maria Lafuente, wat 'n baie indrukwekkende lesing oor koue skade gegee het, is in my opinie die voorste navorser in skildefekte en samewerking met haar sal vir die skilkonsisie navorsings program baie beteken. Ek het ook met dr. David Obenland en dr. Mary-Lou Arpia van USDA in California my navorsing bespreek asook gemeenskaplike probleme soos "peteka" en "pitting" asook die identifikasie van skil defekte. Uit die gesprekke blyk dit dat hulle al meer navorsing wil doen op sagtesitrus en minder op Valencia tipes.

Na afloop van die konferensie is die Holt hawefasiliteit, waardeur al die Suid- Afrikaanse sitrus en sagtevrugte wat na die VSA ingevoer word, besoek. Gedurende die besoek was hulle die gaste van die VSA Produsente Formum. Die hawe is geleë in Gloucester City, New Jersey, reg oorkant Philadelphia, aan die wal van die Dalawer rivier. Tydens die besoek het ’n skip van Suid-Afrika gedok en begin sitrus vrugte aflaai. Dit het die twee studente die geleentheid gegee om die vloei van vrugte deur die hawefasiliteit te sien. Elke dek word individueel deur die USDA-inspekteur vrygestel na afloop van die 24 dae -0.5°C steri-protokol, waarna vrugte afgelaai en in ’n enorme, onverkoelde stoor in PUC-kodes georganiseer word vir inspeksie deur die USDA. Na suksesvolle kwaliteit- en fitoinspeksies word die vrugte in verkoelde store (~1°C) gestoor waar die invoermaatskappye vir skil kondisies (bv. koue skade, oleocellosis en skil afbraak) en interne kwaliteit inspeksies doen. Van daar geskied verspreiding en versending in koeltrokke. Die vrugte word meestal na supermarkte aan die weskus van die VSA versprei.

Tydens die besoek is daar besef dat ’n tekort aan kennis en opleiding van die inspekteurs foutiewe inligting geskep kan word oor vrugkwaliteit, en spesifiek fisiologiese skil defekte, a.g.v. verkeerde identifikasie tydens die inspeksie. Die inligting kan dan tot verwarring en foutiewe afleidings lei by produsente, pakhuise en diensverskaffers in Suid-Afrika. Daar is ook opgemerk dat ’n onderbreking van die koueketting kan voorkom wat ’n negatiewe effek op vrugkwaliteit en bederf sal hê.

Die besoek aan die VSA het ons twee student blootgestel aan wat kan blyk om twee uiteenlopende wêrelde te wees. Maar die kennis van die fundamentele navorsing, soos beoefen en aangebied deur die top navorsers by die konferensie, spreek van die fundamentele fisiologiese probleme aan waarmee die Suid-Afrikaanse vrugte industrie stoei. Slegs deur in te skakel in die stroom van nuwe inligting en denkwyses sal daar geslaag word om probleme soos koueskade, vroegtydige veroudering en fisiologiese defekte te beheer en sodoende meer kompeterend te wees as ‘n uitvoerder van vars vrugte.

Connecticut College, New London Connecticut, waar die Gordon Navorsings kongres gehou was.

By die Holt hawe word pallette volgens PUC kodes gepak voor USDA inspeksies. Let op die plastiek seile en waaier wat gebruik word vir beroking van sagtevrugte. Tussen 4000 en 6000 pallette kan gelyktydig by hierdie fasiliteit berook word. Alles geskied by lug temperatuur.

USA Produsente Forum verteenwoordiger Gerrit van den Merwe, besig in die forum se kantoor by die Holt hawe.

Paul Cronje en Mariana Jooste by die herverpakkings fasiliteit.

Herverpakking geskied a.g.v. skildefekte soos koue skade en “Stem-End-Rind-Breakdown”.

8.4 H.F. LE ROUX

8.4.1 Besoek aan Kalifornië – Mei 2006

‘n Versoek is gedurende 2005 aan CRI gerig om Sun Pacific en Paramount Citrus in Kalifornië te besoek om hulle behulpsaam te wees met sitruswortelprobleme. Die versoek is gerig deur Prof Etienne Rabe en is goedgekeur vir die tydperk 15-19 Mei 2006. Die volgende aspekte is van belang vir die Suid Afrikaanse industrie om van kennis te neem:

(a) Sitrusaalwurm

Die sitrusaalwurmsituasie in Kalifornië stem baie ooreen met die in Suid Afrika. Sitrusaalwurms is ‘n ernstige probleem in ‘n groot persentasie van die ouer aanplantings in Kalifornië terwyl dit glad nie ‘n probleem is in enige van die jong boorde wat op nuwe gronde hervestig is nie.

Biotipes. Die rasse wat daar voorkom verskil van die wat in Suid Afrika voorkom. In Kalifornie kom die “Citrus-“ en “Poncirus” biotipes voor terwyl die Meditireense biotipe in Suid Afrika voorkom. Die “Citrus” biotipe reproduseer swak op *Poncirus trifoliate* maar goed op *Citrus* spp en hibriede soos Carrizo – en

Troyer citrange, olywe, druiwe en persimmons. Die Poncirus biotipe reproduseer goed op *Citus* spp, *P.trifoliata* en druiwe maar nie olywe nie terwyl die Meditireense biotipe soortgelyk aan die *Citrus* biotipe is maar nie op olywe reproduseer nie.

Voorplantbehandelings. Metielbromied word nie in sitrus in Kalifornie gebruik nie maar Telone wel. Hierdie produk is uiters effektief en die herplantboorde wat gevestig is op gronde wat vooraf berook is met Telone se wortelstelsels is almal goed ontwikkel en sonder enige aalwurmprobleme. Die gebruik van Telone in herplantsituasies waar Swingle citrumelo of C35 citrange nie gebruik kan word nie moet in Suid Afrika ondersoek word.

Na-plant aalwurmdoders. Met die uitsondering van Vydate (oksamiel) is alle na-oes chemiese aalwurmdoder- toedienings op sitrus in Kalifornië verbied. Daar is ‘n beperkte voorraad Nemacur (fenamiphos) wat nog gebruik mag word totdat die bestaande voorrade uitgeput is. Geen Nemacur mag verder vervaardig of ingevoer word nie.

Nemacur presteer swak in die boorde in Kalifornië waar dit wel gebruik word ten opsigte van aalwurmpopulasies. Die rede hiervoor is onbekend en omdat die produk onttrek gaan word sal die oorsaak nie verder ondersoek word nie. Die produk het egter oesopbrengste verbeter. Die rede vir die swak prestasie tov die aalwurm-populasies kan waarskynlik toegeskryf word aan die volgende:
1) Enkeltoedienings wat nie die aalwurms se lewenssiklus effektief kon verbreek nie. 2)Vinnige afbraak van die produk agv gronde met hoe kalsium vlakke en relatief hoe pH.
3)Versnelde mikrobiologiese afbraak agv die herhaalde gebruik van dieselfde produk.

Hoewel Vydate nog geregistreer is op sitrus in Kalifornië word dit deur niemand gebruik nie omdat die werking van die produk so swak is. Die metode van toediening verklaar die swak werking. Vydate is slegs geregistreer as ’n toediening deur drupbesproeiingstelsels. Die produk is bekend vir sy gevoeligheid vir versnelde biologiese afbraak en aangesien die toediening van aalwurmdoders deur drupstelsels waarskynlik die beste manier is om te verseker dat versnelde afbraak sal ontwikkel, kan gespekuleer word dat versnelde afbraak waarskynlik weereens die oorsaak is vir die swak prestasie van hierdie aalwurmdoder. Daar behoort gekyk te word na stamtoedienings van Vydate aangesien die produk beide akropetaal en basipetaal deur die plant beweeg.

Onderstamme. Swingle citrumelo kan nie gebruik word nie agv die hoë kalsiumvlakke in die grond. Trifoliaat is in die verlede op ‘n groot skaal gebruik maar omdat die Poncirus biotipe voorkom is dit nie ‘n alternatief om te gebruik om sitrusaalwurmprobleme te oorkom nie. Carrizo en Troyer citrange wat meesal gebruik word doen goed op gronde wat vooraf berook is maar nie in gronde waar geen berokings gedoen is nie.

Omdat die Kaliforniese sitrusindustrie nie oor ‘n organisasie soos CRI beskik wat onafhanklike navorsing vir hulle bedryf doen nie, is geen werk die afgelope aantal jaar gedoen om onderstamme te evalueer nie. C35 citrange doen uitstekend in Suid Afrika as ‘n herplantonderstam. Hierdie inligting kan nie net so van toepassing gemaak word op die Kaliforniese bedryf nie omdat hulle aalwurmbiotipes verskil. Dit kan egter vir hulle die moeitewerd wees om C35 te evalueer.

(b) **Biologiese beheer agente**

Phytophthora

Wortelvrot. *Phytophthora* wortel en kraagvrot is ’n algemene gesig in ouer boorde veral in van die suurlemoenboorde wat besoek is. Al die jonger boorde is *Phytophthora* vry met die uitsondering van een herplantboord wat nie berook is voordat dit herplant is nie. Die Telone berokings skakel die *Phytophthora* in herplantsituasies ook uit nes die aalwurms. Fosfonaatbespuitings word baie algemeen gebruik maar slegs as een en hoogstens twee toedienings per seisoen, teen sowat ‘n derde die aanbevole dosis in Suid Afrika. Die Kaliforniers bluf hulleself wat betref die gebruik van fosfonate vir *Phytophthora* beheer. Hulle sal hoër konsentrasies moet gebruik indien hulle effektiewe beheer van fosfonate teen *Phytophthora* wil bewerkstellig.

Bruinvrot. Slegs koperbespuitings word gebruik en nie fosfonaatbespuitings nie. Hulle sit met probleme wanneer dit reen ten spyte van die koperbespuitings. Die fosfonate behoort hierdie probleem op te los.

Nadorcotts

Saadprobleme. Ernstige probleme word ondervind met saad in die W-Murcotts, in Suid Afrika bekend as Afurers, Nadorcotts of Clemengolds. Dit word veroorsaak deur die gebruik van bye tydens blom en daar

word tans gepoog om wetgewing daar te stel wat byeboere sal verbied om byekorwe binne ’n 8km radius om W-Murcott boorde uit te sit. Die bye mag nie vergif word nie. In Suid Afrika waar dieselfde probleme met saad in die Nadorcotts ondervind word behoort ons ook ‘n waarskuwing aan produsente te rig om nie byekorwe naby hierdie boorde uit te sit nie.

Planttelersregte. In Amerika is daar geen planttelersregte op die W-Murcott soos op die Nadorcott in Suid Afrika nie. Die kultivar word op groot skaal aangeplant en is uiters gewild.

Nawels

Laat oes. Die Kalifornièrs was steeds besig om van die vorige seisoen se nawels te pluk en sal waarskynlik nog tot laat in Junie nawels oes. Dit is ongelooflik om te sien hoe laat hulle die nawels laat hang. Die vrugte is diep oranje en stroopsoet en ’n mens wonder hoe die Wes Kaapse bleek geel ontgroende nawels hiermee sal kompeteer. Die pryse van die nawels het tydens my besoek van U$16 na U$18 per karton gestyg omdat die vrugte begin skaarser raak het.

(c) **Kultivarontwikkelling**

Die Kaliforniese sitrusindustrie beplan om ’n persoon aan te stel om vir hulle bedryf nuwe kultivars te werf en te evalueer.

(d) **Kaliforië vs Florida**

Sitruskanker. Die feit dat Florida van plan is om die handoek in te gooi wat betref sitruskanker het ’n rippel-effek ook in Kalifornië. Hoewel sitruskanker nie ’n patologiese probleem vir die grootste deel van Kalifornië inhou nie, is daar gebiede soos Ventura county waar die siekte wel kan vestig. Dit sal ernstige fitosanitere implikasies he vir Kaliforniese sitrusbedryf en daarom doen die Kaliforniese sitrusbedryf alles in hulle vermoe om te verseker dat Florida vrugte nie naby Kalifornië toegelaat sal word nie.

(e) **Ander**

Granate. Dit word hoofsaaklik vir sap gevestig en is uiters gewild by die gesondheidsbewuste Amerikaners as ’n drankie. Daar moet onthou word dat daar nie VKM in Kalifornië is nie. In Suid Afrika sal daar beslis met VKM rekening gehou moet word.

8.4.2 **Besoek aan Zimbabwe – November 2006**

Aim

Zimbabwe was visited during the end of November 2006. The areas visited included Mid Save, Beitbridge, Chegutu and Mazoe. Chris Maggs from Interfresh accompanied me through the area. The aim of the visit was to give research feedback to growers, to determine the citrus research needs in Zimbabwe and to access the current situation in the different citrus producing areas with regard to their current production potential and the possible increase in phytosanitary problems. An irrigation trial at Mazoe was also visited and assessed.

Grower Feedback Meetings

Three feedback meetings were held. The first with the Beitbridge growers, the second with the Chegutu growers and the third at Mazoe. During these meetings the research presented at the 4th Citrus Research Symposium was presented to the growers. Each of these meetings was handled as workshops and there were lively participation by the growers. The Beitbridge group once again requested to be seen as a different TTG than Tshipise and Northern Zimbabwe and would like to have their own meetings. From the orchard visits it was clear that inputs with regard to nutrition were needed. None of the growers in the area has lost any of their citrus orchards yet. There is a good crop on the trees and the prediction is that the 2007 crop will be much larger than the previous crop. The fruit are clean and the size is good.

At Chegutu the meeting were attended by al but one of the citrus producers in the area. None of the new owners attended. The fruit size and yield are good and a large crop can be expected. The same situation was found at Mid Save and Mazoe. All the areas had good rains and a uniform crop can be expected.

The research needs in Zimbabwe were not determined in time this year. This was as a result of the uncertainties in Zimbabwe and because of Chris Maggs not being available to visit the different areas. However, the research needs are the same as in the past. Grey mite is still the first priority in the northern areas. The only exception is in the south where there is a definite need for work to be done on fertilization.

Production

During the 2006 season the Beitbridge growers produced approximately 2,3 million cartons of citrus compared to 300 000 in the rest of the country. The northern areas are thus down to almost 15% of what they were supposed to produce would a redistribution of farms not have taken place. Most of the fruit exported in the northern areas are from the groves still owned by white farmers. This indicates that the loss in fruit exported from farms that were taken over was more than 90%. This, in spite of the technical knowledge still being available through mentors such as Chris Maggs and John Perrot. What has happened in Zimbabwe sends a clear signal to CRI to take notice of what is happening in South Africa and the effect that land reform could have on levy income.

Processing

A new processing plant has been set up on the Cawoods farm in the Beitbridge area. Though they only processed 2000 ton of fruit this year, their capacity is 20 000 t per year. It can thus be expected that very little fruit will in future be send through the border to be processed at Granor Passi in Pietersburg. Mazoe is down to 14 000t of fruit processed compared to 20 000t during the previous season. This is less than 30% of the plants capacity. Ironically they are sitting with stock which they can not sell. The competition board in Zimbabwe has decided that Coca Cola can not take over Schweppes and as Schweppes Zimbabwe has financial constraints they can not take up concentrate from Mazoe because they can not pay. Mazoe has paid R700/t of fruit to growers which is much better than most of the plants in South Africa. The increased world price for juice concentrate will benefit Mazoe as well as the new plant in Beitbridge.

Pseudocercospora angolensis

During this visit Mazoe and the citrus producing areas south of Harare was visited. I was impressed by the condition of the trees, the crops and the positive attitude of the growers. The areas where *Pseudocercospora angolensis* can be found were not visited. The disease has however not spread to any of the areas that were visited and according to Chris Maggs the incidence in the areas where it reoccurred in the north is very low as a result of a lack of flush in the neglected orchards.

Partial Root-zone Drying (PRD) Irrigation Trial

The PRD irrigation trial at Mazoe was visited. This trial is partially supported by CRI and done by Sebinasi Dzikiti who is a PhD student at the University of Zimbabwe. During a previous visit to the trial certain changes to the treatments were recommended by Graham Barry. This was done and though the purpose of my visit was to do more changes this was not done after Sebinasi explained the reasoning behind the current applications. The trial site is taken good care of. The instruments to take measurements are impressive and Sebinasi is doing a fine job. I was impressed by him as a person and CRI can continue to support his research proposals. There were slight problems with some of the drippers blocking but it is being taken care off. Chris Maggs who took over the responsibility for Mazoe, after Fred du Pont's visum to work in Zimbabwe was not renewed, will ensure that it will be done. The research feedback meeting held at Mazoe was also attended by Barnabas Chipindu who is one of Sebinasi's promoters at the University of Harare. Sebinasi promised to present his work at the 2008 Citrus Research Symposium.

CRI must ensure that the money that is paid for this research comes from the levies paid in Zimbabwe and not directly from South Africa. It is extremely difficult to transfer the levies from Zimbabwe to South Africa. For this reason Chris also covered all my expenses in Zimbabwe and will deduct that from the levies owned to the CGA.

Technology Transfer Groups

As was mentioned previously the Beitbridge growers would like to form their own TTG. It is difficult for them to attend the Tshipise meetings and they are also to far from Harare to join the rest of the growers up north. Paul Bristow is running the TTG in Beitbridge and is doing a fine job.

The northern area is different. This area include Chegutu, Mazoe, Bindura, Mtepatepa, Mvurwi , Shamva and Mid Save. The production in this area has dropped to a level where it actually do not justify the appointment

of a consultant to assist with the TTG in the area. However, I would like to recommend that CRI do obtain the assistance of Chris Maggs from the first of January 2007 to do so. In the original budgets Extension did cater for a consultant in Zimbabwe. The northern areas are much more difficult to organise and without Chris it will be impossible. Chris has been doing this for many years and through him we do not only have access to the growers but to the Zimbabwe citrus industry as a whole. Chris is also monitoring the research work that is conducted at Mazoe and *P. angolensis* in Zimbabwe. I would therefore like to proceed and send the contract that was signed by the rest off the consultants to Chris for consideration.

9 **TECHNOLOGY TRANSFER** (H.F. le Roux [Nelspruit] en J.J. Bester [Port Elizabeth])

9.1 **NAVORSINGSPRIORITEITE / RESEARCH PRIORITIES - 2007**

Die Navorsingsprioriteite van die verskillende sitrusproduserende streke in suider-Afrika vir 2007 is gedurende Augustus en September 2006 bepaal deur Hennie le Roux en Hannes Bester. Dit is gedoen net na afloop van die 4de Sitrusnavorsingsimposium wat in Port Elizabeth gehou is, tydens ’n navorsingsterugvoersessie wat by elk van die Tegnologie-oordragingsgroepe aangebied is. Die navorsingsprioriteite is in wese dieselfde as in 2004 en 2005. Sitrus swartvlek en VKM bly steeds die bo aan die lys van navorsingsprioritite a.g.v. die streng vereistes vir sitrusuitvoere. Die produsente besef dat indien suider Afrika die 100 miljoen kartonne wat voorspel word vir 2010, suksesvol wil bemark, die bedryf instaat sal moet wees om meer kartonne in alternatiewe markte soos China te kan plaas. In die geval van swartvlek word daar steeds gewag op terugvoer van die Europese Unie. Tot tyd en wyl hierdie patogeen sy fitosanitêre status in Europa verloor sal dit die hoogste prioriteit bly vir gebiede soos Letsitele en die Onderberg. Produsente het gevra dat hierdie saak dringend met die EU opgevolg en afgehandel moet word. Skildefekte bly steeds die belangrikste prioriteit binne die Hortologie afdeling met Peteca wat in meer as een gebied nou die hoogste navorsingsprioriteit vir die gebied is.

Soos in die verlede toon Tabelle1-3 die prioriteite van die verskillende areas op ’n skaal van 0-3. Aspekte waaraan ’n 0-waarde toegeken is benodig nie verdere navorsing nie terwyl ’n 3 beteken dat navorsing op daardie betrokke gebied die hoogste prioriteit moet ontvang. Elke navorsingsaspek se prioriteit word vir die verskillende streke aangetoon sodat dit makliker vir die navorsers sal wees om te sien in watter areas die onderskeie onderwerpe hoë navorsingsprioriteite is. Die hoogste navorsingsprioriteit vir elke area word ook aangetoon. Navorsers word daaraan herhinner dat hoewel die geweegde belangrikheid ook gegee word dit misleidend kan wees aangesien daar sekere prioriteite is soos *Pseudocercospora angolensis* wat in geheel gesien ’n lae gewig mag hê, maar wat vir Zimbabwe een van die hoogste prioriteite is.

In die geval van die Siektebestuurprogram val die klem steeds op Swartvlek, *Alternaria*, Na-oes bederf, Vergroening en Tristeza navorsing. Antraknose was die afgelope seisoen ’n ernstige bron van kommer op nawels en moet daarom aangespreek word. Daar moet ’n effektiewe beheerprogram vir *Phytophthora citrophthora* vir die Suid-Kaap ontwikkel word. In die Geintegreerde Plaagbestuur Program is dit valskodlingmot en vrugtevlieg (veral Natalvlieg) wat die hoogste prioriteit het. Grysmyt is steeds deur twee van die sitrusproduserende gebiede as hul hoogste prioriteit aangedui. Die sogenaamde graan “Chinch bug” bly vir die Wes Kaap ’n bedreiging. Die Oesopbrengs en Vruggehalte-program se klem val veral op skilprobleme soos Peteca, Skilafbraak, Gepokte skil en Vruggrootte manipulering. ’n Belangrike opmerking is gemaak dat navorsing veral op die primêre, dieperliggende oorsake van fisiologiese skildefekte gefokus moet word. ’n Oplossing vir kruisbestuiwing van laat mandaryne moet gevind word. Die behoefte vir die aanstelling vir ’n voedingskundige wat ook die veskillende produkte wat op die mark is, wat veronderstel is om die wortelomgewing te verbeter te kan evalueer, is weereens soos in die verlede, herhaaldelik beklemtoon. Hierdie behoefte vereis dringende aandag.

Wat kultivars aanbetref is daar veral ’n behoefte vir groot laat Valencias in die Noorde sowel as vroeë pomelos vir Japan. Dit is vir die sitrusprodusente duidelik dat die Kultivarprogram weer op dreef is. Die opleiding van ’n Suid-Afrikaner in Florida onder dr. Fred Gmitter om ons kundigheid met betrekking tot genetiese manupilering van sitrusmateriaal uit te bou is versoek.

Die Sitrusuitvoerders Forum het versoek dat meer werk gedoen moet word op verskepingstemperature, verpakking en ventilasie en dat dit deur CRI gekoördineer moet word. Hulle ander prioriteite was skildefekte, witluisbeheer en alternatiewe kultivars.

Dit is vir die sitrusprodusente duidelik dat die Kultivarprogram weer op dreef is. Die opleiding van ’n Suid Afrikaner in Florida onder dr Fred Gmitter om ons kundigheid met betrekking tot genetiese manupilering van sitrusmateriaal uit te bou is versoek.

Die opdatering van die Produksie Riglyne bly vir die bedryf 'n prioriteit, veral in die geval van sproeibemesting.

Die aspekte waaraan daar steeds aandag gegee moet word ten opsigte van elk van die verskillende navorsingsprogramme is die volgende:

PROGRAM: SIEKTEBESTUUR

Projek: Swartvlek

In die geval van Weipe, Tshipise en die Benede Oranje rivier is die hoogste prioriteit toegang tot die VSA. Hierdie prioriteite is streng gesproke Marktoegang aangeleenthede maar word genoem omdat dit die aspekt is waarteen CRI se waardetoevoeging vir hierdie gebiede gemeet sal word. Daar word aanbeveel dat die huidige Swartvlekprojek sal voortgaan met al die verskillende fasette wat nog nie afgehandel is nie.

1. *Piknidiospore as moontlike bron van inokulum*:
Piknidiospore kan in sekere kultivars met meervoudige vrugsette soos suurlemoene as bron van inokulum optree. Wat is die klimaatsvereistes wat nodig is vir infeksie om plaas te vind? Op watter stadium is die blare vatbaar vir piknidiospoor infeksie? Van hierdie vrae is reeds beantwoord maar die werk moet nog gepubliseer word. Dit is van kritiese belang dat hierdie werk voltooi sal word sodat SA sy argumente reg het sou Brasilie inligting oor piknidiospoorvrystelling publiseer wat marktoegang van Suid Afrikaanse vrugte in die EU kan bemoeilik.

2. *Epidemilogie*:
(a) Evaluering van verskillende biologiesebeheer agente om die dooie blare op die grond vinniger af te breek en sodoende askospoorvrystelling te voorkom.
(b) Evaluering van die stofsuier wat in die Oos Kaap ontwikkel sou word om dooie blare te verwyder. Alternatiewelik: Die makadamiabedryf in Levubu gebruik 'n stofsuier om blare te verwyder voordat hulle die neute optel. Ondersoek die moontlikheid om hierdie stofsuier in sitrus te gebruik.
(c) Evaluering van grondbewerking (disc) om blare wat in die rye in gevee is effektief te bedek.

3. *Na-oesbeheer*:
(a) 'n Studie om vas te stel wat gedoen kan word om die ontwikkeling van latente infeksies na verpakking in transito te onderdruk. Dit sluit in 'n oudit van wat huidiglik met vrugte gebeur vanaf die pakhuis onderweg na die hawe, by die hawens voordat dit verkoel word, tydens die laaiproses en tydens verskeping.
(b) Effek van warmwaterbehandelings op simptoomontwikkeling.
(c) Chemiese / biologiese onderdrukking van simptoomontwikkeling.
(d) Metodes om simptoomontwikkeling in boorde te stimuleer sodat latente infeksies kan wys voordat vrugte verpak word.
(e) Metode om vrugte op die paklyn te kan "scan " vir latente infeksies.

4. *Kwekerye*:
- Warmwaterbehandeling om swartvlekvry entmateriaal te verseker.
- Finalisering van die protokol om sitruskwekerye te monitor vir die teenwoordigheid van swartvlek in plantmateriaal.
- Swartvlekbeheerprogramme wat in die kwekerye gebruik kan word wat nie 'n gevaar inhou vir die ontwikkeling van weerstand teen swamdoders wat in kommersiële boorde gebruik word nie.

5. *Chemiese beheer:*
- Evaluering van nuwe chemiese middels asook beheerstrategië wat meer bekostigbaar is.
- 'n Sporekill program moet geregistreer word in kombinasie met die strobulirienes, benzimidasole, kopers en mancozeb. Die kombinering van Sporekill met hierdie produkte is tans nie geregistreer nie en daarom is die aanbeveling om hierdie produkte tem eng onwettig.
- Herevaluering van die terugwerkende aksie van die strobilurienes om vas te stel of die terugwerkende aksie dalk langer as 14 dae kan wees.
- Registrasie van strobulirienes op suurlemoene.
- Ondersoek om die verspreiding van Benlate weerstandbiedendheid te bepaal.

6. *Weerstandbiedendheid:*
'n Toets om *Guignardia* weerstandbiedendheid teen die strobilurienes te bepaal soos wat tans vir die benzimidasole gedoen word.

7. *Nuwe PCRs en ’n Diagnostiese “kit”*
   - Verfyning van die PCRs om die teenwoordigheid van CBS op simptoomlose blare en enthout te bepaal.
   - Die ontwikkeling van ’n vinnige toetsapparaat wat deur produsente/pakhuise /PPECB gebruik kan word om te onderskei tussen *G. citricarpa* en *G*. *mangiferae.*

8. *Selektiewe medium*:

’n Selektiewe medium om *Guignardia* op te kweek.

9. *Genetiese weerstand*:
Ondersoek die moontlikhede om geneties gemodifiseerde weerstand in sitrusplantmateriaal te bewerkstellig.

10. *Voorspellingsmode*l:
- Voorspellingsmodel wat kan voorspel wanneer die klimatologiese toestande gunstig sal wees vir spoorontkieming en infeksie vir inokulumbestuur (Katrivier)
- Outomatisering van spoortellings.

11. ’n Versoek dat Prof Kotze alle Swartvleknavorsing sal saamvat sedert sy artikel in Plant Disease verskyn het in die 1970s tot op hede sodat dit in ’n oorsigsartikel in Plant Disease gepubliseer kan word. (Ida Paul is glo tans besig met so ’n oorsigsartikel. Betrek Prof Kotze daarby).

Projek: Entoordraagbare Siektes

Vergroening

1. Opname om vas te stel of *Liberibacter asiaticus* en *L. americanus* in suider Afrika voorkom.
2. Vektorbeheer: Ontwikkeling van alternatiewe middels vir stamaanwending om die organofosfate (methamidofos) te vervang. Mospilan is beskikbaar en moet weer vergelyk word met stamaanwendingsformulasies van Confidor of soortgelyke generiese produkte. Registrasie van hierdie produkte. (Confidor is bv nie geregistreer vir bladvlooi beheer in boorde nie. Dit bemoeilik wettige aanbevelings vir bladvlooibeheer met Confior).
3. Vektorbeheer:Ontwikkeling van lok– en dood middels om bladvlooi op ’n soortgelyke wyse te beheer as wat die M3 vrugtevliegbeheer.
4. Vektorbeheer: Ontwikkeling van alternatiewe bladvlooibeheer strategie soos bv. Predatore, parasiete of paringsontwrigting.
5. Genetiese weerstand: Weerstandsteling deur gebruik te maak van chimeras met vergroeningsverdraagsame sektore (Embrio rescuing).
6. Genetiese weerstand: Weerstandsteling d.m.v hoëvlak biotegnologie en geneties gemanipuleerde weerstand. (Vergelyk met die werk wat in Bordeaux gedoen is teen *Spiroplasma citri s*owel as die werk wat tans aan die gang is in Florida deur dr Fred Gmitter en sy groep).
7. Korrektiewe middels: Alle moontlike middels moet hier ge-evalueer word ongeag aanvaarbaarheid vir die markte. Dit sluit in antibiotikas, middels wat plantweerstand (SAR) verhoog en middels wat vergroeningsimptome onderdruk.
8. Hittebehandelings: Die gebruik van plastiese koepels om d.m.v. solarisasie temperature so te verhoog dat dit die bakterië binne die plant kan vernietig. Ontwikkel ’n model wat aantoon hoe die bakterie titer afneem namate sekere Temperatuur/Tyd behandelings toegepas word.
9. Goedkoop tegniek om kwekerybome te toets vir vergroening (UV lamp wat gentisiensuur uitwys).

Tristeza

1. Identifisering en karakterisering van die deel van die genoom van Tristeza wat verantwoordelik is vir kruisbeskerming en die mate van strafheid.
2. Ondersoek die teorie dat die saamstel van ’n super CTV kruisbeskermingsras nie nodig is nie maar slegs die gedeelte van ’n kruisbeskermingsras se DNA wat die sein vir kruisbeskerming gee.
3. Evaluering van verbeterde kruisbeskermingsrasse vir elk van die verskillende pomeloproduserende areas (Tshipise, Letsitele, Hoedspruit, Malelane, Komatipoort/Swaziland, Nkwaleni, Benede-Oranje). Veral Letsitele voel sterk hieroor.
4. Verdere evaluering van verskillende CTV populasies op TSR. Maak seker dat die kruisbeskermingsras wat huidiglik by die Grondvesblok gebruik word wel die regte een is.
5. Tuinboukundige aspekte wat die insidensie van Tristeza agteruitgang kan vertraag.
6. Alle bestaande proewe wat Faan van Vuuren voortgaan.
7. Instandhouding van groeipuntenting, kruisbeskerming van nuwe plantmateriaal en monitering van Grondvesblok.

Onverenigbaarheid

Ondersoek die rol wat “Citrus leaf blotch “ speel in bo-stam/onderstamonverenigbaarheid.

Ander

- Ondersoek die galvorming op die Clemenpons en maak seker dat hierdie kultivar vry is van ongewensde siektes. Al die sogenaamde “Bulbome” is vry van galle terwyl die wat die sogenaamde regte Clemenpons bome is, almal galle het. Hierdie bome is duidelik gestres wat die vroeër rypwording verklaar. Die bedryf wil die versekering hê dat daar nie ’n risiko bestaan dat die siekte wat die galle op die Clemenpons veroorsaak na ander kultivars kan versprei nie.
- Ondersoek die Lina nawels in die Katrivier en in die Grondvesblok en stel vas of Impietratura nie dalk deur die groeipuntentingsproses geglip het nie.
- Ondersoek onverenigbaarheid by die Fukomoto in Suid Afrika.
- Ondersoek onverenigbaarheid op kumkuarte.

Projek: Na-oes patologie

1. Evaluering van nuwe wakse, oppervlak steriliseerders en swammiddels soos dit beskikbaar raak.
2. Ontwikkeling van strategië om alternatiewe in plek te hê indien weerstand opbou teen die bestaande na-oes swammiddels (GRAS chemikalië, Fisiese behandelings soos osoon en warmwaterstrategië).
3. Na-pak berokings om bederf en fitosanitêre plae te elimineer.
4. Evaluering van bederf wat voorkom tydens kommersiële ontgroenings.
5. Na-oes strategië vir organies geproduseerde sitrus. Kan Sporekill hier Imazalil en Guazatien vervang al is dit nie so effektief nie?
6. Verklaar hoekom die VSA vrugte se wakse in Japan soveel beter vertoon as vrugte wat in SA gewaks is.
7. Monitering (landswyd) van die insidensie en regstelling van imazilielweerstand- biedendheid in pakhuise (Wes Kaap).
8. Effek van humiditeitsbeheer op na-oespatogene in die ontgroeningsproses.
9. Voorkomende beheer van suurvrot. Monitering van produkte wat tans in die handel gebruik word vir suurvrot beheer. SABS resultate wys dat van hierdie produkte nie die gespesifiseerde hoeveelheid aktief bevat nie.
10. Alternatiewe beheer deur gebruik te maak van antagoniste en ander biologiese beheer agente. Die bedryf is van mening dat die kapasiteit binne na-oespatologie verhoog moet word.

Projek: Grondgedraagde siektes

Sitrusaalwurm

1. Evaluering van talle biologiese beheeragente wat in die handel beskikbaar is. (Bv produkte deur Monterey en Micro Life in Amerika)
2. Evaluering van Enzone vir aalwurmbeheer in bestaande boorde.
3. Stimulering van aalwurmeiertjies om uit te broei sodat eenmalige aalwurmdodertoedienings die aalwurm se lewenssiklus meer effektief kan onderbreek.
4. Onderstamevaluerings.
5. Voorplantbehandeling van herplantgronde deur middel van beroking . (Beide gronde met ’n relatiewe hoë klei-inhoud en sanderige grond). Hier moet gekyk word na alternatiewe vir metielbromied soos Vapam, bioberoking, en die kombinasie van bio-beroking en solarisasie.
6. Effek van aalwurms indien enige in OHS boorde. Evaluering van *Paecilomyses lilanicus* onder drup.

Skede-aalwurm (*Hemicycliophora*)

1. Onderstamevaluering.
2. Effek op groeikragtigheid van kwekeryboompies.

Phytophthora

1. Evaluering en registrering van die gebruik van fosfonate deur drupstelsels.
2. Effektiewe beheer van *P. citrophthora.*
3. Faktore wat vrugte meer gevoelig maak vir koper en fosfonaat “stippeling” (Water pH?)

4. Evaluering van nuwe chemiese middels wat op ander gewasse teen *Phytophthora* ontwikkel is in die afsienbare verlede, met die klem op kwekerye.
5. Ontwikkeling van 'n merker om onderstamme vinniger te kan toets vir *Phytophthora* verdraagsaamheid.
6. Evaluering van verskillende fosfonaat formulasies deur Agri Inspect of die DC.

***Fusarium*/Blight**

1. Die rol van *Fusarium* op wortelvrot waar lae koolhidraatvlakke voorkom en alternatiewe drag veroorsaak, soos wat tans met die laat mandaryne ondervind word, moet ondersoek word.
2. Onderstam-evalueringsproef in Letsitele teen Blight moet gemonitor word.
3. Nuwe onderstamme se gevoeligheid vir isomartisien (*Fusarium*toksien) moet bepaal word. Hoe vergelyk C35 met die huidige kommersiële kultivars?

Armillaria

1. Chemiese beheermaatreëls (dit wil voorkom of die Phytex wat by IYSIS in Swaziland gebruik word effektief is. Is dit waar?)
2. Alternatiewe gashere in Patensie.

Projek: Vrug en blaarsiektes

Pseudocercospora angolensis (P.a)

1. Ondersoek na huidige status van siekte sodra daar weer politieke stabiliteit in Zimbabwe is.
2. Residu ontledings van chemikalië wat vir die laaste bespuiting in Februarie/Maart aanbeveel word (hierdie werk kan in SA gedoen word).
3. Klimatologiese kartering van *P.a.*

Alternaria

1. Evaluering van nuwe kultivars teen *Alternaria* soos wat hulle in die land ingebring word.
2. Evaluering van meer koste-effektiewe spuitprogramme en nuwe chemiese produkte a.g.v. nuwe.
3. Voorspellingsmodel vir die sitrusindustrie.

Botrytis

1. Evaluering van chemiese beheer op suurlemoene. Registrasie van produkte sodat wettige aanbevelings op sitrus gedoen kan word.
2. Bepaling van toedieningstye. Hier moet veral gelet word op *Botrytis* wat die vruggies aanval en laat val of mumifiseer vanaf blom tot albastergrootte.

Colletotrichum

1. Faktore wat vrugte predisponeer vir antraknose.
2. Voor-oes beheerstrategieë vir antraknose.
3. Na-oes beheerstrategieë vir antraknose.

Cercospora

Geen navorsing maar monitor die situasie voortdurend.

PROGRAMME: INTEGRATED PEST MANAGEMENT

Project: False Codling Moth (FCM)

1. Evaluation of new chemicals.
2. Evaluation of granuloviruses for FCM control on a commercial scale in all the citrus production areas (Letsitele especially are not going to use the product unless River Bioscience has tested it in the Valley.)
3. Evaluation of *Trichogrammatoidea cryptophlebiae* for FCM control in more areas.

4. Development of SIT on a commercial scale.
5. Evaluation of repellents for FCM.
6. Evaluation of biocontrol of FCM larvae (e.gg *Heterorhabditis & Steinernema*).
7. Evaluation of mating disruption to control FCM.
8. Re-evaluation of the Lorelei and other traps and the meaningfulnessof the threshold levels. Determine threshold levels for traps other than the Lorelei.
9. Effect of Alsystin and Nomolt on red spider repercussions.
10. Effect of higher cold storage temperatures vs time (every raised 0.5°C cold storage temperature has a major effect on colour and rind decay of certain cultivars).
11. Evaluation of post-packing fumigation treatments.
12. Determine whether Meothrin susceptibility exists in Patensie.
13. Evaluation of parasite releases.
14. Cultural practices that can change the severity of FCM, such as discing.
15. Packhouse line detection of infected fruit (IR?).
16. Irradiation of fruit prior to export as an alternative to cold steri.
17. Revise the intervention thresholds of FCM monitoring (all registered traps) in light of its phytosanitary status.

Project: Fruit fly

1. Registration of M3 for organic farming.
2. A model to reduce the number of M3 traps from the outside to the inside of larger orchards to reduce cost. Results to be published in SA Fruit Journal on this aspect.
3. Evaluation of insecticides used in the M3 to last longer.
4. Evaluation of grape varieties for susceptibility in order to be able to concentrate on these vineyards to eliminate fruit fly in areas where both citrus and grapes are grown. Refer to DFPT.
5. Cold sterilization of different varieties for Natal fruit fly.
6. Creation of areas which is for all practical purposes fruit fly free in Patensie and Vaalharts.
7. Evaluation of chemicals to be used in cost-effective bait spray programmes when the use of the OPs are terminated.
8. Alternative chemicals for aerial applications.
9. Alternative fumigation options to control fruit fly post packaging.
10. Re-evaluate threshold values of Sensus traps in comparison to the Delta trap.
11. Other fruit fly aspects:
    - Survey for exotic fruit fly.
    - Cold sterilization of marula fruit fly in oranges.
    - Determine the prevalence of marula fruit fly in citrus.
    - Urban testing of the M3.

Project: Mealybug

1. Evaluation of biocontrol of mealybug in the different citrus producing areas.
2. Determine the natural populations of predators and parasites in the different areas to establish whether the release of biocontrol agents will increase the levels of parasitism.
3. Seek acceptance of the DNA probe developed in SA by the USDA. Implement the PCR for mealybugs at the DC in Nelspruit.
4. Evaluate alternative chemicals to replace the OPs.

Project: Cosmetic pests

Thrips

1. Evaluation of alternative chemicals.
2. Breeding and testing of the predatory thrips collected in the Lower Orange River.

Grain Chinch bug (GCB)

1. Evaluate pre-harvest chemical control options to the point of being registered.
2. Evaluate the residu status of the above chemicals in parallel to avoid wasting time on actives that have no potential from a residu perspective.
3. Evaluate post harvest packhouse controls using pyrethrin and pyrethrin + azadirachtin products.
4. Determine GCB control in areas around the packhouse.
5. Isolate GCB attractants for monitoring and possible control purposes.

6. Determine the timing of the movement of GCB into the orchards and the risk of fruit being infested post packing.

Leafhopper

1. Thresholds on thrips traps.
2. Host range
3. Effect of intercropping.
4. Timing of control sprays.

Project: Production pests

Psylla

1. Alternative chemicals to replace OP stem treatments.
2. Develop biological control options.
3. Develop alternative control strategies especially in older trees (Dimethoate?)

Ants

1. Develop strategies/chemicals to keep ants out of the trees but in the orchard.
2. Donor with alternative active ingredient.

Red scale

1. Determine the effect of oil sprays on colour, yield (effect of 1% summer oil followed by a dry January/February on yield), internal fruit quality, seedless cultivars.
2. Determine the impact of *Aphytis* releases to establish if the positive results after these releases are in fact because of the releases or as a result of the producers abstaining from chemical sprays allowing the natural occurring *Aphytis* to increase.
3. List of alternative hosts to establish predator and parasite colonies.
4. Timing of the release of *Aphytis*-based on scientific principles (monitoring of male flights?).
5. Timing of chemical sprays.
6. Registration of a generic imidacloprid.
7. Rearing of *Aphitis africanus.*

Soft Green scale

1. Investigate biocontrol complex.
2. Chemical control options.
3. Host range.
4. Why more prominent in IPM orchards?

Grey mite

1. Effective control strategies.
2. Determine the possible role of the spiroplasma-like organism found by UP several years ago with the concentric ring blotch caused by grey-mite.
   This mite is the no. 1 priority for both the Rustenburg and Ohrigstad study groups.

Budmite

1. IPM programme for the Vaalharts area.
2. Alternative for Acarol.
3. Late miticide especially on lemons.

Lemon moth

1. Alternative control options.

Snails

Effective, affordable control measures.

Bollworm

Registration of alternative control strategies e.g. virusses.

Stinkprinkaan

Beheermaatreëls.

Project: Biocontrol disruption

1. Keep data-base of non-target effects of the different chemicals updated.
2. Determine which of the OPs are the first to be removed and ensure that alternative control strategies/chemicals are available when needed.
3. Accreditation of insectaries.

Other

1. Times required by the different chemicals to dry before being effective.
2. Effect of oil on the leaves on subsequent chemical sprays. Do these chemicals then need a longer period to dry?

PROGRAM: OESOPBRENGS EN VRUGKWALITEITSBESTUUR

Projek: Oesopbrengs

1. Evaluering van bestaande Oop Hidroponiese Stelsels (OHS) in die verskillende areas vir verskillende kultivars.
2. Riglyne vir die bestuur van besproeiing en bemesting deur OHS om maksimum suikers en kleurontwikkeling te verseker (Produksie Riglyne).
3. Evaluering van puls-besproeiing teenoor minder gereelde drup.
4. Evaluering van boomgrootte beheer en ligbestuur in hoë digtheid aanplantings.
5. Formulering van 'n vrugset-strategie vir nawels in die Oos-Kaap waar groot temperatuur skommelings veroorsaak dat die vrugte afspeen.
6. Vrugsetstrategie vir saadlose suurlemoene.
7. Snoei van suurlemoene in warm areas (Pongola).
8. Verhoogde vrugset op Minneolas om vruggrootte te verbeter en kleiner vrugte te kry (Marble Hall).

Projek: Vruggehalte

Vruggroote

1. Finalisering van vruggrootte model.
2. Verfyning van die gebruik van Corasil en Maxim op vruguitdunning van Ou Kloon Valencias en Pomelos.
3. Oesskattingsmetode vir vruguitdunning.
4. Evaluering van fulviensuur, humiensuur, TopGroei en ander soortgelyke produkte wat tans in die handel verkrygbaar is vir oes en vruggrootte verbeterings.
5. Effek van somerolie gevolg deur warm temperature op vruggrootte.
6. Onderstamevaluering vir onderstamme wat groter vrugte gee op swaarder gronde.
7. Effek van handuitdunning en snoei verskillende tye van die jaar. Soek 'n produk wat hergroei kan onderdruk wat meer gebruikers vriendelik is as Planofix.
8. Is die bemestingsnorme wat in die sewentigerjare op lemoene op growweskilsuurlemoene ontwikkel is steeds relefant vir vandag se kultivars op 'n wye verskeidenheid onderstamme in verskillende klimaatstreke?

Interne vrugkwaliteit

1. Verlaging van suur in sekere areas. Chemies sowel as bestuurspraktyke.
2. Ontwikkeling van 'n plaasvervanger van kalsiumarsenaat om sure te verminder.
3. Metodes om vastestowwe (TSS) te verhoog.
4. Effek van ringelering op interne kwaliteit.
5. Turkey Valencia het probleme in die mark deurdat dit pap word en druk. Kan hierdie probleem d.m.v. bemesting reggestel word?

Oesbestuur

1. Som strategië vir vrugset op Deltas en Midknights op.
2. Snoeistrategië – som bestaande kennis op.

Eksterne vrugkwaliteit

1. Ondersoek die fisiologie van “stippeling” wat sekere tye van die jaar veroorsaak word deur bespuitings van bv. Koper, fosfonate en vrugtevlieglokase. Stel die verband tussen hierdie verskynsel (necrostoma), antraknose en “Swazi-spot” vas. Speel water pH dalk ’n rol? Wat van nagtemperature?
2. Opkleur van suurlemoene vir vroeër vrugte na Japan.
3. Kleurverbetering vir vrugte na die VSA (Gibbereliene , PGRs, OHS)
4. Indusering van langer vrugte by suurlemoene.
5. Effek van osoon op rakleeftyd van vrugte.

Voorspellingsmodelle

1. Ontwikkel ’n toets om oorrypheid van vrugte te bepaal.

Projek: Skilkondisie

Peteca

1. Bepaal die bydraende oorsake.
2. Herbevestig dat hoe CO_2 ’n rol speel. Kan CO_2 gebruik word om ’n produsent vriendelike metode te ontwikkel om suurlemoene te toets vir hulle vatbaarheid vir Peteca?
3. Formuleer strategië om dit te voorkom.
4. Voorspellingsmodel.
5. Effek van wakse en waksaanwending. Herbevestig dat dit wel die swaarder wakse is wat skilafbraak verhoog.
6. Effek van ontgroening.
7. Enige na-oes behandelings? Herbevestig dat swaarder wakse veiliger is.
8. Effek van warmwaterbaddens.
9. Effek van hoë stikstofvlakke
10. Effek van die spoed en intensie waarmee vrugte geborsel word.

Skilafbraak

1. Bepaal die faktore wat ’n bydrae lewer.
2. Formuleer strategië om dit te voorkom (bestuur die fisiologie van skilafbraak).
3. Na-oesbehandelings om skilafbraak te voorkom.
4. Die rol wat die koueketting speel in skilafbraak. Die temperatuur/tyd protokolle van die verskillende kultivars moet opgegradeer word.
5. Bepaal die effek van snoei-intensiteit en die oophou van hergroei op skilprobleme (karotenoides se rol).
6. Rol wat ontgroening speel in skilafbraak (daar kom snaakse goed uit die ontgroeningskamers. ’n Hoër uitpakpersentasie is nodig na ontgroening.)

Gepokte skil (Rindpitting)

1. Bepaal die bydraende faktore.
2. Formuleer strategië om dit te voorkom.
3. Na-oesbehandelings om rindpitting te voorkom

Kraakskil

1. Bepaal die faktore wat aanleiding gee tot kraakskil (na al die jare se navorsing is die probleem steeds nie opgelos nie en lei Sondagsrivier tot R15milj. se verliese per jaar.)
2. Ontwikkel ’n betroubare model om kraakskil te voorspel.
3. Evalueer die bestaande boorde onder die OHS in gebiede waar kraakskil voorkom en stel vas of daar ’n afname in kraakskil in hierdie boorde is.

4. Is die verskil in kraakskil in boorde op growweskilsuurlemoenonderstamme wel soveel laer as op die citranges dat dit 'n terugbeweeg na hierdie onderstam met sy laer interne kwaliteit regverdig?
5. Kan oliestress 'n bydraende rol speel?
6. Bepaal watter varieteite/seleksies kry dit nie.

Oleo

1. Bepaal weereens al die faktore wat 'n rol speel.
2. Voorspellingsmodel.
3. Fisiologiese oorsake.
4. Voorkomingstrategie moet weer 'n slag gepubliseer word.

Rysterigheid

1. Bepaal die faktore wat dit veroorsaak.
2. Stel die fisiologie van die verskynsel vas.
3. Formuleer strategië om dit te voorkom.

"Checker board" effek

Stel vas of ontgroende vrugte nie weer kan vergoen nie. Daar word vrugte gekry waar die skilkleur wissel van oranje na 'n veel ligter geel.

Vrugbars.

1. Faktore wat 'n rol speel.
2. Strategie om dit te voorkom (Letsitele).

Koueskade

1. Ontwikkel verskepingsprotokolle vir nuwe kultivars soos die laat mandaryne.
2. Ontwikkel riglyne om koueskade te voorkom.

PROGRAMME: CULTIVAR DEVELOPMENT AND EVALUATION

Project: Cultivar and Rootstock Evaluations

Cultivars

South

1. Determine the distances needed to prevent cross pollination of late mandarins.
2. Determine the effect of bees on cross pollination.
3. Investigate the alternative bearing of the late mandarins.
4. Evaluate the late mandarins in the Knysna area.
5. Evaluation of Satsumas, Early and Late navels in Ohrigstad.
6. Determine the current status of the Fukomoto in SA with regard to incompatibility.
7. Vaalharts are looking for an earlier navel and Clementine variety for their area and asked that it should also include evaluations for cold hardiness.
8. Continued evaluation of cultivar trials at Richmond, KwaZulu-Natal.
9. Confirm that Impietratura did not pass through shoot-tip grafting into the material that went to the CFB. Test both trees at the CFB and in Kat River.
10. Cultivar workshop in Nkwaleni.
11. Determine the true-to-typeness of the Cambria.
12. Confirm that there is no unidentified organism involved with the Clemenpons that could pose a threat to the industry.

North

1. Letsitele Study group requested that Late navels, Late mandarins, a large Late Valencia and sweeter grapefruit to be sourced.
2. The Groblersdal area is also looking for a large Late Valencia to be picked in August and September and an early maturing navel.
3. Weipe is looking for an early Valencia that ripens before the Bennie for the Chinese market as well as a large late Valencia.
4. Evaluation of Satsumas, early- and late navels in the Ohrigstad area.

5. Swaziland would like to have the Late navels evaluated at Ngonini.
6. Burgersfort wants an early navel as well as a late Valencia.
7. Nelspruit is looking for a navel that will colour up earlier as well as an earlier Valencia.
8. Hoedspruit is looking for a late Valencia.
9. Tshipise and Hoedspruit are looking for an early grapefruit which can be used for Japan if the Florida citrus industry collapses.
10. Letsitele, Hoedspruit and the Onderberg would appreciate grapefruit selections less susceptible to sheepnose.
11. All new cultivar trials should also be evaluated for pests and diseases as well as its susceptibility for ind problems, e.g. Oleo.

Rootstocks

1. Evaluation of rootstocks for drought tolerance.
2. Evaluation of rootstocks to be used where the water quality is deteriorating. High sodium, chlorine and boron (Letsitele).
3. Cold hardy rootstocks (Vaalharts and Marble Hall).

PROJECT: BREEDING

1. Cultivar improvement using chimeras and *in vitro* ovule rescue.
2. Develop the capacity to do inhouse genetic manipulation of citrus cultivars (overseas training of CRI personnel).

PROGRAM: SITRUSVERBETERINSKEMA

1. Daar moet voordurent gesorg word dat die groeipuntenting en pre-immunisasie wat deur die ITSG en die CRI gedoen word altyd volgens protokol sal geskied en dat die hoogste moontlike standaarde gehandhaaf sal word.
2. Daar moet te alle tye gesorg word dat die sitrusgenebronne instand gehou sal word.
3. Daar moet verseker word dat die Grondvesblokstandaarde voortdurend sal verseker dat die entmateriaal wat in die bedryf gebruik word genetiese verantwoordbaar is en dat die materiaal vry sal wees van skadelike peste en plae.
4. Daar moet betyds vooruitbeplanning gedoen word om Thys du Toit te vervang sou hy sy aftree ouderdom nader.

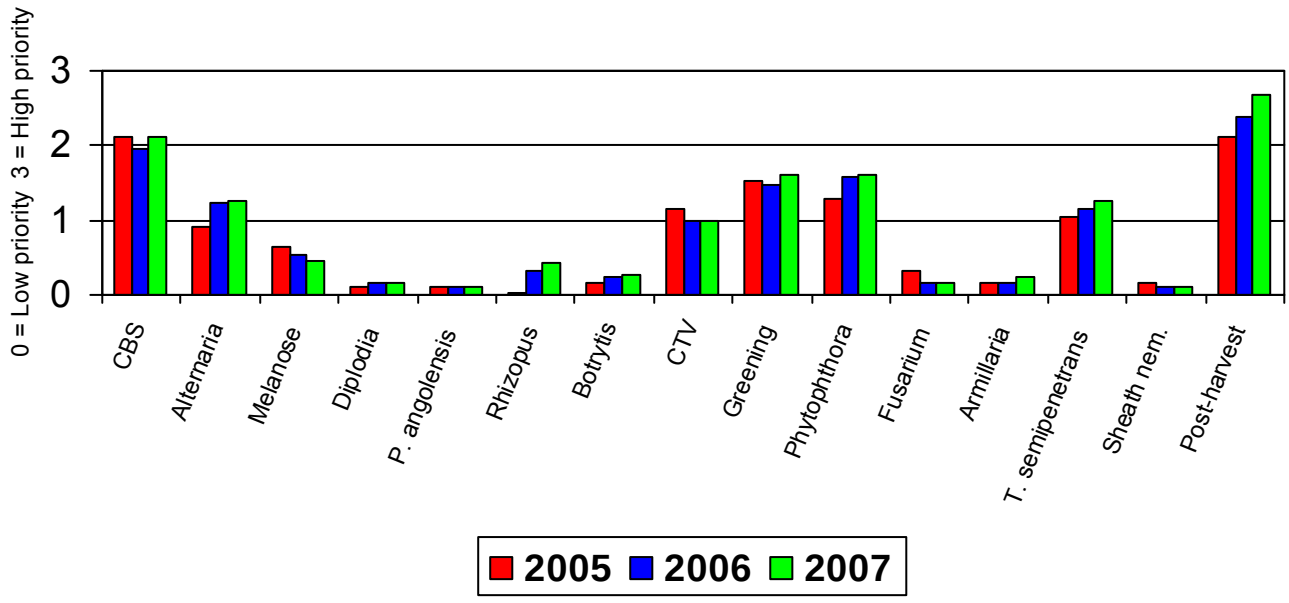
EXPORTERS TECHNICAL PANEL: RESEARCH PRIORITIES 2006/7
Rating: 0-3 (3 = High priority)

Waste/Decay	**2006**	**2007**
Penicillin (complete strategy)	3	0 (imazalil resistance)
Wet waste (sour rot) - Rhizopus	3	3
FCM	3	3
Fruit Fly	1	1
Resistance to imazalil survey	3	3
Replacement to imazalil	3	3
Manual for decay control	2	2
Optimum shipping temp to control waste (protocols)	2	2
Determine over-maturity (puffiness) - quantify	3	3
Reduce industry decay below 3% (ctns)	Challenge	
Rind Disorders / Physiological	***Priority No. 1**	
Rind pitting	3	3
Peteca	3	3
RBD – mainly softs	2	3
Cubing/puffiness	2	0 (covered)
Prevention of creasing	3	3
Optimum shipping temp to control rind disorders (protocols)		3

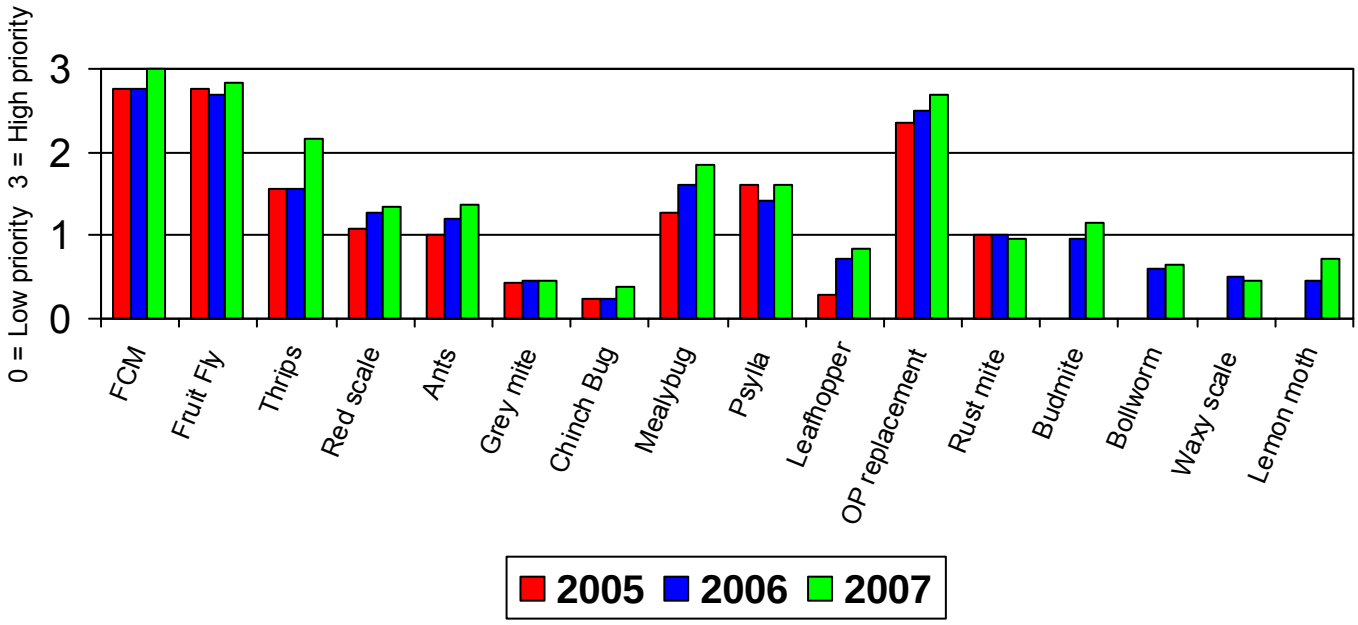
Waste/Decay	2006	2007
Marketability		
New cultivars	2	3
Adaptability of existing cultivars	2	2
Market Access		
FCM	3	3
Fruitfly (Natal+)	3	3
Mealybug (USA/Korea)	2	3
Chinch bug (USA)	2	2
Other hitchhikers (Dusty Surface beetle)	1	1
CBS	3	3
Shipping conditions		
Temp profile/regimes (containers & packaging)	2	2
Temp regimes	2	0 (covered)
CO_2 / CH_4 / Humidity	2	0 (covered under RD)
Neg. effect of low temp on colour/quality	2	0 (covered – Temp)
New container technology	2	2
New Technology		
Irradiation, etc.: Replacement of cold steri	3	3
Packaging and ventilation (CRI to co-ordinate)	2	2
New waxes (to control rind disorders)	2	2
Ozone for decay control	3	3
Technology Transfer		
Co-ordination and interpretation of research results	3	3
Manual for decay ID and control (origin, etc.)	3	3
Comments		
CRI must be involved in all new and existing cultivars for objectivity		
New products for mealybug to be evaluated		
Temperature regimes needed for containers and packaging		
Cartons to be standardised and evaluated for whole industry		
CRI to co-ordinate research on packaging and ventilation		
MRLs to be distributed to all chemical companies, AVCASA members, Exporters, SACNASP, SASCCON, etc.		
No. 1 Priority		
Rind disorders		
Changes		
Rind breakdown 2-3		
New cultivars 2-3		
Mealybug 2-3		
All changes to 0 are covered elsewhere		
New priorities		
Optimum shipping temperature to control rind disorders (protocols)		3

RESEARCH PRIORITIES - NATIONAL AVERAGES FOR THE RESEARCH PROGRAMMES

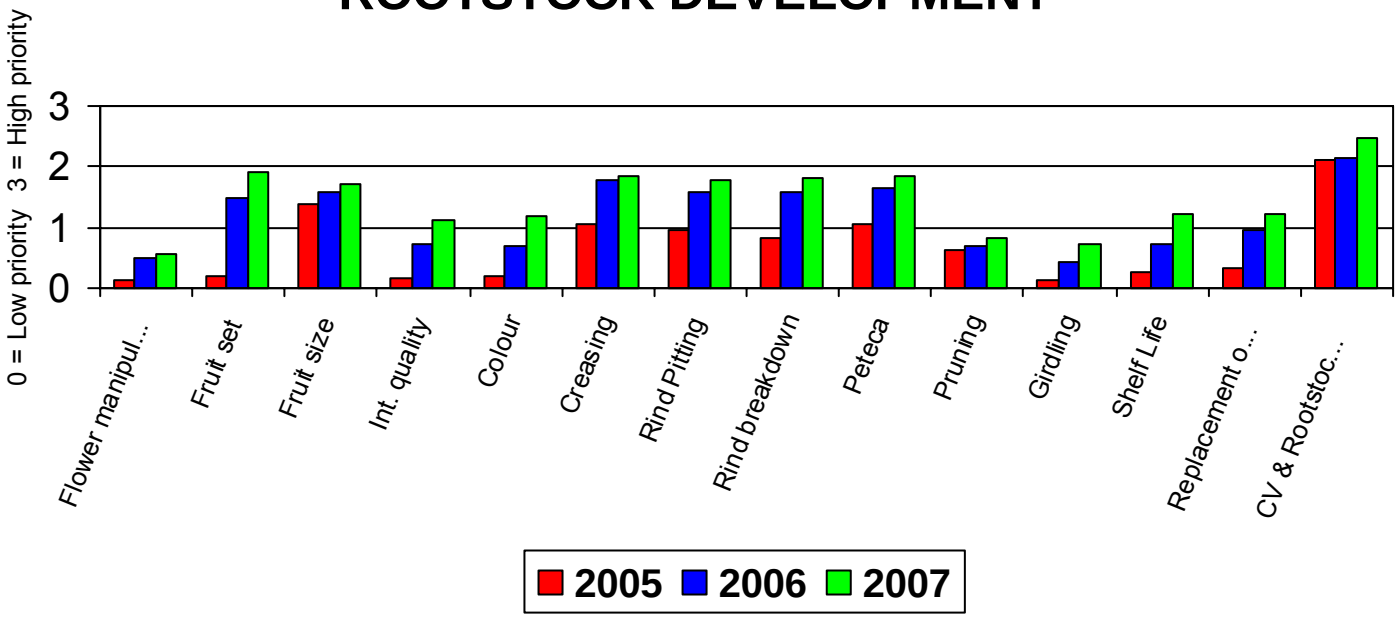
NATIONAL AVERAGES FOR DISEASE MANAGEMENT



NATIONAL AVERAGES FOR INTEGRATED PEST MANAGEMENT



NATIONAL AVERAGES FOR CROP LOAD & FRUIT QUALITY MANAGEMENT & CULTIVAR & ROOTSTOCK DEVELOPMENT



RESEARCH PRIORITIES 2006/2007

DISEASE MANAGEMENT Table 1

Citrus Area	CBS		Alternaria		Melanose		Diplodia		P. angolensis		Rhizopus		Botrytis		CTV		Greening		Phytophthora		Fusarium		Armillaria		Tylenchulus		Sheath nem.		Post Harvest	
	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07
Pongola	3	3	1	0	1	1	1	1	0	0	0	0	0	0	3	3	2	2	2	2	0	0	0	0	0	0	0	0	3	3
Swaziland	3	*3	1	0	1	0	0	0	0	0	0	0	0	0	3	3	2	2	1	1	0	0	1	1	1	1	0	0	2	2
Komatipoort	3	3	1	1	1	1	0	0	0	0	0	0	0	0	3	3	0	0	1	1	0	0	0	0	1	1	0	0	3	3
Malelane	3	3	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0	0	1	1	0	0	0	0	1	1	0	0	2	2
Nelspruit	3	3	1	2	0	0	0	0	0	0	0	0	1	1	1	1	3	*3	1	1	0	0	0	0	1	1	0	0	3	3
Burgersfort &O	3	3	3	*1	1	0	0	0	0	0	0	0	0	0	0	0	3	3	3	2	0	0	0	0	2	2	0	0	3	3
Groblersdal	3	3	2	2	1	1	0	0	0	0	0	0	1	1	0	0	3	3	2	2	0	0	0	0	2	2	0	0	2	3
Hoedspruit	3	3	1	1	0	0	0	0	0	0	0	0	0	0	3	3	3	3	3	3	0	0	0	2	2	2	0	0	3	3
Letsitele	3	*3	1	0	0	0	0	0	0	0	0	0	0	0	2	2	3	3	2	2	1	1	0	0	2	2	0	0	3	3
Limpopo	3	*3	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	1	1	0	0	0	0	2	2	0	0	2	2
Rustenburg	3	3	2	2	1	1	0	0	0	0	0	0	1	1	0	0	3	3	1	3	2	2	0	0	2	2	0	0	3	3
Weipe	3	*3	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	2	2	0	0	0	0	0	0	0	0	2	2
Zimbabwe	3	3	0	0	3	3	0	0	3	3	0	0	1	1	0	0	3	3	1	1	1	1	0	0	1	1	0	0	3	3
Citrusdal	0	0	3	3	0	0	0	0	0	0	3	3	0	0	0	0	1	1	2	2	0	0	0	0	2	3	0	0	3	3
Swartland		0		2		0		0		0		2		0		0		1		1		0		0		1		0		3
Stellenbosch	0	0	2	2	0	0	0	0	0	0	3	3	0	0	0	0	3	3	1	1	0	0	0	0	1	1	0	0	3	3
Breederivier		0		2		0		0		0		1		1		0		*3		1		0		0		1		0		3
Swellendam	2	2	3	3	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3	3	0	0	0	0	0	0	0	0	2	2
Knysna	2	2	1	1	2	2	3	3	0	0	0	0	0	0	0	0	1	1	3	2	0	0	0	0	0	0	0	0	2	2
Patensie	1	3	2	3	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	1	0	0	3	3	2	3	1	1	3	3
Sondagsrivier	2	3	2	2	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	2	1	0	0	3	3
Katrivier	2	2	2	2	0	0	0	0	0	0	2	2	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	3	3
Oranjerivier	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	1	1	0	0	0	0	0	0	0	0	2	2
Vaalharts	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	2	2
Suid-Natal	1	1	1	1	1	1	0	0	0	0	0	0	1	1	0	0	2	2	2	2	0	0	0	0	2	2	0	0	3	3
Nkwaleni	2	*3	1	2	2	2	0	0	0	0	0	0	0	0	3	3	2	2	3	3	0	0	0	0	2	2	2	2	2	3
Weight	**51**	**55**	**32**	**33**	**14**	**12**	**4**	**4**	**3**	**3**	**8**	**11**	**6**	**7**	**26**	**26**	**38**	**42**	**41**	**42**	**4**	**4**	**4**	**6**	**30**	**33**	**3**	**3**	**62**	**70**
Average	**1.96**	**2.11**	**1.23**	**1.27**	**.54**	**.46**	**.15**	**.15**	**.11**	**.11**	**.31**	**.42**	**.23**	**.26**	**1**	**1**	**1.46**	**1.61**	**1.57**	**1.61**	**.15**	**.15**	**.15**	**.23**	**1.15**	**1.27**	**.11**	**.11**	**2.38**	**2.69**

*** Highest Priority; All Changes are in red font.**

INTEGRATED PEST MANAGEMENT | Table 2

Citrus Area	FCM		Fruit Fly		Thrips		Red scale		Ants		Grey mite		Chinch bug		Mealy-bug		Psylla		Leaf-hopper		OP replacement		Rust mite		Bud-mite		Boll-worm		Waxy scale		Lemon moth	
	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07
Pongola	3	*3	3	3	2	2	2	2	3	3	0	0	0	0	3	3	2	2	0	0	3	3	1	1	1	1	1	0	2	2	1	0
Swaziland	3	3	3	3	2	3	1	1	1	1	0	0	0	0	1	1	2	2	0	0	3	3	1	1	2	1	0	0	0	0	0	0
Komatipoort	3	3	3	3	2	2	1	1	1	1	0	0	0	0	1	1	0	0	0	0	3	3	1	1	0	0	0	0	3	1	0	0
Malelane	3	3	3	3	2	2	1	1	2	2	0	0	0	0	1	1	0	0	0	0	3	3	1	1	0	0	0	0	2	2	0	0
Nelspruit	3	3	3	3	2	2	1	1	1	1	0	0	0	0	1	1	3	3	1	1	3	3	2	3	1	2	0	0	0	0	2	2
Burgfrt & O	3	3	3	3	2	3	2	2	1	1	3	3	0	0	2	2	3	3	1	1	3	3	1	1	3	3	1	1	0	0	0	0
Groblersdal	3	*3	3	3	2	2	1	1	1	1	3	3	0	0	2	2	3	3	0	0	3	3	1	1	1	2	1	1	0	0	2	2
Hoedspruit	3	*3	3	3	2	2	1	1	1	2	0	0	0	0	0	0	3	3	1	1	3	3	1	2	2	2	2	2	1	1	0	0
Letsitele	3	3	3	3	2	2	1	1	2	2	0	0	0	0	2	2	3	3	0	0	3	3	1	1	0	0	1	1	2	2	0	0
Limpopo	3	3	3	3	3	3	1	1	1	1	0	0	0	0	2	2	0	0	0	0	3	3	0	0	0	0	0	0	0	0	0	0
Rustenburg	3	3	3	3	2	2	2	2	1	1	3	*3	0	0	1	2	3	3	0	0	3	3	0	0	1	1	1	1	0	0	0	0
Weipe	3	3	3	3	1	3	2	2	0	0	0	0	0	0	1	1	0	0	2	2	3	3	2	2	0	0	0	0	0	0	0	0
Zimbabwe	3	3	3	3	1	1	1	1	0	0	3	*3	0	0	0	0	3	3	0	0	2	2	0	0	0	0	0	0	0	0	0	0
Citrusdal	3	*3	2	2	1	2	2	2	1	1	0	0	3	3	3	3	0	0	1	1	3	3	2	2	2	3	2	2	0	0	0	3
Swartland		*3		3		1		1		2		0		3		3		2		1		3		0		2		1		0		2
Stellenbosch	3	*3	3	3	1	1	1	1	1	1	0	0	3	3	3	3	3	3	1	1	3	3	0	0	2	2	2	1	0	1	0	2
Breederivier		3		2		0		1		1		0		1		3		3		1		2		0		2		2		0		0
Swellendam	3	*3	3	3	1	1	1	1	3	3	0	0	0	0	3	3	3	3	0	0	3	3	0	0	0	0	0	0	2	2	0	0
Knysna	3	3	3	3	1	1	1	1	1	1	0	0	0	0	1	1	2	2	2	2	3	3	3	0	0	0	0	0	0	0	0	0
Patensie	3	3	2	2	2	1	1	1	1	2	0	0	0	0	2	2	2	1	1	1	3	3	1	1	2	2	0	0	0	0	2	2
Sondagsrivr	3	3	3	2	2	2	1	1	1	0	0	0	0	0	2	1	0	0	1	0	3	3	1	0	2	1	0	0	0	0	1	2
Katrivier	3	3	3	3	2	2	1	1	3	3	0	0	0	0	2	2	0	0	2	2	3	3	1	1	2	2	1	1	0	0	2	2
Oranjerivier	3	*3	3	3	0	0	3	3	1	2	0	0	0	0	1	1	0	0	0	2	0	0	2	2	0	0	0	0	0	0	0	0
Vaalharts	3	3	3	3	1	1	2	2	1	1	0	0	0	0	3	*3	0	0	2	2	0	0	2	2	1	1	1	1	0	0	0	0
Suid-Natal	3	3	3	3	2	2	1	1	1	1	0	0	0	0	2	2	1	1	2	2	3	3	1	1	2	2	2	2	0	0	2	2
Nkwaleni	3	3	3	3	3	3	2	2	2	2	0	0	0	0	3	3	1	2	2	2	3	3	1	2	1	1	1	1	1	1	0	0
Weight	**72**	**78**	**70**	**74**	**41**	**56**	**33**	**35**	**31**	**36**	**12**	**12**	**6**	**10**	**42**	**48**	**37**	**42**	**19**	**22**	**65**	**70**	**26**	**25**	**25**	**30**	**16**	**17**	**13**	**12**	**12**	**19**
Average	**2.76**	**3**	**2.69**	**2.84**	**1.57**	**2.15**	**1.27**	**1.34**	**1.19**	**1.38**	**.46**	**.46**	**.23**	**.38**	**1.61**	**1.84**	**1.42**	**1.61**	**.73**	**.84**	**2.5**	**2.69**	**1**	**.96**	**.96**	**1.15**	**.61**	**.65**	**.50**	**.46**	**.46**	**.73**

*** Highest Priority; All Changes are in red font.**

CROP LOAD & FRUIT QUALITY MANAGEMENT	Table 3

Citrus Area	Flower Manip		Fruit set		Fruit size		Internal Quality		Colour		Creasing		Rind Pitting		Rind breakdown		Peteca		Pruning		Girdling		Shelf life		Replacem of Ca-ars.		CULTIVAR DEVELOP-MENT	
	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07	06	07
Pongola	0	0	3	3	3	3	1	1	1	1	2	1	0	0	1	1	1	1	2	2	0	0	0	0	1	1	2	2
Swaziland	2	0	2	3	3	3	0	0	0	0	0	0	1	1	2	0	1	0	1	1	0	0	0	0	2	2	2	2
Komatipoort	0	0	2	2	2	2	2	3	2	2	3	*3	3	3	3	3	1	1	0	0	0	0	0	0	1	1	2	2
Malelane	0	0	0	0	2	2	3	3	0	0	3	*2	3	3	3	3	1	0	0	0	0	0	2	2	0	0	2	2
Nelspruit	0	0	3	2	1	1	0	0	2	2	1	1	3	3	0	0	3	3	0	0	0	0	0	1	3	3	3	3
Burgfort & O	1	1	1	2	1	2	1	2	0	0	3	*3	3	1	3	3	0	0	1	0	1	2	0	2	3	3	3	3
Groblersdal	0	0	0	0	1	1	2	2	1	2	3	3	1	1	0	2	3	3	1	1	1	1	3	3	1	1	2	2
Hoedspruit	1	1	3	3	2	2	1	2	3	3	3	3	1	1	0	0	3	3	1	1	0	0	0	1	1	1	2	3
Letsitele	0	0	0	2	3	3	2	2	0	2	3	3	3	3	3	3	0	0	0	3	0	2	0	2	1	1	3	3
Limpopo	0	0	0	2	0	0	0	0	0	0	0	0	2	2	2	2	0	2	0	0	0	0	0	0	0	2	3	3
Rustenburg	1	1	2	3	1	1	1	1	0	0	1	1	1	1	0	0	2	2	1	1	1	1	0	0	2	0	2	2
Weipe	3	3	3	3	3	3	1	2	3	3	2	2	2	2	2	2	0	0	1	1	0	0	2	2	1	1	0	2
Zimbabwe	0	0	2	2	1	1	0	0	3	3	0	0	0	0	3	3	2	2	0	0	2	2	0	0	0	0	0	0
Citrusdal	0	0	2	2	0	1	0	3	0	3	3	3	3	3	2	3	1	1	1	1	0	0	2	2	3	3	3	3
Swartland		2		2		2		2		1		3		3		3		3		1		1		2		2		3
Stellenbosch	2	2	2	2	2	2	0	1	0	1	3	3	3	3	3	3	3	3	0	0	0	0	2	2	0	1	3	3
Breederivier		2		2		2		1		1		1		2		2		2		1		2		2		0		2
Swellendam	0	0	3	3	0	0	0	0	0	0	3	3	2	2	2	2	3	3	0	0	1	1	3	3	0	0	3	3
Knysna	0	0	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	3	3*
Patensie	0	0	0	2	2	1	2	1	0	*3	3	3	2	2	2	2	3	3	0	0	1	2	2	2	0	3	3	3
Sondagsrivier	0	0	2	1	2	1	0	0	1	1	3	3	2	1	2	2	3	3*	0	0	0	1	0	2	1	2	3	3
Katrivier	0	0	1	1	0	0	0	0	1	1	2	2	3	3	3	3	3	3	0	0	0	0	0	0	0	0	3	3
Oranjerivier	3	3	3	3	3	3	0	0	0	1	1	1	1	1	1	1	3	3	3	3	2	2	2	2	0	0	0	0
Vaalharts	0	0	0	0	1	1	0	0	0	0	2	2	1	1	1	1	3	3	1	1	1	1	0	0	2	2	3	3
Suid-Natal	0	0	3	3	3	3	1	1	0	0	2	2	1	1	1	1	2	2	1	1	0	0	0	0	0	0	3	3
Nkwaleni	0	0	2	2	2	2	2	2	1	1	0	0	0	3	2	3	2	2	2	2	1	1	1	2	3	3	3	3
Weight	**13**	**15**	**39**	**50**	**41**	**45**	**19**	**29**	**18**	**31**	**46**	**48**	**41**	**46**	**41**	**47**	**43**	**48**	**18**	**22**	**11**	**19**	**19**	**32**	**25**	**32**	**56**	**64**
Average	**0.5**	**.57**	**1.5**	**1.92**	**1.57**	**1.73**	**.73**	**1.11**	**.69**	**1.19**	**1.77**	**1.84**	**1.57**	**1.77**	**1.57**	**1.81**	**1.65**	**1.84**	**.69**	**.84**	**.42**	**.73**	**.73**	**1.23**	**.96**	**1.23**	**2.15**	**2.46**

***Highest Priority;** **All Changes are in red font.**

NAVORSINGSPRIORITEITSVERGADERINGS - Augustus & September 2006

BREEDERIVIER

Program: Siektebestuur

- No 1 = Greening.
- Vergroening is wesenlike probleem.

Algemeen

- Nuwe studiegroep – eerste bepaling van navorsingsprioriteite.

CITRUSDAL

Program: Geïntegreerde Plaagbestuur

- No 1 = FCM.
- Rooispinmyt is nie gelys nie – prioriteit hiervoor moet 3 wees, veral met nuwe beperkings op produkte (Tedion).
- Effektiewe beheer van koringstinkluis. Organo Z ondersoek. Xterminator geregistreer.
- Evaluate pre-harvest chemical control options for Grain Chinch Bug (GCB), to the point of being able to take out registration (evaluate residue status in parallel to avoid wasting time on actives that have no potential acceptance from a residue perspective).
- Evaluate post-harvest (packhouse) controls for GCB, using pyrethrin and pyrethrin + azadirachtin products.
- Is there any sense in considering attempts to control GCB in areas around the packhouses?
- There is a need to isolate GCB attractants for monitoring and possible control purposes.
- More clarity is required regarding the timing of GCB movement into orchards and the risk of packed fruit being infested post-packing.
- There is a request to revise the intervention thresholds for FCM monitoring (all registered traps) in light of its phytosanitary status.

Program: Siektebestuur

- Soek nuutste info oor Alternaria.
- Antrachnose was erg hierdie seisoen en is 'n bron van kommer.
- Aalwurm word toenemend 'n groter probleem.
- Alternaria: Meer effektiewe beheermaatreëls. Prioriteit verhoog van 1 na 3 op grond van nuwe mandaryn kultivars wat aangeplant word.

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Kapasiteitskepping t.o.v. kunsmisaanbevelings en onafhanklike bemestingsnavorsing. Die bedryf benodig weer 'n Hannes Coetzee.
- Alternatiewe drag: Probleme met laat mandaryne.

Program: Kultivar en Onderstamontwikkeling

- Kultivarontwikkelling: Saadprobleme op laat mandaryne wat veronderstel is om saadloos te wees. Veral op Afourers tov 600m buffer.

KATRIVIER

- No 1 = Scientific Analysis and Quantification of the various Carbon based materials on the Market and to set up some standard against which to measure the products e.g. Humic and Fulvic acid).

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Hoogste prioriteit: Skildefekte (rind pitting, rind breakdown, peteca, zebra skin).

- Vrugsetprobleme: Lisbon suurlemoene en nawels.

Program: Siektebestuur

- Alternaria kernvrot. Enige korrelasiemet knopmyt en bolwurm.
- Alternaria werkswinkel. Nuutste rakende chemise beheer programme.
- Raklewe: Verwerking van data van retensiemonsters opgebou oor jare om oorsake van bederf te evalueer.
- Lina nawels: Stel vas of Impietratura nie dalk deur groeipuntenting gekom het nie. Toets beide die CFB moederbome en bome in Katrivier.

Program: Geïntegreerde Plaagbestuur

- Bladspringerprobleme op suurlemoene. Stel vas wat die oorsaak is: Lemon piercing moth of *Prays citri?*
- VKM: Effek van grondbewerking (disc/rotavation) op die lewenssiklus van VKM. Kan dit nie as 'n kulturele praktyk toegepas word om bv. die November vrugval te begrawe nie. Dit behoort die papies in die grond ook te versteur (terselfde tyd dooie blare wat swartvlekspore vrystel te begrawe).

KNYSNA

Program: Kultivar en Onderstamontwikkeling

- No 1 = Cultivar development
- Hoogste prioriteit: Geskikte kultivars.
- Kultivar opsies baie beperk.

Program: Oesgrootte en Vrugkwaliteitsbestuur

- Interne gehalte marginaal.

Program: Geïntegreerde Plaagbestuur

- Beheerprogram vir rooidopluis.
- Beheerprogram vir rooimyt.
- Identifiseer plaag: Witvlieg vs. Bladspringer + beheerprogram (S. Moore of M. Fry).

Program: Siektebestuur

- Beheerprogram op skrif vir *Phytophthora citrophthora* (T. Schutte).
- Effek van Sporekill op melanose (Swazi spot).

NKWALENI

Program: Siektebestuur

- No 1 = CBS

Program: Geïntegreerde Plaagbestuur

- Nr. 1 prioriteit is FCM-beheer.
- 'n Swart letsel op die skil van suurlemoene, wat 'n spesifieke reuk afgee wanneer dit gesny word, kom in die area voor. Daar moet bepaal word wat dit veroorsaak. Die vermoede is dat dit of 'snite beetle' of 'carob moth' is.
- Plantluise speel 'n groot rol by die verspreiding van STV. Beheermaatreëls en die belangrikheid van plantluis t.o.v. STV moet gekommunikeer word.
- Daar is 'n sterk behoefte in die area vir leiding oor hoe om die regte balans tussen 'n chemiese en biologiese benadering te volg.

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Skilafbraak op pomelos was erger op vrugte wat na Japan versend is onder koue-sterilisasie as op vrugte wat na ander markte versend is onder normale temperature. Vrugte ondergaan geforseerde lugverkoeling i.g.v. Japan, maar statiese verkoeling i.g.v. ander markte. Ondersoek moet ingestel word om te bepaal of vrugte skilafbraak kry a.g.v. koue-sterilisasie of a.g.v. geforseerde lugverkoeling.
- Vrugset op Marsh, Star Ruby en Delta Valencia is 'n probleem.
- Interne gehalte is 'n probleem. Lae TSS en hoë suur veroorsaak dat die TSS/suur verhouding swak is.
- Die toediening van Ca-arsenaat skep verwarring. Die dosis, asook die toediening jaar op jaar moet uitgeklaar en gekommunikeer word.
- Vrugsplit op Delta's is 'n probleem in die area en moet aangespreek word.
- Daar bestaan 'n leemte vir besproeiings- en bemestings-aanbevelings.

Program: Kultivar en Onderstamontwikkeling

- Behoefte bestaan vir 'n werkswinkel oor kultivars en onderstamme. Die versoek is gerig dat die hoofstuk oor kultivars en onderstamme in die Produksieriglyne opgedateer moet word, met duidelike omskrywings en fotos.

Ander

- Die Produksieriglyne behoort gereeld op die website opgedateer te word vanwaar produsente die nuutste inligting kan trek.

ORANJERIVIER

Program: Geïntegreerde Plaagbestuur

- No 1 = FCM
- FCM raak groot probleem – kan toegang tot VSA kelder.
- Vrugtevlieg-getalle buitengewoon hoog. Druiweprodusente wat nie beheer toepas nie is 'n probleem.
- Sonbrand: Surround reperkussiest t.o.v. dopluis is onaanvaarbaar. Vind oplossings.
- Knopmyt: Ondersoek om vas te stel of dit wel 'n probleem is in die gebied.

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Kleurontwikkeling vroeg in seisoen is stadig.
- Hoogste prioriteit: Vrugset van bestaande kultivars aangeplant in Benede Oranje.
- Alternaria op druiwe word al groter probleem. Hou dit enige gevaar in vir sitrus?
- Vrugset: Effek van snoei op vrugset. Hoe moet boomgrote beheer word sonder dat die noei die vrugset negatief affekteer.

Ander

- Toegang tot VSA.

PATENSIE

Program: Oesgrootte en Vrugkwaliteitsbestuur

- No 1 = Colour
- Kleur van vroeë kultivars 'n probleem – wil kleurontwikkeling vervroeg.
- Vrugset 'n probleem by Midknight en Mor.
- Clementines gee skurwe skil met verhewe selle (witsel).
- Kraakskil werk deur Clive Kaiser. Resultate voordat hy emigreer.
- Kraakskil middels moet op groter skaal geevalueer word.
- Clemenpons galle en sogenoemde "bul bome" moet ondesoek word. Bedryf moet verseker dat 'n organisme die dalk deur groeipuntenting gekom het wat 'n gevaar vir die industrie inhou nie. Fitosanitêre status van die Clemenpons moet ondersoek word.

- Interne kwaliteit: Hoe kan swak kwaliteit van Midknights op Volckameriana verbeter word?

Program: Geïntegreerde Plaagbestuur

- Hoogste navorsingsprioriteit: VKM beheer.
- Nuwe vrugtevlieg middels, onthoudingsperiodes en residue.
- Suurlemoenmotbeheerprogram.

Program: Siektebestuur

- Aalwurmbehandelings is baie duur – kyk na alternatiewe metodes en middels.
- Terugsterwing van Clementines. Tian moet vasstel of dit ook *P. Citrophthora* is.
- Armillaria beheerprogram.
- Raklewe: Effektiwiteit van humiditeit op raklewe tydens verkoeling.
- Alternaria: Besoek van Tian om beheerstrategië te bespreek (Sporekill?).

Algemeen

- Ondersoek hergebruik van chemiese middels soos Benlate en Dithane vir marktoegang en residue.
- Registrasie van chemiese middels wat veilig is op sagte sitrus.
- Versoek dat aandag gegee moet word aan bestuur van Navorsingsproefplaas – plaas is in baie swak toestand.
- China heffings moet heronderhandel word. Tans is China mark finansiël onaantreklik.

Program: Kultivar en Onderstamontwikkeling

- Tipe-egtheid van Cambria word bevraagteken. Die feit dat ronde en oval vrugte geproduseer word, impliseer dat die kultivar se patentregte teruggetrek moet word. CRI ingryping word vereis.
- Tipe-eg seleksie van Robyn moet skoongemaak en by Grondvesblok gevestig word.

Marktoegang

- Ondersoek van VKM besmetting vs. rypwordingsperiode van sekere kultivars (m.a.w. VKM mag 'n problem wees op nawels maar nie op Midknights op dieselfde plaas wat dan na China uitgevoer kan word).

SONDAGSRIVIER

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Hoogste prioriteit: Oorsake van Peteca op suurlemoene op boordvlak.
- Kraakskil. Afgelope aantal jare se resultate het onvoldoende resultate gelewer.
- Midknight vrugset strategie.
- No 1 = Peteca
- Grondverbeteringsprodukte. Evaluering van produkte soos humien en fulviensure op grondstruktuur, wortelontwikkelling, penetrasie ens.
- Evaluering van die gehalte van grondverbeteringsprodukte.
- Sure: Middel om kalsiumarsenaat te vervang.
- Opmerking: Al die Martines stelsels het misluk. Groot negatiwiteit t.o.v. OHS bestaan.

Program: Siektebestuur

- Voorkoms van CBS neem toe.
- Alternaria nawelentverrotting. Beheerprogram.

Program: Geïntegreerde Plaagbestuur

- Lemon moth raak problematies.
- Knopmyt. Plaasvervanger vir Acarol veral in Confidor boorde.
- VKM beheer : Navorsing vir alternatiewe ekonomies aanvaarbare strategie.
- Botrytis: Terugvoer van Tian rakende sy navorsing op suurlemoene in die Wes Kaap.

Algemeen

- Meeste veranderinge in prioriteite is afwaarts aangepas.

PAARL / STELLENBOSCH

Program: Geïntegreerde Plaagbestuur

- Hoogste prioriteit: Effektiewe beheer van VKM.
- Abamectin: 60 ml/100l water registrasie op sitrus moet gedoen word.

Program: Siektebestuur

- Vergroening is 'n bron van kommer.
- Vergroening: PCRs moet nie alleen jaarliks in Jul/Aug gedoen word nie maar ook in Mei vir sagtesitrus.
- Rhizopus: Effektiewe beheer op Satsumas.
- Effek van klimaatsveranderinge op sitrusverbouing en potensiële patologiese en pesgevare in die Wes Kaap moet bestudeer word.
- Verhoog Na-oespatologie kapasiteit.

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Saadprobleme op laat mandaryne wat veronderstel is om saadloos te wees. Navorsingsbefondsing van probleme op patentkultivars.
- Verfyn aanbevelings vir Gibb bespuitings op laat hang van kultivars en bepaal effek op raklewe.
- Ringelering: Ondersoek effek op nuwe kultivars.
- Blommanupilasie en vrugset: Riglyne vir nuwe mandaryn kultivars soos Mor en Or.

Program: Geïntegreerde Plaasbestuur

- VKM: Surround se effek op FCM moet ondersoek word.
- Bestudeer die effek van koue sterilisasie op VKM na toepassing van "step down" temperatuur protokolle.
- Knopmyt en bolwurmbeheer vereis meer navorsing a.g.v. skade op nawels.
- Onafhanklike evaluering van Maxim.

Algemeen

- LNR teëlprogram. Die verwydering tussen ITSG en CGA moet besleg word.
- Residukontaminasie tussen sitrus en sagtevrugte dmv kratte. Nie navorsing, maar waarskuwing moet uitgaan na kratverskaffers en produsente.
- Plaas Produksieriglyne op website en hou dit opgedateer. Slegs vir SA sitrusprodusente.
- Algemeen: Opvolger vir Hendrik Hofmeyr moet opgelei word.

SUID-NATAL

Program: Geïntegreerde Plaagbestuur

- No 1 = Red mite (not listed in table).
- Suurlemoene: Word die motskade veroorsaak deur "Honeydew moth" of *Pray citri*?
- Fullers Rose Weevil: Potensiële "hitch hiker" gevaar na Japan. Versoek beheermaatreëls.
- Abamectinbespuitings: Hoe om weerstand te vermei? 2x10ml bespuiting teenoor 1x20ml.

Geen ander veranderinge in navorsingsprioriteiete.

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Hoogste prioriteit: Vrugset en vruggrootteverbetering van Rustenburg nawels.
- Vruggrootteprobleme op Rustenburg nawels, suurlemoene en Midknights moet aangespreek word.

- Skildikte/sappersentasie probleme op Turkeys.
- Rustenburg: Nie alleen swak vruggrootte, ook swak set en vrugsplitprobleme.

Program: Siektebestuur

- Sogenaamde virus probleem op nawels moet opgevolg word. (Kontak Ballie Wahl en hoor waarom hy van mening is dat dit 'n virus is).
- Suurlemoenprobleem: Botrytis of windskade?
- Vergroeningswerkswinkel om voorkomende aksies te kan neem en die gebied vergroeningsvry te hou.

SWARTLAND

Program: Geïntegreerde Plaagbestuur

- No 1 = FCM.

Algemeen

- Nuwe studiegroep.
- Eerste keer dat hulle navorsingsprioriteite bepaal het.

SWELLENDAM

Program: Geïntegreerde Plaagbestuur

- No 1 = FCM.
- Insidensie van FCM was nog altyd laag, maar neem vinnig toe.
- Beheer van wasdopluis.

Program: Siektebestuur

- Hoogste prioriteit: Vergroeningsnavorsing.
- Bevestig dat die fosfonate op die mark se aktiewe bestandele vergelykbaar is. Kyk na effek van onsuiwerhede.
- Bevestig of Surround wel maklik afwas in pakhuis. Produsente beweer dat dit nie die geval is in teenstelling met Graham se opinie.

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Effek van ringelering op raamtakke vs hoofstam op terugsterwing / boomtoestand en alternatiewe drag van laat mandaryne.
- Kyk na koolhidraatvlakke in wortels en die effek daarvan en Fusariumwortelvrot op die agteruitgang van laat mandaryne.
- Protokol t.o.v. verskepingstemperature vir verskillende kultivars.

Program: Kultivar en Onderstamontwikkeling

- Herondersoek van kruisbestuiwingseffek van al die nuwe kultivars. (Laat mandaryne het heelwat probleme gegee met saad in 2005).

VAALHARTS

Program: Geïntegreerde Plaagbestuur

- Hoogste prioriteit: Witluisbeheer.
- Kry heelwat afkeurings na VSA vroeg in die seisoen a.g.v. witluis.
- Kouebestuur is 'n groot probleem in die area.

- Fitosanitêre plae is nie oral goed onder beheer nie – sommige produsente voer nie meer uit nie en beheer nie hul plae nie.

Program: Siektebestuur

- Alternaria kernverrotting op nawels. Voorspellingsmodel en beheer.

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Vruggrootte was meesal vanjaar groot landswyd. In Vaalharts was dit nie die geval nie. Na Mei maand is daar geen vruggroei meer, waarskynlik a.g.v. koue. Hoe kan vruggrootte voor Mei maand vinniger toeneem?
- Hoë suur op Valencia tipes.

Algemeen

- Gebied is tevrede met navorsing, nie met voorligting. Hoop dat Areavoorligtingsbestuurder se aanstelling die situasie sal verbeter.

Additional Research Priorities 2006/2007: priority 3 allocated for each priority listed below.

Programme: Disease Management

- Sudden decline in Clementines in the Eastern Cape. Positively ID the pathogen and set up a spray program to prevent it.
- Citrus Black spot – the timing of sprays and the materials to be used in the Cape Midlands specifically.
- Post-Harvest – alternative natural products that can be used. Also the alternatives to Guazatine into the Japan market.
- Alternaria in navels and Clementines.
- Control measures for Rhizopus: pre- and post-harvest.
- More funding for post-harvest research.

Programme: Integrated Pest Management

- Alternative ant control.
- Effective female attractant for Fruit Fly and the determination of the thresholds for control.
- Control of melybug needed.
- Alternative for Acarol needed to control budmite.

Programme: Crop Load and Fruit Quality Enhancement

- Scientific Analysis and Quantification of the various Carbon based materials on the Market and to set up some standard against which to measure the products.
- Cross-pollination of the Late Mandarin varieties with other fertile cultivars.
- Rind disorders: pitting, peteca, RBD, etc. More basic research needed to find out what triggers it and what happens to affected plant material. Evaluation of different waxes, temperatures, etc. not sufficient. Get to the root of the problem.

Programme: Cultivar and Rootstock Development

- Find an early Clementine and navel that is suitable.
- Find a disease and cold resistant rootstock.

BURGERSFORT / OHRIGSTAD

Program: Siektebestuur

- Hoogste prioriteit: Vergroeningsbeheer
- Vergroening: Nuwe middels om bladflooie te beheer.
- Vergroening: Korrektiewe behandelings.
- *Phytophthora* beheer: Strenger toetse deur CIP. Alternatiewe produkte.

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Kraakskil: Effektiewe beheer.
- Bemesting: Oplei van 'n persoon om dr Hannes Coetzee te vervang. Bedryf moet 'n onpartydige persoon hê vir bemestingsaanbevelings.

Program: Geïntegreerde Plaagbestuur

- FCM: Effektiewe beheer.
- Vrugtevlieg: Alles dui daarop dat daar op sekere plase (Elbert de Kock, Willie en PLM Boerdery) weerstandbiedendheid ontwikkel het teen huidige lokase.
- Werkswinkel: Vrugtevlieglugbespuitings.

Algemeen

- Evaluering van chemiese produkte om te verseker dat generiese produkte op standaard is.
- Opdatering van produksieriglyne.

GROBLERSDAL/MARBLE HALL

Program: Geïntegreerde Plaagbestuur

- Hoogste prioriteit: Effektiewe beheer van VKM.
- Grysmyt: Voorkomende beheermaatreëls moet ontwikkel word.
- Roesmyt: Voorkomende beheermaatreëls en middels.
- *Prays citri*: Monitering en drempelwaardes vir bespuiting nodig.
- Bladspringer: Die katoenaanplantings in die omgewing vererger die probleem. Spuitaanbevelings is nodig. Geen registrasies op sitrus.
- Biologiese spuitprogramme: Die evaluering van die gebruik van biologiese beheer produkte soos EM en ander tees vir insek en siektebeheer.
- Knopmyt: Alternatiewe vir Acarol
- Rooimyt: Beheer veral waar meer Confidor toegedien word.

Program: Siektebestuur

- CBS: Toets vir strobulirienweerstandbiedendheid.
- Voedingswetenskappe: Die evaluering van die nuwe golf van voedingsteorie wat die klem plaas op produkte wat die wortelomgewing verbeter.

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Peteca: Vinniger vordering op hierdie gebied uiters noodsaaklik. Riglyne aan podusente om dit te beheer.
- Alle na-oesskildefekte.
- Kraakskil: Probleem op Bahianinas veral op Troyer. Vind oplossings.
- Vrugkleur: Vroeër kleur op vroeë vrugte.
- Stresverligters (Hitte, droogte, koue).

Program: Kultivar en Onderstamontwikkeling

- Kultivarontwikkelling: Bepaal die beste laat nawel vir die area.
- Kultivarontwikkeling: Vind 'n goeie vroee nawel vir die area.
- Kultivarontwikkeling: Vind 'n goeie laat Valencia vir die area.

HOEDSPRUIT

Program: Geïntegreerde Plaagbestuur

- Hoogste prioriteit: Beheer van VKM sodat toegang tot China 'n kommersiele realiteit kan word.
- Vrugtevlieg: Alternatiewe beheer.

- Beheer van stinksprinkaan.
- Evaluering van generiese chemiese produkte.

Program: Siektebestuur

- Vergroening: Meer effektiewe middels om organofosfate wat verlore gaan te vervang. Bevestiging dat vergroening in Hoedspruit wel deur *Liberibacter africanus* veroorsaak word en dat die ander *Liberibacters* nie teenwoordig is nie.

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Vrugset: Vrugset van saadlose suurlemoene, Deltas, Midknights en pomelos.
- Vrugkwaliteit: Turkeys en Bennies word sag in die mark. Hoe kan dit vermeil word.
- Alternatiewe drag: Maniere om dit uit te skakel.

Program: Kultivar en Onderstamontwikkeling

- Kultivarontwikkeling: Goeie kwaliteit laat Valencias.
- Kultivarontwikkeling: Vroeër pomelos vir Japanese mark sou Florida se sitrus tot niet gaan.
-

KOMATIPOORT

Program: Siektebestuur

- *Sitrus Swartvlek:* 'n Diagnostiese toets vir weerstandbiedendheid teen die strobulirienes.
- *Na-oes Patologie*: TBZ probleme met vrugte wat na die sapfabrieke toe gestuur word.

Program: Oesopbrengs en vrugkwaliteitsbestuur

- Hoogste prioriteit: Kraakskil op Valencias.
- Ringbrand: Effek van die verskillende buffers, kleefmiddels, benatters en abamectin formulasies op die ringbrand letsels wat die afgelope seisoen gevind is (generiese produkte).
- Skilgebreke: Kraakskil is 'n probleem op die Valencias en vrugsplit op die Deltas.
- Vastestowwe: Verhoging van vastestowwe op pomelos is nodig. Vastestowwe is baie wisselvallig. Na een reenbui val vastestowwe van 10 na 'n 8 en herstel nie weer.

Program: Geïntegreerde Plaagbestuur

- VKM: Vervolmaak die Cryptogran spuitprogram vir spesifieke boorde vir uitvoere na China.
- Witluis: Ontwikkel 'n metode om te onderskei tussen verskillende witluis spp. met die oog op effektiewe parasietvrylating.
- Rooidopluis: Ontwikkel 'n diagnostiese toets vir weerstandbiedendheid teen Nemesis.
- Blaaspootjie: Ontwikkel 'n diagnostiese toets vir weerstandbiedendheid teen abamectin.

Program: Kultivar en Onderstamontwikkeling

- *Kultivarontwikkeling:* Pomelokultivars wat minder gevoelig vir skaapneus is.

Algemeen

- Databank met afbraakkurwes vir alle chemiese produkte tot op 0.01 dpm. Sodat krissese nie ontstaan wanneer MRL valkke verlaag word nie.
- Verpakkingsnavorsing (Ifco kratte is R1-30/karton goedkoper as karton verpakking). Watter ander moontlikhede bestaan?
- (a) Palletmakers se monopolie moet gebreek word.
  (b) PPECB is nie konsekwent nie en hulle koste is te hoog.

LETSITELE/CONSTANTIA

1. Hoogste prioriteit is om CBS se fitosanitêre status in die EU te verander sodat die siekte weer slegs 'n kosmetiese probleem sal wees. Plaas druk op die EU om te reageer op die verslae wat aan hulle

voorgele is met die nodige navorsingsresultate om te bewys dat swartvlek nie ’n fitosanitere risiko vir die EU inhou nie.

2. Program: Siektebestuur

CBS

Jaarlikse terugvoering oor marktoegang. Hoe beinvloed die aansluiting van Oos-Europese lande by die EU ons marktoegang tot hierdie lande.

Citrus Tristeza Virus

Evaluering van nuwe kruisbeskermingsrasse vir pomelos in Letsitele.

Na-oes patologie
- Ontwikkel alternatiewe beheerstrategiee en nuwe chemiese produkte insluitende ”GRAS chemicals”.
- Monitering van imazaliel en guazitien bestandheid in pakhuise.

Vergroening
- Manupilasie van genetiese material om plantweerstand teen Liberibacter te bewerkstellig.
- Korrektiewe beheer van die siekte.
- Opdatering van gasheerlys deur die voorkoms in alternatiewe gashere met PCR te bevestig.

Phytophthora
- Evaluering van biologiese beheer middles, middles wat sistemies die weerstand in die plant verhoog (SAR), die humiensure en fulviensure op *Phytophthora* wortelvrot.

Tylenchulus semipenetrans
- Evaluering van biologiese beheermaatreels.
- Laboratoriumtoetse om eieruitbroei te manipuleer.

3. Program: Geintegreerde plaagbeheer

VKM
- Evaluering van granulovirus in Letsitele. Cryptogran sal nie in Letsitele verkoop alvorens RiverBioscience nie sy effektiwiteit in die gebied bewys het nie (proewe moet op Turkeys gedoen word omdat hulle meer gevoelig is vir VKM).

Vrugtevlieg
- Alternatiewe middles vir gebruik saam met lokase. Vrugtevlieg: Plaasvervangers vir Malathion en Dipterex.
- Ondersoek Hymlure fitotoksisiteit op Nadorcot. (Hymlure + Koper maar ook Hymlure op sy eie wat stippling veroorsaak)
- Toedieningsmetodes wat kontak met die vrug vermei.
- ’n Meer bekostigbare M3. Evaluering van ’n vermindering in die aantal M3s wat per ha gebruik word namate die blokke groter word.

Blaaspootjie
- Nuwe GPB chemiese middels.
- Alternatief vir Agrimec.
- Evaluering van verskillende abamectin formulasies.

Witluis
- Maklike metode om tussen Sitrus en Oleander witluis te onderskei.
- Parasietnavorsing.
- Nuwe chemiese middles om OPs te vervang.

Psylla
- Alternatiewe gashere bo en behalwe sitrus.

Miere
- Ontwikkel om miere op grond maar uit die boom te hou.

Rooidopluis
- Toets van generiese produkte.
- Opdatering van oliefeitelys.

Wasdopluis
- Oorsaak vir toename in voorkoms

OPs
- Alternatiewe produkte.

4. Program: Oesopbrengs en Vrugkwaliteitbestuur

Interne kwaliteit
- Voorspellingsmodel.

Vruggrootte
- Voorspellingsmodel.

Opbrengs
- Voorspellingsmodel.

Bymiddels
- Evaluering van blaarbespuitings en grondtoedienings wat buite die registrasie van misstowwe vereniging val.

Skilprobleme
- Vind oorsake en oplossings.

Vrugset
- Som riglyne vir Deltas en Midknights op. Stresvermindering tydens vrugsetperiode.

Skaapneus op pomelos
- Watter klimatologiese toestande predisponeer vrugte vir skaapneus? Is daar praktyke wat die voorkoms daarvan kan verminder?

Snoei
- Navorsing van snoei in warm areas moet voltooi word en in riglyne opgesom word. Masjiensnoei sowel as verwydering van individuele takke om boomhoogte af te bring en/of ligpenetrasie te verbeter moet bespreek word .

Ringelering
- Die gebruik van takringelering ipv die ringelering van stamme moet met mekaar vergelyk word om te verseker dat bome nie in ’n alternatiewe dragpatroon in gaan nie.

OHS
- Riglyne vir die gebruik van die stelsel moet voltooi word. Dit moet riglyne insluit wat sal verseker dat die vastestowwe nie nadelig beinvloed word nie.

Grondverbetering
- ’n Onafhanklike persoon met ’n grondkundige en bemestingsagtergrond moet kyk na die opbou van die organise komponent in die grond sowel as die interaksie tussen die biologiese en chemiese komponente in die grond.

Kultivarevaluering
- Plukdatum, hantering en verskepingsprotokol van Turkey. (Pap vrugte is ’n probleem)
- Evaluering van onderstamme vir Cl, B en Na gevoeligheid. (Benton / Sunki) Die kwaliteit van die besproeiingswater is besig om al swakker te word.
- Groot laat valencia (met en sonder saad).

Algemeen
- Meer gereelde opgradering van internet met jongste inligting.
- Produksieriglyne moet op webwerf beskikbaar wees
- Meganisering van oes. Plukkostes moet aangespreek word. Vergelyk SA met lande soos Australie.

Hoe kry hulle dit reg om teen soveel hoer koste as SA te oes en steeds kompeterend te bly?

- Aanstel van 'n grondkundige.

LIMPOPO/TSHIPISE

Program: Marktoegang

- Hoogste prioriteit: Marktoegang tot die VSA.

Program: Geïntegreerde plaagbestuur

- Blaaspootjie: Vergelyk verskillende abamectin formulasies met Agrimec. Daar word beweer dat sekere van die generiese abamectin produkte swak resultate lewer. Stel vas of dit die waarheid is.
- VKM: Oorkom die fitosanitêre bedreiging wat hierdie organisme vir die sitrusindustrie inhou.
- Skaapneus: Metode om te bepaal of 'n vrug skaapneus het of nie (meetbaarheid van skildikte nie net gevoel).

Program: Siektebestuur

- Na-oes patologie: Pakhuisbestuursprogram (Bv Chloor by warmwaterbad, Opbou van Penicillium spore in imazililbad. Pakhuisbehandelingsstrategiesessie aan begin van pakseisoen. – Keith)
- Na-oes patologie: Terugvoer van ondersoek na *Penicillium* weerstandbiedendheid teen imazalil en guazatien in die verskillende pakhuise.

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Bemestingsaanbevelings: Die bedryf benodig weer soos in die verlede 'n onafhanklike persoon om bemestingsaanbevelings te maak. Veral dringend in die lig van die feit dat Hannes Coetzee nie meer van aftrede is nie.
- Bemestings/voedingskundige om onafhanklike opinie te lewer oor kunsmisstowwe en middels wat veronderstel is om die wortelomgewing (rhisosfeer) te verbeter.
- Skilafbraak: Bepaal oorsake en oplossings. TBZ se invloed. Is daar 'n verskil tov skilafbraak op vrugte wat op verskillende kleurvlakke gepluk is.
- Peteca: Bepaal oorsake en oplossings. Metode om gevoeligheid van vrugte te bepaal. (CO_2?).
- Metodes om plukseisoen te rek. GA3. Wat is die effek op volgende jaar se oes.
- Skildikte van pomelos. Hoe om dit uit te skakel in jare met 'n lae oes.

Program: Kultivar en Onderstambestuur

- Kultivarontwikkeling: Laat Valencia (Saad en aadlose kultivar).
- Kultivarontwikkeling:Vroeer rooi pomelos om die opening te vul wat na verwagting gaan ontstaan omdat die Florida uitvoere na Japan gaan afneem a.g.v. sitrus kanker en vergroening.
- Verandering in prioriteitsstatus.

MALELANE

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Hoogste prioriteit: Lae vastestowwe (Advance Cap, K2SO4 bespuiting ??).
- Skilgebreke: Die effek van anorganiese en organise bemesting op skilgebreke. Kraakskil, vrugsplit, vrugbars en vrugset.
- Bemesting: 'n Onafhanklike persoon om organise bemesting en die verbetering van die grondstruktuur te ondersoek.
- Aanplantingsriglyne.

Ander

- (a) PPECB se konsekwentheid moet bevraagteken word.
  (b) CGA studie oor winsgewendheid van sitrusverbouing moet gedoen word. (Produksieriglyn hoofstuk).

NELSPRUIT / HAZYVIEW

Program: Siektebestuur

- Hoogste prioriteit: Vergroening, meer spesifiek korrektiewe behandelings om siek bome gesond te dokter.
- Vergroening: Die ontwikkeling van biotegnologie om die geen wat die plant se weerstand kan aanskakel die oomblik wat die patogeen die plant infekteer in bestaande sitrusgenemateriaal in te bou.
- Vergroening: Genetiese manupilasie van sitrusvoortplantingsmateriaal om weerstand in kommersiele sitruskultivars in te bou.
- CBS: EU aanvaarding van skrywe aan hulle gerig essensieël om te verseker dat die patogeen sy fitosantêre status verloor.
- Evalueer die gebruik van Smartfresh op sitrus vir raklewe.
- Endokserose: Oorsake en oplossings.

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Gepokte skil: Bepaal die oorsake en stel ondersoek in na paklyne waar borsels nie gebruik word nie. Simptome word gedokter nie oorsake.
- Skilafbraak: Is die Benny valencia meer gevoelig as ander kultivars.
- Peteca: Dringende vordering nodig om produsente toe te rus om die probleem uit te skakel in die toekoms.
- Ca arsenaat vervanger. Dringend nodig voordat hierdie produk verbied word.
- Kraakskil: Het Corasil enige effek op kraakskil en vrugsplit?
- Bemesting: 'n Spesfieke bemestingsprogram is nodig vir Turkeys om vrugte wat sag word in die mark uit te skakel (Werklik nodig? Nie dalk omdat Turkeys te laat ge-oes word nie. Turkey is 'n midseisoen nie 'n Valencia).
- Bemesting/verbeterde grondstruktuur: Voordele van humate en fulviensure moet wetenskaplik ge-evalueer word.
- Kleur: Ondersoek die gebruik van natuurlike kleurstowwe soos paprika olie in wakse (word algemeen in Mexico gedoen).

Program: Geïntegreerde Plaagbestuur

- VKM: Verfyning van toedieningsaanbevelings op laat kultivars.
- Sitrusbladvlooi: Alternatiewe beheermaatreëls. Poog om Citrimet te behou.
- Myte: Verskeie myte word nie meer deur abamectin beheer nie. Nuwe middels moet gevind word (toename in myte waar Confidor gebruik word).
- Carobmot monitorsisteme om vruguitvoere na China moontlik te maak.

Program: Kultivar en Onderstamontwikkeling

- Kultivarontwikkelling: 'n Nawel wat vroeër kleur moet vir die area gevind word. 'n Vroeër Valencia moet ook gevind word wat die gebruik van Ca arsenaat onnodig sal maak.

PONGOLA

Program: Geïntegreerde Plaagbestuur

- Hoogste prioriteit: VKM beheer.
- Evaluering van generiese produkte.
- Witluisbeheer.

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Evaluering van grondverbeteringsprodukte insluitende die humiensure en fulviensure.
- Organiese bemestingsriglyne.

Program: Siektebestuur

- Na-oespatologie: Ontwikkeling van alternatiewe beheerstrategie.

- Evaluering van onderskeie buffers.
- Evaluering van onderskeie kleefmiddels.
- Na-oesbeheer. Alternatiewe beheerstrategie.

Algemeen

- Databank vir afbraakkurwes van chemiese produkte.
- Produksieriglyne oor Oorwerking insluitende 'n ekonomiese oorsig.
- Versnelde toegang tot meer uitvoermarkte om 2010 se 100 miljoen uitvoerkartonne te kan absorbeer.

RUSTENBURG

Program: Geïntegreerde Plaagbestuur

- Hoogste prioriteit: Grysmyt beheer.

Program: Siektebestuur

- Vergroening: Korrektiewe beheermaatreëls.
- Vergroening: Alternatiewe beheermaatreëls vir bladvlooi.
- Toetse om die vlakke van die verskillende na-oes behandelings in die diptenks in die pakhuis te bepaal (Imazilil & Sporekill).
- TBZ in sapfabrieke.

Program: Kultivar en Onderstamontwikkeling

- Kultivarontwikkeling: Ondersoek die verskynsel waar 6 jaar oue Bahianina nawels pitte ontwikkel (in proses).

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Kumkwat skildefek: Oorsaak? Skilafbraak of grysmyt?
- Ontwikkel metodes om koolhidraat vlakke in die wortelstelsel te bepaal en hoe om dit vinnig te verhoog.

SWAZILAND

Programme: Disease Management

- *Citrus greening*: Survey to determine the presence of *Liberibacter asiaticus* and *L. americanus* in the hotter citrus production areas of southern Africa.
- *Citrus Black Spot*: Alternatiewe beheermaatrëels en die verandering van die status van CBS van fitosanitêr na kosmeties.
- *Armillaria*: Bevestiging dat Phytex wel *Armillaria* beheer.
- *Tylenchulus semipenetrans*: Enzone moet ge-evalueer word vir aalwurm en *Phytophthora* beheer.

Programme: Crop Load and Fruit Quality Management

- Hoogste prioriteit: Bemestingsnavorsing (onafhanklike evaluering van humiensure, fulviensure en ander middels wat veronderstel is om die wortelomgewing te verbeter).
- Bemestingsnavorsing: Sproeibemesting aanbevelings (kan doen en produksieriglyne kan skryf).

Programme: Integrated Pest Management (IPM)

- FCM: Beheermaatrëels wat toegang tot markte soos China sal verseker.

WEIPE

Program: Marktoegang

- Hoogste prioriteit: Marktoegang tot die VSA.

Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Skilafbraak: Bepaal oorsake en maniere om dit te oorkom.
- Skaapneus: Her-evalueer standaarde vir afkeurings. Kyk na ander faktore soos karton gewig, skildikte en sappersentasie.

Program: Kultivar en Onderstambestuur

- Valencia kultivars wat vroëer kleur as die Bennie Valencia vir China.
- Later Valencia kultivars.

Program: Siektebestuur

- Verwerkingsvrugte: Wat is die stand t.o.v. produkte soos Tecto, imazalil en guazatien? TBZ verminder skilafbraak en moet gebruik word. Aankopers van sitruskonsentraat vereis TBZ-vry vrugte al is dit nie in ooreenstemming met EU regulasies nie.

Program: Geïntegreerde plaagbestuur

- Biologiese maniere om blaaspootjie te beheer.

ZIMBABWE

Programme: Integrated Pest Control

- Highest priority: Grey mite control.
- Red mite: Alternative chemicals to control this pest.
- Thrips: Development of more chemicals to control this pest.
- FCM: Registration of Cryptogran in Zimbabwe.

Programme: Disease Management

- *Pseudocercospora angolensis*: Its control and possible eradication in neglected or abandoned orchards once the situation in Zimbabwe has normalised.
- Greening: Alternative chemicals to replace the organophosphates.

Programme: Crop Load and Fruit Quality Management

- Confidor: Does this product stimulate fruit size and yield?
- Fruit colour: Early colour development is an issue and must be addressed.

General

Pseudocercospora angolensis: The SA Department of Agriculture must pressurise the Zimbabwe Department of Research and Specialist Services to remove neglected citrus orchards that pose a phytosanitary threat as a result of *P. angolensis*.

The SA Citrus industry must compile an action plan to determine the spread and eradication of this disease in a post-Mugabe era. This will include finding the financial recources to assist in eradicating the disease.

CRI must, via the NDA, establish negotiations with the Angolan government to ensure that no citrus planting material from Brazil enters Angola. Angola must source its citrus budwood and seed from the CFB. Diseases that can be introduced from Brazil into Africa includes Citrus canker, Citrus Variegated Chlorosis, Sudden Death, Leprosis, Rubiloses, *Liberibacter asiaticus* and *Liberibacter americanus* to name a few.

Kommentaar (nie navorsing)

Die volgende kommentaar wat nie direk met navorsing verband hou nie maar waarvan die CRI moet kennis dra is deur die onderskeie studiegroepe gelewer. Van die kommentaar soos punt een is vir die eerste keer gelewer terwyl van die ander punte by herhaling gelewer is. Dit is steeds geldig:

1. Daar is tans ’n groot aantal sitrusplase in die Noorde waar die eienaars a.g.v. die huidige regeringsbeleid hulle plase waarskynlik gaan verloor. Hierdie plase is meesal in die Strydom Blok, Komatipoort, Malelane, Hoedspruit, Letsitele, Tshipise en Weipe omgewings en verteenwoordig sowat 10 miljoen uitvoerkartonne. Van die vooruitgeskatte 100 miljoen uitvoerkartonne wat geskat word vir 2010, verteenwoordig dit meer as 12,5 miljoen kartonne. Indien daar nie met initiatief te werk gegaan word om te verseker dat hierdie plase vorentoe effektief bestuur word nie, kan hierdie vrugte afgeskryf word vir uitvoere. Die enigste manier waarop hierdie plase wel volhoubaar bestuur kan word in die mediumtermyn is as die bestaande produsente nadat hulle uitgekoop is sou voortgaan om hierdie besighede (boerderye) te bestuur. Sou dit nie gebeur nie sal al hierdie plase waarskynlik vyf jaar vorentoe vir die sitrusprosesseringsbedryf vrugte produseer. Dit sal ’n redelike gedeelte van die uitvoervrugte uit die mark verwyder wat goed kan wees vir die pryse van die vrugte wat wel uitgevoer sal word. Met die huidige vooruitskouing vir versappingsvrugte wat goed behoort te presteer oor die volgende 5-8 jaar kan dit ook goeie inkomstes vir die nuwe eienaars verseker. CRI moet egter nouer kontak maak met die proseseringsbedryf sodat ons in die toekoms ook ’n heffing op verwerkte vrugte kan probeer daarstel.
2. Die behoefte aan “Scout “ en Sitruskortkursesse bestaan steeds.
3. Meganisering van sitrusverbouing. Die uitweking van HIV word in feitlik al die streke as ’n realiteit ervaar. Die gemiddelde plukvermoë per plukker is aan die afneem.
4. CRI moet betrokke raak by navorsing van verpakkingsmateriaal.
5. Die CRI web moet meer gereeld opgedateer word.
6. Die PPECB se rol moet heroorweeg word. Sy monopolie moet gebreek word en hulle moet ’n groter rol speel met om te help met stelsels soos EUREPGAP, Natures Choice, ICMS en BRC eerder as net gehalte inspeksies.

9.2 TEGNOLOGIE OORDRAGINGSGROEPE – TOG’s (SITRUSSTUDIEGROEPE)

Gedurende Januarie 2006 is die tweede fase van die uitbreiding van die CRI Voorligtingsnetwerk in werking gestel. Die eerste fase wat in 2005 afgehandel is, was die aanstel van Hannes Bester as Area-voorligtingsbestuurder vir die suidelike provinsies. Die tweede fase het behels die kontraktering van konsultante wat lid is van SASSCON om betrokke te raak by sekere van die sitrusstudiegroepe. Chris Kellerman sal in die toekoms betrokke wees by die Pongola/Swaziland en die Komatipoort/Malelane studiegroepe. Tom van der Meulen is betrokke by Hoedspruit en Clive Pountney by Burgersfort/Ohrigstad. Dr Isak Bruwer is betrokke by die Breederivier sitrusstudiegroep. Die koördinering van die Zimbabwese studiegroepe is ook steeds ‘n probleem.

Na aanleiding van die ernstige gehalteprobleme en meegaande verliese wat gedurende die voorafgaande seisoen in die markte voorgekom het en die ontwikkeling van *Penicillium* weerstandbiedendheid teen imazaliel, het daar ’n prioriteit ontstaan om plukker-opleiding, boordpraktyke en pakhuispraktyke voor die aanvang van die nuwe seisoen aan te spreek. Studiegroepvergaderings wat bederf en raklewe aanspreek is met al die studiegroepe gereël. Met die uitsondering van Vaalharts en Weipe is al die vergaderings goed bygewoon en was bywoning in al die gevalle verteenwoordigend van ten minste 70% van die totale hoeveelheid uitvoerkartonne in daardie bepaalde area. In Vaalharts het die situasie ontstaan dat agv hoë kostes en lae winsmarges heelwat produsente besluit het om nie meer uit te voer nie, maar lokaal te bemark en dit gevolglik nie meer ’n prioriteit ag om die studiegroepvergaderings by te woon nie. In Weipe se geval moet die gebied nog as ’n tegnologie-oordragingsgroep saam begin funksioneer.

Hennie le Roux en Hannes Bester het vanaf 3-7 Julie die Swaziland, Malelane, Constantia en Rustenburg studiegroepe se voorsitters besoek om die vasstel van die 2007 navorsingsprioriteite met hulle uit te klaar. Tydens die besoek is vrugtevlieglokaas fitotoksisiteit op die Nadorcotts in Burgersfort en Vivo ondersoek terwyl besoeke ook aan Esselen-, Witkrans- en Casmar kwekerye gebring is.

Twee nuwe studiegroepe is in die Suide gestig. Swartland Studiegroep omsluit die hele Swartland area met Wietze Post wat as voorsitter gekies is. In die Gamtoos het die privaat pakkers hul eie studiegroep gestig met Phillip Dempsey as voorsitter, wat bekend sal staan as Patensie Privaat Pakkers.

Gedurende Augustus is die Wes- en Noord-Kaap besoek waartydens verskeie onderwerpe gedek is. Onder andere het Sean Moore in sy hoedanigheid as CEO van River Bioscience en Hannes Coetzee van CAL aanbiedinge gelewer.

Na afloop van die Sitrusnavorsingsimposium is die hoogtepunte van die simposium saamgevat en is dit by elk van die Tegnologie oordragingsgroepe in suider Afrika aangebied. Na afloop hiervan is elke area se sitrusnavorsings-prioriteite vir 2007 bepaal.

Die betrokkenheid van konsultante by die vergaderings is uitstekend. Mark Fry het 'n gedeelte van die aanbiedinge by verskeie van die studiegroepe hanteer en Sakkie Bruwer het deur die jaar waardevolle insette by studiegroepe in die Wes-Kaap gelewer. Chris Kellerman, Tom van der Meulen en Clive Pountney het almal suksesvolle byeenkomste met elk van hulle studiegroepe gereel. Die struktuur van die Tegnologieoordragingsgroepe sien tans as volg daaruit:

VOORSITTERS VAN TEGNOLOGIEOORDRAGINSGROEPE 2006/7 CHAIRMAN OF TECHNOLOGY TRANSFER GROUPS 2006/7				
TTG/TOG	**NAME/NAAM**	**TEL. NO/NR**	**FAX/FAKS NO/NR.**	**EMAIL/EPOS**
Baviaans (Patensie)	Phillip Dempsey	082 498 2778		phillipdempsey@southernfruit.co.za
Beitbridge	Paul Bristow	072 701 9227	09263 862434	bristow@mweb.co.za
Benede-Oranjerivier	Francois Reyneke	082 771 6758	054-4310780	francois@karsten.co.za
Breederivier	Sakkie Bruwer	083 226 2540		subtrop@netactive.co.za
Burgersfort	Elbert de Kock	013-2317757	013-2318334	moronesitrus@intekom.co.za
Citrusdal	Otto Frielingsdorf	082 804 9054	022-9212511	otto@ghcitrus.com
Groblersdal/ Marble Hall	Gerda Burger	082 388 1041	013 262 6602	gerda@moosrivier.co.za
Hoedspruit	Pierre Malherbe	084 517 3378		driehoek@lantic.net
Katrivier	Bruce Knott	082 877 1164	046-6452345	j&bcitrus@bosberg.co.za
Komatipoort	Dirk Horn	013-7937536 / 083 259 3359	013-7937536	sommerreg@soft.co.za
Knysna	John Stanwix	082 789 5051	044-3884611	knycit@mweb.co.za
Letsitele	Pieter Vermaak	015 386 8718 082 491 7743	015-386 8718	nic@mweb.co.za
Limpopo	Bennie Nicholson	015-5390763 / 083 306 0552	015-5390718	alicedale@lantic.net
Malelane	Leon Esselen	013-7900160	013-7900492	esselenk@mweb.co.za
Marble Hall	Pierre v. Rensburg (Midknight Study Grp) Gerda Burger	013-2611203 / 082 388 3101 082 388 1041	013-2611203	avant@lantic.net gerda@moosrivier.co.za
Nelspruit	Graham Piner	013-7538000 072 804 6495	013-7522560	crocval@mweb.co.za
Nkwaleni	Shane Dellis	083 256 3650	035-4600634	valfarm@corpdial.co.za
Paarl/Stellen-bosch	Stephan Venter	083 670 8030		stephan@insectscience.co.za
Patensie	Ilze du Plessis	082 926 8086	042-2830893	ilzed@gamnet.co.za
Pongola	André Barnard	083 229 8539	034-4351083	mhlati@idhweb.com
Richmond	Peter Button	082 488 8537		pbutton@futurenet.co.za
Rustenburg	Johan-Chris Grobler	082 922 1579	014-5733036	witkrans1@mweb.co.za
Sondagsrivier vallei	Dave Gerber	072 495 3162	042-2331037	technical@srcc.co.za
Swartland	Wietse Post	082 804 9054		wietse@clearsky.co.za
Swaziland	Gerd Höppner	09268-3232311	09268-3232317	ghoppner@iysiscitrus.co.sz

VOORSITTERS VAN TEGNOLOGIEOORDRAGINSGROEPE 2006/7 CHAIRMAN OF TECHNOLOGY TRANSFER GROUPS 2006/7				
TTG/TOG	**NAME/NAAM**	**TEL. NO/NR**	**FAX/FAKS NO/NR.**	**EMAIL/EPOS**
Swellendam	Sarel Neethling	028-5123606 / 082 551 2357	028-5123659	sarel@thornlands.net
Vaalharts	Tom Fouché	053-4710277 / 082 783 4842	053-4710277	marithaminnie@mweb.co.za
Weipe	Danie Erasmus	083 236 7798	015 5330056	depoweipe@lantic.net
Zimbabwe	Chris Maggs	09263 11419624	09263 11419624	technical@interspan.co.za

9.3 **THE RELATIVE FUNDING SUPPORT FOR RESEARCH PROGRAMMES AND PROJECTS FOR 2006-7**

By Tim G. Grout (CRI)

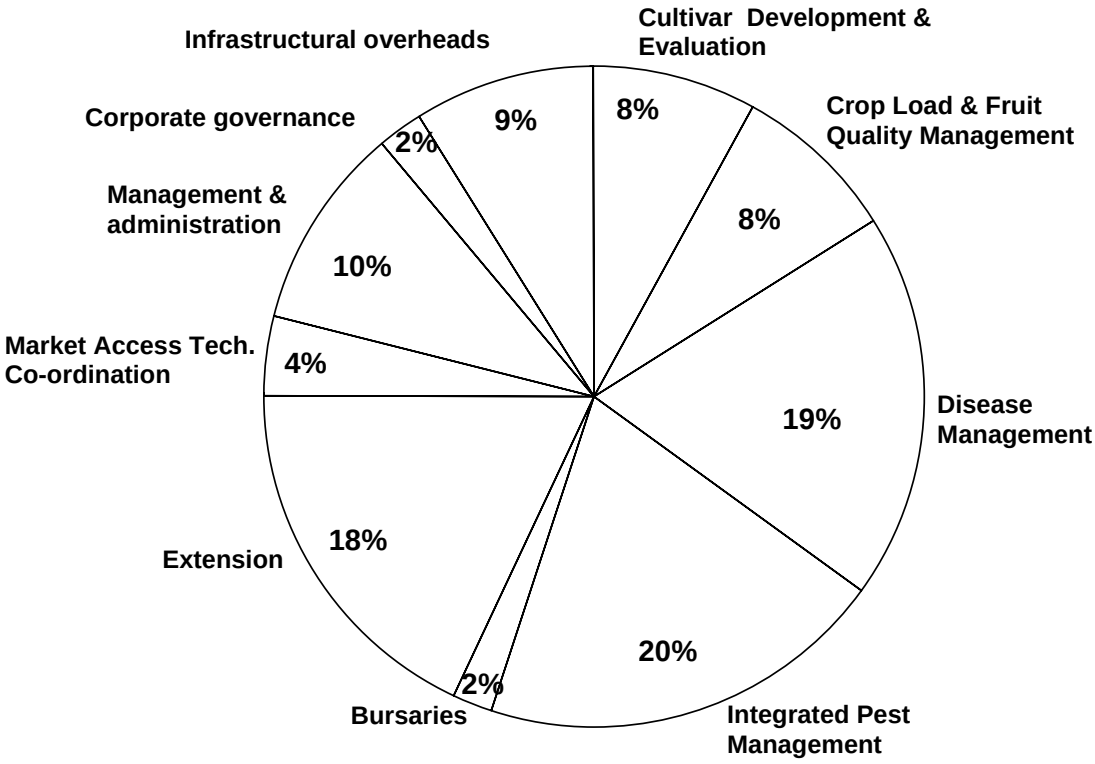


Fig. 9.3.1. Percentage funding in each CRI programme and rest of budget.

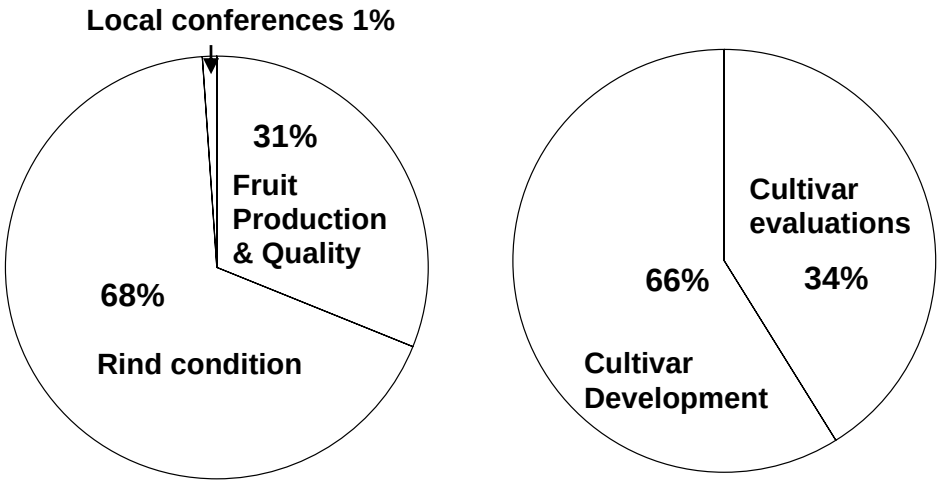


Fig. 9.3.2. Percentage funding to projects in the CRI Research Programmes: Crop Load & Fruit Quality Management (left) and Cultivar Development & Evaluation (right) for 2006-7

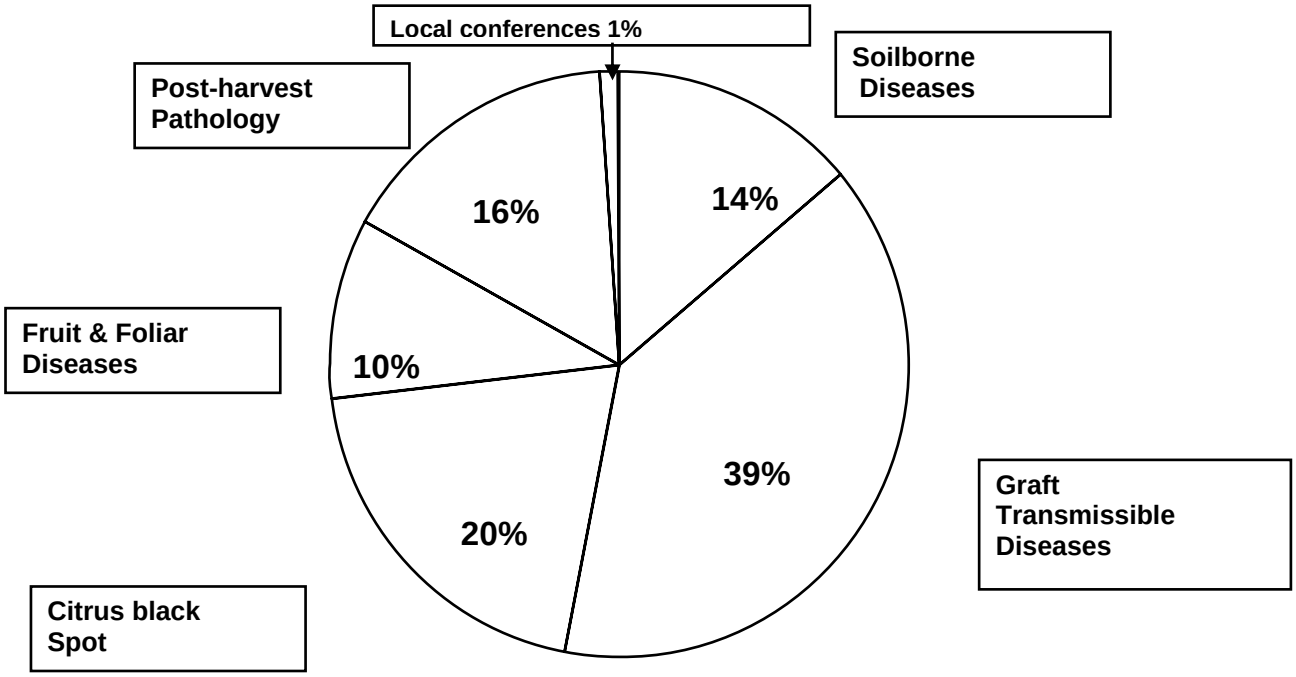


Fig. 9.3.3. Percentage funding to projects in the CRI Research Programme: Disease Management for 2006/7.

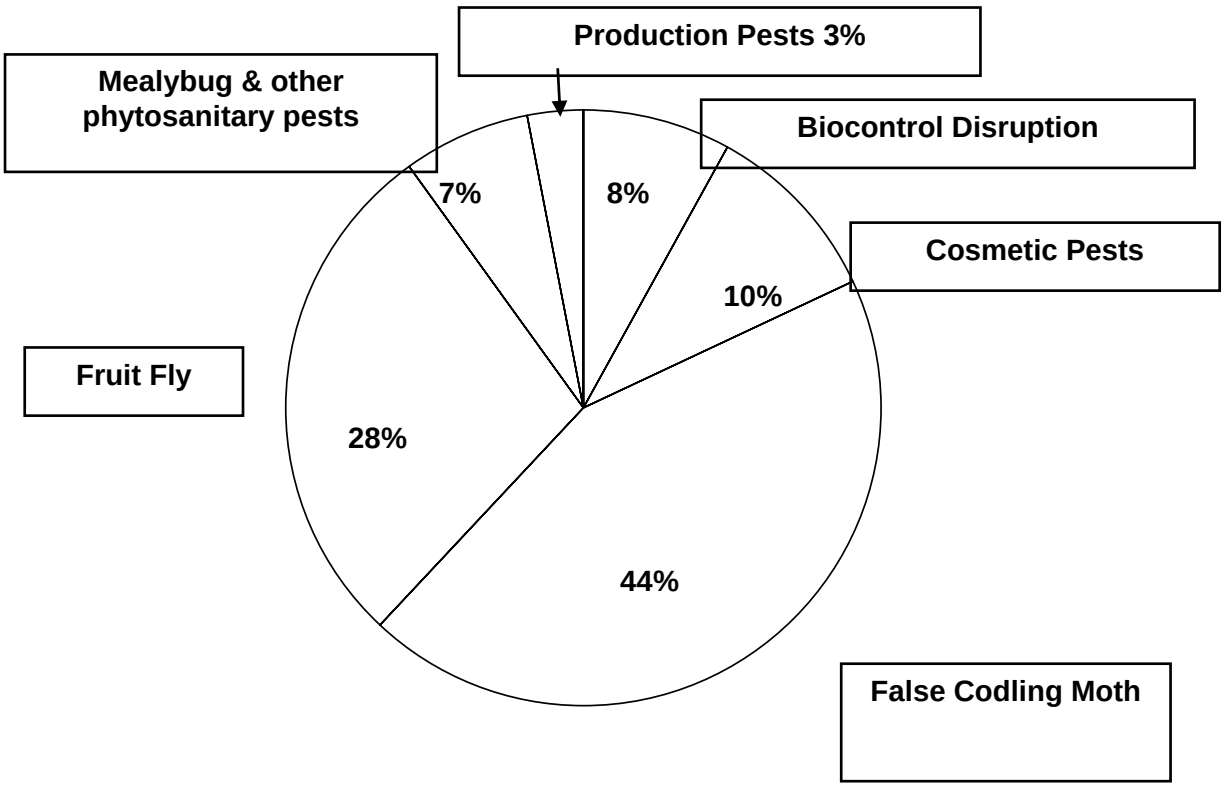


Fig. 9.3.4. Percentage funding to projects in the CRI Research Programme: Integrated Pest Management for 2006/7.

9.4 EXTENSION PRESENTATIONS BY CRI GROUP RESEARCHERS IN 2006

RESEARCH			
Name	**Date**	**Place**	**Topic**
Barry, G.H. (CRI)	22/05/2006	Tshipise Citrus Study Group	What is the future of Tshipise citrus?
			Cultivar Innovation: Effective sourcing and commercialisation of citrus cultivars in Southern Africa
	18/05/2006	Nexus Chemical Representatives, Paarl	Overview of PGR usage in citrus
	05/06/2006	Kirkwood	Cultivar options for the E. Cape citrus production regions
	20/07/2006	Terason Chemical Representatives	The citrus tree and its fruit
	02/08/2006	Vaalharts Citrus Study Group, Hartswater	Frost Management workshop
	10/08/2006	Senwes Citrus Study Group, Schoeman Boerdery, Marble Hall	Fruit quality management: How can I optimise fruit quality in the market?
	21-23/08/06	4th Citrus Research Symposium, PE	Rind colour enhancement
			Rind Breakdown of Clementine mandarin: with special reference to mineral nutrients and carbohydrates
			Rind pigments and antioxidant capacity association with rind disorders in 'Clementine' mandarin fruit as influenced by canopy position and cultivar
			Controlled water deficit can improve sugar accumulation in a drip irrigation system

RESEARCH			
Name	**Date**	**Place**	**Topic**
			Cultivar Innovation: Effective sourcing and commercialisation of citrus cultivars in Southern Africa
			Current status of citrus cultivars and their characteristics
Breytenbach, J.H.J. (CRI)	21-23/08/06	4th Citrus Research Symposium, PE	The effect of single-aphid transferred *Citrus tristeza virus* sub-isolates on the growth of young Marsh and Star Ruby grapefruit trees.
			The response of citrus rootstocks to Citrus Blight after 15 years.
Cronjé, P.J.R. (CRI)	20/03/2006	Citrus Exporters Forum, Capespan	Rind Condition Research 2005.
	21-23/08/06	4th Citrus Research Symposium, PE	Rind breakdown of Clementine mandarin: with special reference to mineral nutrients and carbohydrates
			The different citrus waxes on the development of peteca spot on lemons
			Identification of post-harvest rind disorders of citrus fruit
	05/12/2006	Goedehoop boeredag, Citrusdal	Waarnemings in market: Faktore wat skilkwaliteit beinvloed
Grout, T.G. (CRI)	19/04/06	Nelspruit	Fruit fly, FCM, mites, fruit piercing moth.
	10/08/06	Hoedspruit	Pest management strategies
	21-23/08/06	Citrus Research Symposium, PE	Research funding, citrus thrips, citrus psylla.
	03/10/06	Nelspruit	Spray machine calibration
	10/10/06	Citrus Short Course	Pest management
Kirkman, W. (CRI)	21-23/08/06	4th Citrus Research Symposium, PE	An investigation of alternative hosts for false codling moth in the Eastern Cape
	11/09/06	Swellendam Breederivier	Symposium feedback
	12/09/06	Paarl/Stellenbosch Swartland Citrusdal	Symposium feedback
	13/09/06	Benede-Oranjerivier	Symposium feedback
	14/09/06	Vaalharts	Symposium feedback
Lesar, K.H. (CRI)	21/02/06	Hoedspruit Study Group	Post-Harvest Decay
	23/02/06	Swaziland/Pongola Study Group	Post-Harvest Decay
		TSB Packhouses, Tecklenburg at Rooigras & Capespan	Waste & rind condition Workshop
	06/03/06	Patensie Study Group	Post-Harvest Decay
	07/03/06	SRCC Study Group	Post-Harvest Decay
	08/03/06	Kat River Study Group	Post-Harvest Decay
	09/03/06	Richmond Study Group	Post-Harvest Decay
	10/03/06	Nkwaleni Study Group	Post-Harvest Decay
	22/03/06	Paarl/Stellenbosch Study Group	Post-Harvest Decay
	23/03/06	Robertson & Swellendam Study Groups	Post-Harvest Decay

RESEARCH			
Name	**Date**	**Place**	**Topic**
	04/04/2006	Kakamas Study Group	Post-Harvest Decay
	05/04/06	Vaalharts Study Group	Post-Harvest Decay
	11/04/06	Groblersdal/Marble Hall Study Group	Post-Harvest Decay
	18/04/06	Malelane/Onderberg Study Groups	Post-Harvest Decay
	21/04/06	Burgersfort Study Group	Post-Harvest Decay
	25/04/06	Tshipise/Weipe Study Groups	Post-Harvest Decay
	02/05/06	Letsitele Study Group	Post-Harvest Decay
	21-23/08/06	4th Citrus Research Symposium, PE	The effect of different citrus waxes on the development of peteca spot on lemons
			The History of Post-Harvest fungicide Resistance and the present day management and strategies to reduce the risk of a potential disaster in the Southern African Citrus Industry
Malan, A. (SU)	21/08/06	4th Citrus Research Symposium, PE	Potential of entomopathogenic nematodes of the familes Steinernematidae and Heterorhabiditidae to control FCM
Moore, S.D. (CRI)	02/08/2006	Hartswater grower study group	FCM management
			Fruit Fly management
			Mealybug management
			Leafhopper management
	03/08/2006	Benede-Oranjerivier grower study group	FCM management
			Fruit fly management
			Mealybug management
			Leafhopper management
	07/08/2006	Citrusdal grower study group	Cryptogran for FCM control
	08/08/2006	Paarl/Stellenbosch grower study group	Cryptogran for FCM control
		Swartland grower study group	Cryptogran for FCM control
	09/08/2006	Swellendam grower study group	Cryptogran for FCM control
	10/08/2006	Breederivier grower study group	Cryptogran for FCM control
	21-23/08/06	4th Citrus Research Symposium, PE	FCM alternative hosts
			Cryptogran
			Entomopathogenic nematodes for FCM control
			Host status of lemons for FCM
			Virus for bollworm control
	19/09/2006	Knysna grower study meeting	General pest management
	20/09/2006	CGA/DoA Special markets workshop, PE	FCM protocols and GAPs for China
	21/09/2006	Kat River Valley grower study group	Red scale management
			Mealybug management
			Thrips management
			FCM management
		SRCC grower study group	FCM management
	22/09/2006	Gamtoos River Valley grower study group	FCM management
	20/10/2006	Stellenbosch – Workshop with researchers & XSIT	Implementation of SIT
	07/11/2006	Marble Hall grower study group	FCM management

RESEARCH			
Name	**Date**	**Place**	**Topic**
	08/11/2006	Letsitele grower study group	FCM management
	14/11/2006	Benede-Oranjerivier grower study group	FCM management for the USA market
Pietersen, G. (CRI at UP)	21-23/08/06	4th Citrus Research Symposium, PE	Progress towards establishment of a comprehensive diagnostic capability at CRI against Citrus graft-transmissible pathogens
Pretorius, M.C. (CRI)	05-06/2006	Marble Hall Study Group	Phytophthora & Nematodes
	05/2006	Burgersfort Study Group	Phytophthora & Nematodes
	21-23/08/06	4th Citrus Research Symposium, PE	The current status and management strategies of Huanglongbing (citrus greening disease) in the Western Cape province of South Africa
	09-10/2006	Letsitele Study Group	Phytophthora & Nematodes
		Marble Hall Study Group	Phytophthora & Nematodes
	11/10/2006	Hoedspruit Study Group	Phytophthora & Nematodes
	09/11/2006	Malelane/Komatipoort Study Group	Phytophthora & Nematodes
Schutte, G.C. (CRI)	03/08/2006	Nelspruit Study Group	Citrus Black Spot
	04/08/2006	Hectorspruit Study Group	Citrus Black Spot
	21-23/08/06	4th Citrus Research Symposium, PE	New spray programmes for the control of citrus black spot
			Phytophthora citrophthora – the death sentence for Clementine production in South Africa
			New spray programmes for the control of Alternaria brown spot on mandarins in the summer and winter rainfall regions of South Africa
	21/09/2006	Fort Beaufort Study Group	Citrus Black Spot, Alternaria, Phytophthora
	21/09/2006	Addo Study Group	Citrus Black Spot, Alternaria, Phytophthora
	22/09/2006	Patensie Study Group	Citrus Black Spot, Alternaria
	28/09/2006	Swellendam Study Group	Alternaria, Phytophthora
	03/10/2006	Letsitele Study Group	Citrus Black Spot
	04/10/2006	Marble Hall Study Group	Citrus Black Spot
	12/10/2006	Hoedspruit Study Group	Citrus Black Spot
Stewart, K.A. (UP)	21-23/08/06	4th Citrus Research Symposium, PE	Development and implementation of PCR and Microarray based methods to differentiate *Citrus tristeza virus* strains
Verreynne, J.S. (CRI)	18/05/2006	Nexus Chemical Representatives	Fruit size improvement in citrus
	21-23/10/06	4th Citrus Research Symposium, PE	The role of hormones in alternate bearing in citrus
			Evaluation of potential benefits of hand thinning on Nules Clementine mandarins
			Evaluation of alternative means of controlling creasing (albedo breakdown)
	25/08/2006	Colors Citrus Workshop, Kirkwood	Creasing, crop load and alternate bearing

RESEARCH			
Name	**Date**	**Place**	**Topic**
	31/08/2006	UAP Citrus training, Simondium	Creasing in citrus
	19/09/2006	Clanwilliam Farmer's Association	Creasing in citrus
Van Vuuren, S.P. (CRI)	21-23/08/06	4th Citrus Research Symposium	*Citrus tristeza virus* cross-protection of Star Ruby grapefruit
			Citrus tristeza virus cross-protection of sweet orange
EXTENSION			
Name	**Date**	**Place**	**Topic**
Le Roux, H.F. (CRI)	26/01/06	USA Workshop	Agenda (Hannes Bester)
	07/02/06	Grondvesblok	CIP (Hennie le Roux)
	20/02/06	CEF-meeting	Agenda (Hannes Bester)
	21/02/06	Hoedspruit	Boord en Pakhuispraktyke (Keith Lesar) Navorsingsbefondsing (Hennie le Roux)
	06/03/06	Patensie	Boord- en Oespraktyke (Hannes Bester) Pakhuispraktyke (Keith Lesar) Wortelgesondheid (MC Pretorius)
	07/03/06	Sondagsrivier Privaat Pakhuise	Pakhuispraktyke (Keith Lesar)
	07/03/06	SRCC Pakhuise en Tegniese Afdeling	Pakhuispraktyke (Keith Lesar)
	07/03/06	Sondagsrivier SG	Boord- en Oespraktyke (Mark Fry) Pakhuispraktyke (Keith Lesar) Wortelgesondheid (MC Pretorius)
	08/03/06	Katrivier	Boord- en Oespraktyke (Mark Fry) Pakhuispraktyke (Keith Lesar) Wortelgesondheid (MC Pretorius)
	09/03/06	Suid-Natal	Boord- en Oespraktyke (Mark Fry) Pakhuispraktyke (Keith Lesar) Wortelgesondheid (MC Pretorius)
	10/03/06	Nkwaleni	Boord- en Oespraktyke (Mark Fry) Pakhuispraktyke (Keith Lesar) Wortelgesondheid (MC Pretorius)
	20/03/06	Citrusdal	Boord- en Oespraktyke (Hannes Bester) Pakhuispraktyke (Keith Lesar) Wortelgesondheid (MC Pretorius) VSA-GAP's (Sakkie Bruwer)
	20/03/06	Exporters Technical Panel	Navorsingsterugvoer (Keith Lesar) Bederf en raklewe (Keith Lesar) Navorsingsterugvoer oor Skildefekte (Paul Cronje)
	22/03/06	Paarl/Stellenbosch	Boord- en Oespraktyke (Hannes Bester) Pakhuispraktyke (Keith Lesar) Wortelgesondheid (MC Pretorius) VSA-GAP's (Sakkie Bruwer) Sitruswakse (Ossie Bronkhorst)
	23/03/06	Breederivier	Boord- en Oespraktyke (Mark Fry) Pakhuispraktyke (Keith Lesar) Wortelgesondheid (MC Pretorius) VSA-GAP's (Sakkie Bruwer)
	23/03/06	Swellendam	Boord- en Oespraktyke (Mark Fry)

RESEARCH			
Name	**Date**	**Place**	**Topic**
			Pakhuispraktyke (Keith Lesar) Wortelgesondheid (MC Pretorius)
	31/03/06	CMF-meeting	Agenda (Hannes Bester)
	04/04/06	Benede-Oranjerivier	Boord- en Oespraktyke (Hannes Bester) Pakhuispraktyke (Keith Lesar) Wortelgesondheid (MC Pretorius)
	05/04/06	Vaalharts	Boord- en Oespraktyke (Hannes Bester) Pakhuispraktyke (Keith Lesar) Wortelgesondheid (MC Pretorius)
	01/04/06	Rustenburg	Vrugtevliegbestuur (Hennie le Roux) Besproeiingsbestuur (Hannes Coetzee)
	11/04/06	Oos-Kaap CTA	Agenda (Hannes Bester)
	11/04/06	Marble Hall/ Grobersdal	Agenda (Hennie le Roux)
	12/04/06	CFB Evaluasie	Kultivarevaluerings (Hannes Bester)
	21/04/06	Burgersfort	Agenda (Hennie le Roux)
	23/04/06	Tshipise	Agenda (Hennie le Roux)
	16/05/06	CEF-meeting	Agenda (Hannes Bester)
	18/05/06	CMF-meeting	Agenda(Hannes Bester)
	26/05/06	Vergroeningswerkswinkel	Agenda (Hannes Bester, MC Pretorius)
	05/06/06	Oos-Kaap CTA	Kultivarwerkswinkel (Hannes Bester)
	02/08/06	Hartswater	Eksotiese Siektes (Hannes Bester) FCM (Sean Moore) Vrugtevlieg (Sean Moore) Witluis (Sean Moore) Bladspringers (Sean Moore) Kouebestuurswerkswinkel (Graham Barry/Ballie Wahl)
	03/08/06	Benede-Oranjerivier	Eksotiese Siektes (Hannes Bester) FCM (Sean Moore) Vrugtevlieg (Sean Moore) Witluis (Sean Moore) Bladspringers (Sean Moore) Kouebestuurswerkswinkel (Ballie Wahl)
	07/08/06	Exporters Technical Panel Citrusdal	Navorsingsprioriteite 2007 (Hannes Bester) Eksotiese siektes (Hannes Bester) Vergroeningsimptome Cryptogran (Sean Moore) Bemesting / CAL (Hannes Coetzee) Eksotiese siektes (Hannes Bester) Vergroening (Hannes Bester / Sakkie Bruwer)
	08/08/06	Paarl / Stellenbosch Swartland	Cryptogran (Sean Moore) Bemesting / CAL (Hannes Coetzee) Eksotiese siektes (Hannes Bester) Vergroening (Hannes Bester / Sakkie Bruwer)

RESEARCH			
Name	**Date**	**Place**	**Topic**
			Cryptogran (Sean Moore) Bemesting / CAL (Hannes Coetzee) Eksotiese siektes (Hannes Bester) Vergroening (Hannes Bester / Sakkie Bruwer)
	09/08/06	Swellendam Stellenpak Pakhuis	Cryptogran (Sean Moore) Bemesting / CAL (Hannes Coetzee) Eksotiese siektes (Hannes Bester) Vergroening (Hannes Bester / Sakkie Bruwer) Peteca spot (Paul Cronje)
	10/08/06	Breederivier	Cryptogran (Sean Moore) Bemesting / CAL (Hannes Coetzee) Eksotiese siektes (Hannes Bester) Vergroening (Hannes Bester / Sakkie Bruwer)
	11/08/06	Knysna area	Slakmonsters vir *P. citrophthora* ontledings
	20-23/08/06	4de Sitrus Navorsingssimposium	Onderwerpe op program
	28/08/06	Rustenburg	Navorsingshoogtepunte vir 2005/6 soos aangebied by die 4de Sitrusnavorsingsimpsium (NHP) Bepaling van Navorsingsprioriteite vir 2007 (NP)
	29/08/06	Marble Hall	Sitrusnavorsingsimposiumterugvoer NP 2007
	29/08/06	Burgersfort/Ohrigstad	Sitrusnavorsingsimposiumterugvoer NP 2007
	30/08/06	Nelspruit	Sitrusnavorsingsimposiumterugvoer NP 2007
		Malelane	Sitrusnavorsingsimposiumterugvoer NP 2007
	31/08/06	Komatipoort	Sitrusnavorsingsimposiumterugvoer NP 2007
	31/08/06	Swaziland	Sitrusnavorsingsimposiumterugvoer NP 2007
	01/09/06	Pongola	Sitrusnavorsingsimposiumterugvoer NP 2007
	04/09/06	Suid-Natal	Sitrusnavorsingsimposiumterugvoer Eksotiese siektes
	05/09/06	Constantia/Letsitele Nkwalini	NHP NP2007 Sitrusnavorsingsimposiumterugvoer Eksotiese siektes
	06/09//06	Tshipise Katrivier	NHP NP 2007 Sitrusnavorsingsimposiumterugvoer Eksotiese siektes Vergroeningsimptome
	06/09/06	Weipe	Sitrusnavorsingsimposiumterugvoer NP 2007
	07/09/06	Hoedspruit Sondagsrivier Patensie PSB Patensie Privaat Pakkers	Sitrusnavorsingsimposiumterugvoer NP 2007 Sitrusnavorsingsimposiumterugvoer Eksotiese siektes Sitrusnavorsingsimposiumterugvoer Eksotiese siektes Sitrusnavorsingsimposiumterugvoer

RESEARCH			
Name	**Date**	**Place**	**Topic**
			Eksotiese siektes
	11/09/06	Swellendam	Simposium Terugvoer Eksotiese siektes Vergroeningsimptome
		Breederivier	Simposium Terugvoer Eksotiese siektes Vergroeningsimptome
	12/09/06	Paarl / Stellenbosch	Simposium Terugvoer Eksotiese siektes Vergroeningsimptome
		Swartland	Simposium Terugvoer Eksotiese siektes Vergroeningsimptome
		Citrusdal	Simposium Terugvoer Eksotiese siektes Vergroeningsimptome
	13/09/06	Benede Oranjerivier	Simposium Terugvoer Eksotiese siektes VSA-Toelating
	14/09/06	Vaalharts	Simposium Terugvoer Eksotiese siektes
	15/09/06	Kruger Hek	SASSCON vergadering met Du Pont
	19/09/06	Knysna	Simposium Terugvoer Eksotiese siektes Vergroeningsimptome Plaagbeheer (Sean Moore)
	21/09/ 06	Katrivier	Swartvlek (Tian Schutte) *Alternaria* (Tian Schutte) *Phytophthora citrophthora* (Tian Schutte)
		Sondagsrivier	Plaagbeheer (Sean Moore) Swartvlek (Tian Schutte) *Alternaria* (Tian Schutte) *Phytophthora citrophthora* (Tian Schutte) FCM (Sean Moore)
	22/09/06	Patensie	Swartvlek (Tian Schutte) *Alternaria* (Tian Schutte) *Phytophthora citrophthora* (Tian Schutte) Plaagbeheer (Sean Moore)
	26/09/06	Richmond	Swartvlek (Hennie Le Roux) *Phytophthora* (Hennie Le Roux) Sitrusaalwurms (Hennie Le Roux) Cryptogran (Hannes Bester)
	27/09/06	Nkwaleni	Swartvlek (Hennie Le Roux) *Phytophthora* (Hennie Le Roux) Sitrusaalwurms. (Hennie Le Roux) Cryptogran (Hannes Bester)
	28/09/06	Swellendam / Breederivier	*Phytophthora citrophthora* (Tian Schutte) Besoek aan proefblok (Tian Schutte)
	09–13/10/06	Sitrus-kortkursus	Background to the Industry Climatic requirements Cultivars and rootstocks Soil preparation Fertilization and irrigation Planting and care of young trees Disease control

RESEARCH			
Name	**Date**	**Place**	**Topic**
			Pest control Weed control Pruning Scouting
	19/10/06	Crop Load and Fruit Quality Management Meeting	Agenda
	26/10/06	Citrus Marketing Forum	Agenda
	02/11/06	Greening Feedback Meeting	Prof Bové
	07/11/06	Post-Harvest Handling Working Group Meeting	Agenda
	14/11/06	Benede-Oranjerivier Studiegroep	Bestuur van VSA-program (Piet Smit) VSA-GAP's (Hannes Bester) Plaagbeheer (Sean Moore) Nuwe M3 (Danie Kriek)
	24/11/06	Dendron	CRI-Voorligting (Hannes Bester) Rypheidsindeksering en verskepings-temperatuur (Hannes Bester) Pholokwane lughawe (G du Plessis) Logistiek (JC Strauss) Verpakking (Kallie Calitz)

9.5 **OTHER MEANS OF TECHNOLOGY TRANSFER**

9.5.1 **SA Fruit Journal** by Tim G Grout (CRI)

The SA Fruit Journal is distributed to every citrus grower who is paying the levy on export citrus because the subscription is paid out of the levy funds. It therefore is one of the best means of transferring technology on technical issues. Bimonthly Extension Briefs are provided as reminders for growers of practices that need to be implemented at that time and in-depth research articles are also included. The citrus articles published in the SA Fruit Journal during 2006 are listed in Table 9.5.1.1. A misleading advertisement for the unregistered Ecotronics device that claimed to control pests and diseases in an area of 300 ha was taken up with the Advertising Standards Authority and no further advertisements were published. Due to the lag time of two months between submission of the articles and circulation of the journal, urgent information is circulated to growers as Cutting Edge or Snykant articles via CRInet and emails to the technology transfer groups.

Table 9.5.1.1. S.A. Fruit Journal articles by CRI group members during 2006.

2006 Issue	Article	Author
Dec 05/Jan	Factors causing fruit drop in navel oranges	S.D. Moore, G.C. Schutte & G.H. Barry
Feb/March	Post-Harvest Fungicide Resistance on Citrus Fruit	K.H. Lesar
	Pyrethroid resistance in citrus thrips	T.G. Grout
April/May	Newsflash: Keep your eyes Peeled!	Graham H. Barry
	Cultivar vs Variety – Terminology Guideline in the Citrus Industry	Graham H. Barry
June/July	The order of benzimidazole and strobilurin applications in a spray programme for the control of citrus black spot	G.C. Schutte
	Die prys van sitrus voorplantingsmateriaal	Thys du Toit
	4th Citrus Research Symposium	H.F. le Roux
Aug/Sept	Citrus thrips control with abamectin: 10 years on	Tim G. Grout & Peter R Stephen
	Managing Huanglongbing (Citrus Greening Disease) in the Western Cape	M.C. Pretorius & S.P. van Vuuren
Oct/Nov	Huanglongbing/Greening International Workshop	G. Pietersen & H.F. le Roux
	Voëlent: Bedreiging vir Sitrus	H.F. le Roux
Dec/Jan	4th Citrus Research Symposium	H.F. le Roux

9.5.2 **CRI website** by Tim G Grout (CRI)

The Integrated Production Guidelines Volume 3 for Integrated Pest and Disease Management was made available on the website to members during 2006. The monthly page requests have stabilised at about 4500 (Figure 9.5.2.1) which is a further increase on 2005. The highest numbers of requests were received from dot-com domains followed by dot-net. South African domains were the next highest with 10.5% of the bytes requested. Other countries in order of decreasing visits to our website were Australia, Japan, Turkey, Netherlands, Brazil, Argentina, Germany and the United Kingdom.

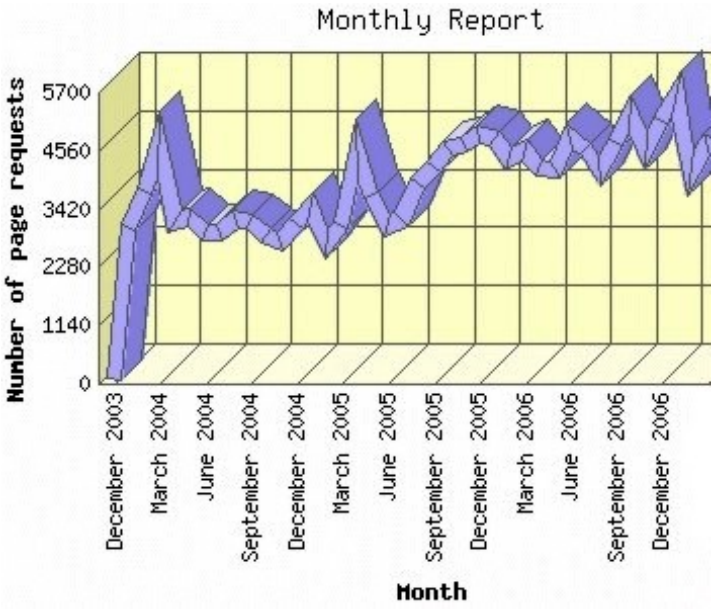


Figure 9.5.2.1. Monthly page requests since December 2003.

9.5.3 **CRInet** by Tim G Grout (CRI)

The number of messages circulated on CRInet during 2006 declined further compared to the two previous years (Table 9.5.3.1). This decline is due to fewer emails from people outside of CRI which hopefully indicates that other methods of technology transfer are being more effective than in the past. The number of people belonging to CRInet is 280.

Table 9.5.3.1. Numbers of messages circulated per month on CRInet.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2006	18	3	1	2	13	9	9	2	1	2	13	2	75
2005	14	11	3	3	3	14	8	3	23	5	11	5	103
2004	7	26	13	28	27	26	12	9	15	12	12	0	187
2003	1	4	6	14	22	4	3	6	5	6	11	3	85

9.5.4 **Cutting Edge** by Tim G Grout (CRI)

During 2006, issues 36 to 51 were circulated via email and made available on the CRI website. The titles covered during this period are given in Table 9.5.4.1.

Table 9.5.4.1. Cutting Edge issues during 2006.

No.	Title	Issue	Author
36	*Phytophthora* bruinvrot bespuitings na goeie deurdringende reëns	January	M.C. Pretorius
37	The use of benomyl (Benlate) in the pre-degreening drench	February	K.H. Lesar
38	Changes to Cryptogran registration and Cryptogran recommendations	March	Sean Moore
39	Non-registered usage of abamectin and thrips resistance	May	Sean Moore & Tim Grout

No.	Title	Issue	Author
40	Update: Thiabendazole (TBZ) use on juicing fruit	May	Paul Hardman
41	Update: Carbendazim EU MRL situation	May	Paul Hardman
42	Update: Implementation of the Food Sanitation Law (Positive List System) in Japan and the Carbendazim (Benomyl) MRL	May	Paul Hardman
43	Penicillium spore (green and blue mould) sampling procedure for imazalil resistance screening	May	K.H. Lesar
44	Strategie vir Oes, Verpakking en Hantering van Sagte Sitrus en Nawels om Verliese te Beperk	May	J.J. Bester
45	Tetradifon USA notification	May	Paul Hardman
46	Update: Withdrawal of tetradifon (tedion) MRL in the USA	May	Paul Hardman
47	Post-Harvest Decay warning	May	K.H. Lesar
48	Some guidelines for reducing the risk of chilling injury on grapefruit exported under extended cold disinfestation conditions	June	K.H. Lesar
49	Precautionary Notice: Buprofezin (Applaud) and Methiocarb (Mesurol)	September	Paul Hardman
50	Effek van Laat Stikstoftoediening op Vruggehalte en Produksie	December	Hannes Bester, Paul Cronje & Ballie Wahl
51	Notice of change to the Fenpropathrin (Meothrin) usage restriction for citrus.	December	Paul Hardman

9.5.5 **4de Sitrusnavorsingsimposium** deur H.F. le Roux (CRI)

Gedurende Augustus 2006 is die 4de Sitrusnavorsingsimposium vanaf 20 -23 Augustus in Port Elizabeth by die Boardwalk Casino aangebied. Die simposium was ’n groot sukses. Dit is bygewoon deur sowat 350 belangstellendes en die navorsers het hulle deeglik van hul taak gekwyt om die afgelope twee jaar se navorsingsresultate aan die bedryf oor te dra. Die klem was veral op die vordering wat gemaak is met Valskodlingmot en Swartvlekbeheer maar daar was talle ander lesings soos die van Tian Schutte oor die terugsterwing van Clementines in die Knysna area wat veroorsaak word deur die swam *Phytophthora citrophthora* wat groot belangstelling gewek het. Drie oorsese besoekers het as sprekers opgetree. Dr Pete Timmer het gepraat oor die bedreiging wat sitruskanker en vergroening vir die Florida sitrusindustrie inhou, Steve Burdette het ’n oorsig oor die Australiese sitrusindustrie gegee en dr Fred Gmitter het die Kultivarontwikkeling wat in Florida plaasvind, bespreek. Daar was twee werkswinkels gedurende die simposium aangebied. Die eerste oor Sitrusswartvlek en die tweede oor Kultivarontwikkeling.

Feitlik alle Uitvoerders is genader om deur middel van borgskappe en advertensies by die simposium betrokke te raak. Daarmee saam is verskeie instansies wat 'n rol in die bedryf speel genader vir borgskappe by die simposium, nl kartonvervaardigers, verskepingsmaatskappye, SAPPI, PPECB, chemiese maatskappye, depots, vervoermaatskappye, asook verskeie instansies wat nie regstreeks in die bedryf betrokke is nie. Hoewel die gesindheid van almal baie goed is teenoor CRI, raak dit toenemend moeiliker om fondse of borgskappe te bekom agv die finansiële druk waaronder almal verkeer. Om hierdie rede moet dit ernstig oorweeg word of die nodige fondse om die simposium in die toekoms aan te bied nie deur die CGA voorsien moet word nie en dit bloot as 'n verlengstuk van Voorligting binne CRI te hanteer. ICA International Chemicals / Hygrotech en Corinth Capital het as Platinum Borge opgetree en Makteshim-Agan en Standard Bank as goue borge. Die Silver borgskappe het bestaan uit: BASF, Bayer Cropscience, Budget, Capespan, Fresh Produce Terminals, Green Trading, Illovo Sugar, Ingwe Print, Insect Science, Katco, Katope, Magalies Sitrus, River Bioscience, SASCCON, UAP Crop Care en Villa Crop Protection. Die Brons borge het ingesluit: Ag-Chem Africa, APL Cartons, Cape Citrus, Colors Fruit, Dow Agrowscience, Du Roi Kwekery & IPM, Glenrand MIB, H&R GSP Sales, Houers Kooperatief, Improcrop, Intertrading Fruit, Jansen Pharmaceutica, Konica Minolta, Lona Trading, Microbial Solutions, Miller Chemicals, Netafim SA, Netstoring Solutions, Philagro South Africa, Plaaskem, Qwemico, RT Chemicals, Sappi, Sinclair, Southern Fruit Growers en Unifrutti SA.

Die Program en lesings wat aangebied is was die volgende:

4th Citrus Research Symposium	
Programme	
VENUE / PLEK: TSITSIKAMA HALL, BOARDWALK CASINO, PORT ELIZABETH – 20-23 AUGUST 2006	
SUNDAY – 20 AUGUST 2006	
17:00 – 19:00	***REGISTRATION AT PROTEA MARINE HOTEL, PE***
19:00 – 21:00	***WELCOME BY VAUGHAN HATTINGH (CRI)*** ***CHEESE AND WINE in the Skyroof Room*** ***Protea Marine Hotel***
MONDAY – 21 AUGUST 2006	
07:30 – 08:30	***REGISTRATION AT TSITSIKAMA HALL, BOARDWALK CASINO, PE***
	SESSION 1: INTRODUCTORY SESSION ***CHAIRPERSON: V. HATTINGH***
08:30 – 08:50	Opening address: Overview of the Research & Technical Support services in the SA citrus industry J. Danckwerts, Chairman of CRI
08:50 – 09:20	Guest speaker: Greening, canker and other diseases threatening to the citriculture of Florida and South Africa L.W. (Pete) Timmer, University of Florida, Lake Alfred, Florida, USA
09:20 – 09:35	How are citrus growers' funds being spent on research? T.G. Grout
	SESSION 2: CITRUS BLACK SPOT ***CHAIRPERSON: J.M. KOTZÉ***
09:40 – 10:00	Overview of citrus black spot research at the University of Pretoria L. Korsten
10:00 – 10:15	The practical application of PCR technology in the citrus industry L. Meyer
10:15 – 10:35	The Critical Infection Period – new information J.M. Kotzé
10:35 – 11:15	***TEA***
11:15 – 11:30	Mysteries of *Guignardia citricarpa* – the switch from green to mean M. Truter, J.M. Kotzé, L. Meyer and L. Korsten
11:30 – 11:45	Seasonal variation in ascospore availability of *Guignardia* spp. in citrus orchards under different climatic conditions S.H. Swart and J.M. Kotzé
11:45 – 12:00	Additional information for effective management of citrus black spot under abnormal conditions S.H. Swart, V. Phalandwa and W. van der Pypekamp
12:00 – 12:15	New spray programmes for the control of citrus black spot G.C. Schutte
12:15 – 12:30	Progress on the development of a Citrus Black Spot disease forecasting model C.M. van Ginkel, J.M. Kotzé and L. Korsten
12:30 – 13:30	***LUNCH***
	SESSION 3: FALSE CODLING MOTH ***CHAIRPERSON: T. GROUT***
13:30 – 13:50	Ontwikkeling van 'n Steriele Insektegniek vir Valskodlingmot: Kommersiële bestryding deur die vrystelling van steriele motte J. Hendrik en Marsheille Hofmeyr, J.E. Carpenter en S. Bloem
13:50 – 14:05	Improvements in the field usage of the *Cryptophlebia leucotreta* granulovirus (CrelGV), CRYPTOGRAN, for control of false codling moth on citrus S.D. Moore and W. Kirkman
14:05 – 14:20	An investigation of alternative hosts for false codling moth in the Eastern Cape W. Kirkman and S. Moore
14:20 – 15:00	***TEA***
15:00 – 15:15	Potential of entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae for control of false codling moth Antoinette P. Malan and Sean D. Moore
15:15 – 15:30	Mating disruption as a method to control false codling moth *Cryptophlebia leucotreta* in citrus A.N. Hanekom
15:30 – 15:45	The host status of lemons for false codling moth S.D. Moore, B. Tate and W. Kirkman

	SESSION 4: INTEGRATED PEST MANAGEMENT ***CHAIRPERSON: K. DE KOCK***
15:45 – 16:00	No silver bullet for citrus psylla control T.G. Grout, B.A. Tate and P.R. Stephen
16:00 – 16:15	The efficacy of a nucleopolyhedrovirus (HearNPV) for control of bollworm on citrus S.D. Moore, W. Kirkman, T. Pittaway, J. Fourie and P. Stephen
16:15 – 16:30	Further investigation of IPM-compatible thripicides for Scirtothrips aurantii T.G. Grout, P.R. Stephen & B.A. Tate
16:30 – 16:45	Changes to the labels of agricultural remedies in an effort to enhance practical resistance management in South Africa H.G. van der Westhuizen
15:00	***BREAK AWAY GROUP*** *South African Citrus Nursery Association (SACNA) Annual General Meeting at CFB* *(Depart for CFB after lunch. Meeting commences at 15:00)*
	TUESDAY – 22 AUGUST 2006
	SESSION 5: INTRODUCTORY SESSION ***CHAIRPERSON: J. CHADWICK***
08:00 – 08:30	Overview of the Australian Citrus Industry S. Burdette, Australia
08:30 – 08:45	Controlling the future of your citrus enterprise J.P. Hughes and L.A. von Broembsen
08:45 – 09:00	Consolidating international food safety and social accountability standards into a one-stop industry service bureau K. Hartman
	SESSION 6: CROP LOAD & FRUIT QUALITY MANAGEMENT ***CHAIRPERSON: S.F. DU PLESSIS***
09:00 – 09:15	The effect of climate on yield and fruit size of citrus in the Nelspruit area S.F. du Plessis
09:15 – 09:30	Fruit set improvement with GA3 sprays I. Garden
09:30 – 09:45	Evaluation of potential benefits of hand thinning on Nules Clementines S. Verreynne
09:45 – 10:30	***TEA***
10:30 – 10:45	Controlled water deficit can improve sugar accumulation in a drip irrigation system J.A. Prinsloo, E. Rabe and G.H. Barry
10:45 – 11:00	The role of hormones in alternate bearing in citrus S. Verreynne and Carol Lovatt
11:00 – 11:15	Rind colour enhancement S. le Roux and G.H. Barry
11:15 – 11:30	Organic material and citrus, another perspective J.G.K. Coetzee
11:30 – 11:45	Besproeiing en bemesting van sitrus: Vrae en antwoorde J. Kruger
	SESSION 7: RIND CONDITION ***CHAIRPERSON: J.J. BESTER***
11:45 – 12:00	Evaluation of alternative means of controlling creasing (albedo breakdown) S. Verreynne
12:00 – 12:15	The influence of canopy position on rind condition of Nules Clementine mandarins P.J.R. Cronjé, G.H. Barry and M. Huysamer
12:15 – 12:30	Rind pigments and antioxidant capacity association with rind disorders in 'Clementine' mandarin fruit as influenced by canopy position and cultivar P.N. Khumalo, A. de Kock, M. Huysamer and G.H. Barry
12:30 – 12:45	The effect of different citrus waxes on the development of peteca spot on lemons K.H. Lesar and P.J.R. Cronjé
12:45 – 14:00	***LUNCH***
14:00	CGA Board meeting starts
	SESSION 8: POST-HARVEST HANDLING ***CHAIRPERSON: L. KORSTEN***
14:00 – 14:15	The history of post-harvest fungicide resistance and strategies to reduce the risk of potential disaster in the southern African citrus industry K.H. Lesar

14:15 – 14:30	*In vitro* sensitivity of Southern African isolates *Penicillium italicum* and *Penicillium digitatum* to imazalil and guazatine J.P. Mildenhall, L.A. Trollope and K.H. Lesar
14:30 – 15:15	***TEA***
15:15 – 15:30	Verbetering van sitrus verpakking F. van Wyk
15:30 – 15:45	Citrus rejection factors (PPECB) C. Julius
15:45 – 16:00	The importance ofmanaging airflow in citrus cartons ad shipping transport M. Dodds
16:00 -16:15	Cold Disinfestation of Medfly-infested Clementines A.B. Ware (ABRC), J-H. Daneel, B. Tate, P. Stephen (CRI) and T. Misumi (MAFF) (VIDEO PRESENTATION)
17:00	Annual General Meetings of CRI, River Bioscience & CGA
20:00 – 23:00	***GALA DINNER – PROTEA MARINE HOTEL (Skyroof room)***
	WEDNESDAY – 23 AUGUST 2006
	SESSION 9: INTRODUCTORY SESSION ***CHAIRPERSON: H.F. LE ROUX***
08:00 – 08:15	Competitiveness of the South African citrus industry J. Chadwick
08:15 – 08:30	South African citrus production trends P. Hardman (CGA)
08:30 – 08:45	Market Access – Status of industry endeavours to Gain, Retain and Optimise Market Access V. Hattingh
08:45	Continuation of CGA Board meeting
	SESSION 10: GRAFT TRANSMISSIBLE DISEASES ***CHAIRPERSON: G. PIETERSEN***
08:45 – 09:00	Citrus Tristeza Virus cross-protection of Star Ruby grapefruit S.P. van Vuuren, B.Q. Manicom and J.H.J. Breytenbach
09:00 – 09:15	The effect of single-aphid transferred Citrus Tristeza Virus sub-isolates on the growth of young Marsh and Star Ruby grapefruit trees J.H.J. Breytenbach and S.P. van Vuuren
09:15 – 09:30	Citrus Tristeza Virus cross-protection of sweet orange S.P. van Vuuren, B.Q. Manicom and J.H.J. Breytenbach
09:30 – 09:45	Progress towards establishment of a comprehensive diagnostic capability at CRI against citrus graft-transmissible pathogens G. Pietersen, K.A. Stewart and M.N.B. Phahladira
09:45 – 10:30	***TEA***
10:30 – 10:45	The current status and management strategies of Huanglongbing citrus greening disease in the Western Cape province of South Africa M.C. Pretorius, S.P. van Vuuren and H. la Grange
10:45 – 11:00	Development and Implementation of PCR and Microarray based methods to differentiate Citrus Tristeza Virus (CTV) strains K.A. Stewart and G. Pietersen
	SESSION 11: FRUIT, FOLIAR AND SOILBORNE DISEASES ***CHAIRPERSON: H.F. LE ROUX***
11:00 – 11:15	*Phytophthora citrophthora* – the death sentence for Clementine production in South Africa? G.C. Schutte and W.J. Botha
11:15 – 11:30	The evaluation of a range of compounds for the control of nematodes and *Phytophthora* spp. M.C. Pretorius and G.C. Schutte
11:30 – 11:45	New spray programmes for the control of *Alternaria* brown spot on mandarins in the summer and winter rainfall regions in South Africa G.C. Schutte
11:45 – 12:00	Commercial fungicide spray efficacy in grapevines: do sprays reach the target? J.C. Brink, G. Holz and P.H. Fourie
	SESSION 12: CULTIVAR DEVELOPMENT & EVALUATION ***CHAIRPERSON: P. WAHL***
12:00 – 12:30	Breeding Approaches to Scion Cultivar Development at UF-CREC

	F.G. Gmitter, University of Florida, Lake Alfred, Florida, USA
12:30 – 12:45	Cultivar Innovation: Effective sourcing and commercialisation of citrus cultivars in southern Africa G.H. Barry
12:45 – 14:00	***LUNCH***
13:45	CRI Board Meeting starts
14:00 – 16:45	***WORKSHOP: CULTIVAR CHARACTERISTICS AND STATUS*** ***CHAIRPERSON: G.H. BARRY***
	Navel oranges C.J. Alexander
	Valencia oranges J. Joubert
	Clementine mandarins G.H. Barry
	Satsuma mandarins C.J. Alexander
	Mandarins G.H. Barry
	Lemons G.H. Barry
	Grapefruit J. Joubert
	Rootstocks J. Joubert
16:45 – 17:00	Closing Session – H.F. le Roux

Daar is 22 plakate met die jongste navorsingsresulate aangebied terwyl daar ’n verdere 20 plakkate in die Argief gedeelte vir ’n tweede keer uitgestal was.

PROGRAMME / PROGRAM ***20-23 AUGUST 2006***	
POSTERS	
1	Post-harvest Chemical Control of Grain Chinch Bug *Tim G. Grout & Bruce A. Tate*
2	The response of citrus rootstocks to Citrus Blight after 15 years *J.H.J. Breytenbach & S.P. van Vuuren*
3	Citrus Research International Diagnostic Centre: Know the status of your Orchards! *L. Huisman*
4	Mode of action of a new antagonist for the control of *Guignardia spp*, *Colletotrichum gloeosporioides* and *Penicillium digitatum* *P.M. Ndou, T. Regnier, L. Halueendo, K. Zeeman, L. Korsten*
5	Storage temperature and storage duration response curves for ‘Eureka’ lemon harvested at different physiological maturities, with special reference to Peteca spot (poster) *P.N. Khumalo, Jerome Davids & Arrie de Kock*
6	Rearing of the lacewing predator *Chrysoperla pudica* and its susceptibility to key pesticides used on citrus *P.R. Stephen & T.G. Grout*
7	*South African Citrus Improvement Programme (CIP)* *M.N. du Toit*

8	Dancy tangerine, the ancestral maternal link for *Alternaria* brown spot susceptibility of new tangerines and their hybrids *G.C.Schutte*
9	Post harvest control of Alternaria black core rot with pre-harvest chemical sprays *G.C. Schutte & K. Lesar*
10	*In vitro* and *in vivo* evaluation of maneb and mancozeb against Guignardia citricarpa, the cause of citrus black spot on Valencia oranges *G.C. Schutte*
11	Ontwikkeling van 'n SIT-program vir Valskodlingmot: Bestralingsbiologie, Oorgeërfde Steriliteit en Hokproewe *J.H.en M. Hofmeyr, Stephanie Bloem en James E. Carpenter*
12	Ontwikkeling van 'n SIT-program vir Valskodlingmot: Kommersiële bestryding deur die vrystelling van steriele motte *J.H. en M. Hofmeyr, James E. Carpenter en Stephanie Bloem*
13	Effect of hydro-cooling and hot water-dipping on the visual flavedo colour and pigment changes of 'Navel' and 'Turkey' oranges *M. Mosoeunyane, I. Bertling and J.P Bower*
14	*Pseudocercospora angolensis,* the cause of fruit and leaf spot disease of citrus in Zimbabwe *M.C. Pretorius*
15	Distribution and control of *Pseudocercospora angolensis* on citrus in Zimbabwe and Mozambique *M.C. Pretorius*
16	Identification of post-harvest rind disorders of citrus fruit *P.J.R. Cronjé*
17	What do fruit fly trap catches signify? *A.B. Ware (ABRC), J-H. Daneel (CRI) and R. Beck (CRI)*
18	Extension *J.J. Bester & H.F. le Roux*
19	Rearing of *Ceratitis rosa* Karsch (Natal Fruit Fly) *John-Henry Daneel*
20	Selective medium for *Guignardia citricarpa*, the causal agent of citrus black spot *T. Regnier, P.M. Ndou, K. Zeeman, M. Truter and L. Korsten*
21	The evaluation of phosphonates for the post-harvest control of *Phytophthora* brown rot *K.H. Lesar*
22	The CRI Group of Researchers *H. Skinner*

PROGRAMME / PROGRAM 20-23 AUGUST 2006	
ARCHIVE POSTERS (from previous local and international symposiums and congresses)	
1	The Chinch Bug: a real tough bugger *A.B. Ware and B.A. Tate*
2	Field trials demonstrating the effectiveness of the M3 bait station in controlling fruit fly *A.B. Ware*
3	Spiders in citrus orchards in South Africa (Arachnida: Araneae) *A.S. Dippenaar-Schoeman, A.M. van den Berg and P. Stephen*
4	The monitoring and control of fruit fly in South Africa *C.H. Buitendag, W. Naudé and A.B. Ware*
5	Control of graft transmissible diseases in southern Africa *S.P. van Vuuren, M. Luttig and B.Q. Manicom*
6	Evaluation of *Citrus tristeza virus* isolates in Clementine *S.P. van Vuuren and J.G.J. Maritz*
7	Effects of *Citrus tristeza virus* isolates on Palmer navel and Delta Valencia on different rootstocks *S.P. van Vuuren*
8	Strain prevalence of *Citrus tristeza virus* cross-protecting isolates altered by red grapefruit hosts *J.B. van der Vyver, S.P. van Vuuren, M. Luttig, B.Q. Manicom and J.V. da Graca*
9	Changes in the *Citrus tristeza virus* status of pre-immunized grapefruit field trees *J.B. van der Vyver, S.P. van Vuuren, M. Luttig and J.V. da Graca*
10	Differentiation of single aphid cultured sub-isolates of two South African *Citrus tristeza closterovirus* isolates from grapefruit by single strand conformation polymorphism *M. Luttig, S.P. van Vuuren and J.B. van der Vyver*

11	The response of clementine to tristeza virus infection *S.P. van Vuuren, J.B. van der Vyver and J.G.J. Maritz*
12	The effect of the rootstock and *Citrus tristeza virus* isolates on Huanglongbing (greening) infection in Palmer navel and Delta Valencia *S.P. van Vuuren*
13	The effect of pruning and graft transmissible isolates on Huanglongbing infection *S.P. van Vuuren and B.Q. Manicom*
14	The association of group III citrus viroids with gum pocket disease in South Africa *S.P. van Vuuren, J.B. van der Vyver, M. Luttig and B.Q. Manicom*
15	Detection of sequence variants in citrus Viroid III isolates by SSCP analysis *M. Luttig and S.P. van Vuuren*
16	Factors affecting citrus production and fruit quality *David P.H. Tucker, Graham H. Barry and Renee M. Goodrich*
17	Efficacy of Phytex (potassium phosphonate) in controlling *Phytophthora* brown rot in citrus *H.F. le Roux, M.H. Mason and M.C. Pretorius*
18	Comparison of accelerated degradation rates between nematicides applied through drip and those applied through micro irrigation *H.F. le Roux,M.C. Pretorius and L. Huisman*
19	Glasshouse evaluation of Beltsville Nartia *Citrus Tristeza Virus* sub-isolates *J.H.J. Breytenbach, S.P. van Vuuren, M. Luttig and L.J. Marais*
20	Comparison of promising field and single aphid *Citrus Tristeza Virus* isolates in the glasshouse *J.H.J. Breytenbach, S.P. van Vuuren and L.J. Marais*

9.6 **Industrie-verwante Vergaderings** deur H.F. le Roux (CRI)

’n Uitvoerders Tegniese Paneel is op die been gebring om te verseker dat kommunikasie met die uitvoerders deurlopend geskied om sodoende hul tegniese behoeftes in CRI se navorsing te integreer. Die vergaderings wat gehou is, is goed bygewoon en is baie positief ervaar. Die ondersteuning van die navorsers is nodig om sodoende die uitvoerders deurlopend van inligting te kan voorsien. Die navorsingsprioriteite is vir die Exporters Technical Panel bepaal waartydens hul dank vir die werk wat die CRI doen, weereens uitgespreek is. Die behoefte aan 'manuals' om na-oes bederf en fisiologiese skildefekte te kan identifiseer, is ook weer beklemtoon.

Bywoning van beide die Citrus Exporters Forum en Citrus Marketing Forum vergaderings is vir die uitvoerders en prominente rolspelers in die bedryf nie net ’n aanduiding van CRI se breë betrokkenheid en belangstelling in alle fasette en op alle vlakke van die sitrusbedryf nie, maar toon ook CRI se beskikbaarheid en bereidwilligheid om broodnodige ondersteuning te verleen soos dit benodig mag word. Selfs al gaan daar vergaderings verby waar daar nie noodwendig insette gelewer word nie, is CRI sigbaar en is dit vir alle rolspelers ’n aanduiding van ons verbintenis tot die bedryf. Die vergaderings is ook ’n bron van inligting om onsself beter te posisioneer ten opsigte van navorsingsprioriteite en tegnologie oordraging, bv. kultivar navorsing en aanbevelings vir nuwe aanplantings, oesmanipulasie t.o.v. vruggrootte, interne gehalte en kleur om aan markvereistes te voldoen, temperatuur protokolle, ens.

Die Sitrus Tegniese Vereniging (CTA) in die Oos-Kaap is weer van die grond af gekry en vergaderings en werkswinkels is gereël om lede van die CTA tegnies te ondersteun. Onder andere is ’n kultivar werkswinkel en ’n inligtingsdag vir volhoubare grondbestuur gehou.

'n Daadwerklike poging is op versoek van die CMF, Exporters Technical Panel en verskeie studiegroepe aangewend om alle navorsing op na-oes verwante onderwerpe te koördineer. 'n Post-Harvest Handling Working Group, wat uit verskeie rolspelers in die bedryf bestaan, het vergader om 'n strategie uit te werk om sinvol na die belange van die bedryf om te sien. Twee vergaderings met alle betrokke rolspelers word vir Januarie en Februarie 2007 beplan om 'n Packaging Forum te stig wat na alle na-oes belange sal omsien.

9.7 **Siekte- en plaagbeheer** deur H.F. le Roux (CRI)

Verskeie probleme in die Wes-Kaap, wat op die Wes-Kaap CTA vergadering geïdentifiseer is, is saam met Hennie Le Roux opgevolg. Simptome van vergroening is wyd opgemerk en dis kommerwekkend dat die omvang daarvan reeds in so gevorderde stadium is. ’n Vergroeningswerkswinkel is vir Vrydag 26 Mei 2006 geskeduleer om ’n strategie uit te werk om die siekte onder beheer te kry. *Phytophthora citrophthora* kom ook wydverspreid op Clementines in die Wes-Kaap voor. Verskeie boorde met hierdie probleem is besoek en aanbevelings is gemaak. Dit is tydens die studiegroepvergaderings aangespreek. Verskeie gevalle van boomagteruitgang is ook opgevolg en dis duidelik dat wortelsorg in verskeie gebiede die afgelope paar jaar verwaarloos is. Dit is ook met die studiegroepvergaderings weer aangespreek.

Weerstandbiedendheid teen na-oes swamdoders raak ’n algemene probleem in verskeie areas. ’n Strategie om dit te bestuur is in plek gesit en alle pakhuise is versoek om monsters te neem om te laat toets vir weerstanbiedendheid. Die produsente en uitvoerders is ook deeglik van die probleem verwittig en versoek om ’n rol te speel deur druk op hul pakhuise te sit om uit te vind wat hul status ten opsigte van weerstandbiedendheid is.

Hannes Bester het 'n vierdag-kursus in 'Sustainable Agriculture' in Pietermaritzburg bygewoon. Daar is 'n redelike sterk dryf in veral die Oos-Kaap om 'n meer langtermyn, sagte benadering in verbouiingspraktyke te volg. Hoewel dit 'n nie-wetenskaplike benadering is, sal CRI met groot omsigtigheid toenemend hierby betrokke moet begin raak om objektief en met die nodige verantwoordelikheid in die behoeftes van die produsentebasis te voorsien. Cryptogran is 'n uitstekende voorbeeld van wat in hierdie verband binne CRI vermag kan word.

Hennie het saam met Prof Gerhard Pietersen ’n internasionale werkswinkel oor Vergroening (Huanglongbing) in Ribeirao Preto, Brazilië, bygewoon vanaf 14–25 Julie. Tydens die werkswinkel is twee referate gelewer. Die eerste het gehandel oor die huidige stand van Vergroening in suider Afrika en die tweede oor die bestuur van vergroening. Terugvoer oor die werkswinkel is reeds gepubliseer in die Oktober/November uitgawe van die SA Vrugtejoernaal, bl 66-67. Na afloop van die werkswinkel is besoeke gebring aan boorde met CVC, Sudden Death, Rubilose en Leprosis. Prof Bové van Bordeaux, Frankryk, was ook teenwoordig.

'n Besoek deur Prof Bové van Frankryk en twee navorsers van Fundecitrus in Brazilië om 'n opname van Vergroening deur die land te doen, het die gevare van die siekte en die belangrikheid om dit onder beheer te kry, beklemtoon. 'n Strategie om die voorkoms en verspreiding van vergroening deurentyd te monitor en die inokulum te verwyder sal so gou moontlik in plek gesit moet word.

9.8 **Tuinboukundige en kultivar aspekte** deur H.F. le Roux (CRI)

'n Geval waar saad in Bahianina nawels voorgekom het, is ondersoek in Noord-Wes. Die oorsaak daarvan is waarskynlik 'n kombinasie van jong groeikragtige bome langs Novas wat sterk kruisbestuiwers is.

In die noorde is heelwat probleme met skaapneus ondervind op die pomelos en verskeie pomeloprodusente het besluit om eerder hulle vrugte vanjaar te versap. Peteca was weer 'n ernstige probleem in vroeë suurlemoene vanaf Limpopo, Mpumalanga en die Oos Kaap.

Verskeie probleme op Afourer is die afgelope seisoen gerapporteer en opgevolg. Dit wil voorkom asof hierdie kultivar baie sensitief is vir skildefekte, chemiese brand, *Alternaria* en, wat baie kommerwekkend is, vergroening. Hierdie probleme sal dringend aandag moet geniet om soortgelyke probleme op hierdie gesogte kultivar in die toekoms te beperk.

'n Besoek is saam met Paul Cronjé aan Stellenpak Pakhuis gebring waartydens 'n vergadering met hul produsente gehou is om Peteca indringend te bespreek. Uit hierdie vergadering het inligting gekom wat gelei het tot die ontdekking dat CO_2 peteca baie vinnig uitwys. Dit kan met die nodige verfyning as 'n baie handige hulpmiddel aangewend word om die omvang van die probleem op 'n baie vroeë stadium vas te stel.

9.9 **Fitosanitêr en ekonomies** deur H.F. le Roux (CRI)

Die ekonomiese situasie waarin die sitrusbedryf bedryf vroeg in die seisoen was, het meegebring dat veral kleiner produsente die handdoek, wat betref die uitvoermark, ingegooi het. Die resultaat hiervan is dat hulle nie meer praktyke toepas om fitosanitêre siektes en plae te beheer nie en toenemend 'n risiko vir ander produksie-eenhede en die bedryf in geheel word. Op die oomblik is daar nie maatreëls in plek om die bedryf hierteen te beskerm nie. Die mees sinvolle strategie sal waarskynlik statutêre ingryping vereis om skuldige produsente of te dwing om minimum GAP's toe te pas, of hul bome uit te trek.

9.10 **Algemeen** deur H.F. le Roux (CRI)

Die terugvoer uit die mark in terme van bederf was bemoedigend. Dit moet egter in ag geneem word dat die mark sterk was en vrugte vinnig verkoop het. Fisiologiese skildefekte op verskeie variëteite is wel later in die seisoen gerapporteer en daar is 'n dringendheid onder produsente dat oplossings vir hierdie probleme gevind word. PPECB het ingestem om alle afkeuringsresultate van elke area op 'n gereelde basis beskikbaar te stel, wat ons in staat sal stel om oor tyd die oorwegende probleme van elke area te bepaal en daarop te fokus.

Daar word op die oomblik 'n positiewe gevoel onder produsente in die Sitrusbedryf ervaar. Die verdienste was bo verwagting goed vanjaar en dit kan hoofsaaklik toegeskryf word aan laer voorsiening aan die markte, asook pogings van die produsente om volumes en kwaliteit beter te beheer, soos veral in die geval van die VSA en Japan. Die gevolg hiervan is dat daar weer nuwe aanplantings deur verskeie produsente beplan word.

Die werk wat CRI die afgelope seisoen vir die bedryf gedoen het, word hoog deur produsente aangeslaan en die deurbrake wat op verskeie gebiede gemaak is, veral VKM en marktoegang, dra saam met die goeie verdienstes vanjaar by dat die toekoms in die Sitrusbedryf rooskleurig lyk. Die groot sukses van die Sitrusnavorsingsimposium is wyd bekend in die Suid-Afrikaanse vrugtebedryf en voorligters van die Sagtevrugtebedryf het reeds kom kers opsteek oor die voorligtingsmodel binne die Sitrusbedryf.

Vir die eerste keer vanjaar het CRI 'n stalletjie, op versoek van die organiseerders, by die Bien Donne Expo by Simondium gehad waar die navorsing en voorligting dmv plakkate en elektroniese aanbiedinge vertoon is. As gevolg van swak weer was die bywoning swakker as verwag, maar die versoek is dat CRI volgende jaar weer 'n stalletjie by die Expo sal beman. Dis interessant dat verskeie besoekers aan die stalletjie, vanuit ander bedrywe, opgemerk het dat hulle gehoor het dat die Sitrusnavorsingsimposium 'n baie groot sukses was.

10 PUBLICATIONS IN 2006

10.1 Refereed publications (or ISI ranked journals)

Meyer, J.B., van Vuuren, S.P., Luttig, M., Manicom, B.Q. and da Graça, J.V. 2004. Strain prevalence of *Citrus tristeza virus* cross-protecting isolates altered by red grapefruit hosts. Proc. 16th Conf. IOCV: 205-212.

Truter, M., Labuschagne, P.M., Kotzé, J.M., Meyer, L. & Korsten, L. 2007. Failure of *Phyllosticta citricarpa* pycnidiospores to infect Eureka lemon leaf litter. Australian Plant Pathology. 36: 87-93.

Van Vuuren, S.P. & Manicom, B.Q. 2004. The response of Star Ruby grapefruit to different *Citrus tristeza virus* isolates. Proc. 16th Conf. IOCV: 112-116.

Van Vuuren, S.P. & Manicom, B.Q. 2004. The effect of pruning, a *Citrus tristeza virus* and a citrus Viroid isolate on Huanglongbing infection. Proc. 16th Conf. IOCV: 362-365.

Van Vuuren, S.P. & Manicom, B.Q. 2004. The effect of the rootstock and *Citrus tristeza virus* isolates on the percentage of Huanglongbing-affected fruits from Palmer navel and Delta Valencia. Proc. 16th Conf. IOCV: 366-369.

Van Vuuren, S.P., Meyer, J.B., Luttig, M. & Manicom, B.Q. 2004. Search for a dwarfing isolate of Citrus Viroid III for high density plantings and the possible association of CVd-III with gum pocket disease in South Africa. Proc. 16th Conf. IOCV: 301-311.

10.2 Semi-scientific publications

Barry, G.H. Newsflash : Keep your eyes peeled! S.A. Fruit J. 5(2): 30.

Barry, G.H. Cultivar vs Variety – Terminology Guideline in the Citrus Industry. S.A. Fruit J. 5(2):31.

Du Toit, Thys. 2006. Die prys van sitrus voorplantingsmateriaal. S.A. Fruit J. 5(3): 41.

Grout, T.G. 2006. Pyrethroid resistance in citrus thrips. S.A. Fruit J. 5(1):40.

Grout, T.G. & P.R. Stephen. 2006. Citrus thrips control with abamectin: 10 years on. S.A. Fruit J. 5(4): 55,57-58.

Le Roux, H.F. 2006. Voëlent: Bedreiging vir Sitrus. S.A. Fruit J. 5(5):69.

Le Roux, H.F. 2006. 4th Citrus Research Symposium. S.A. Fruit J. 5(6): 30-33.

Lesar, K.H. 2006. Post-Harvest Fungicide Resistance on Citrus Fruit. S.A. Fruit J. 5(1):37.

Meyer, L. & Korsten, L. 2006. Citrus black spot detection. Afgriland. 50: 58-59.

Pietersen, G. & H.F. le Roux. 2006. Huanglongbing/Greening International Workshop. S.A. Fruit J. 5(5) :66-67.

Pretorius, M.C. & S.P. van Vuuren. 2006. Managing Huanglongbing (Citrus Greening Disease) in the Western Cape. S.A. Fruit J. 5(4):59-62.

Schutte, G.C. 2006. The order of benzimidazole and strobilurin applications in a spray programme for the control of citrus black spot. S.A. Fruit Journal. June/July, 9(3):36-39.

11 PRESENTATIONS AT SOCIETAL AND INTERNATIONAL CONGRESSES

Barry, G.H. & Coetzee, H.E. Factors affecting rind oil content in lemons. Australian Citrus Growers 58th Annual Conference, Perth, Australia, 27 March 2006.

Barry, G.H. & Le Roux, S. Effects of prohexadione-calcium on rind colour and rind pigments of citrus fruit. Southern African Society for Horticultural Science Conference, Stellenbosch, 29-30 May 2006.

Barry, G.H., Cronjé, P.J.R. & Huysamer, M. Factors affecting rind breakdown of 'Nules Clementine' mandarin. Southern African Society for Horticultural Science Conference, Stellenbosch, 29-30 May 2006.

Barry, G.H. & van Wyk, A.A. Low temperature cold shock induces rind colour development of 'Nules Clementine' mandarin *(Citrus reticulata* Blanco) fruit. Southern African Society for Horticultural Science Conference, Stellenbosch, 29-30 May 2006.

Barry, G.H., Khumalo, P.N., A. de Kock & Huysamer, M. Effect of storage temperature and storage duration on the post-storage quality of 'Nules Clementine' mandarin *(Citrus reticulata* Blanco) with special reference to rind breakdown and chilling injury. Southern African Society for Horticultural Science Conference, Stellenbosch, 29-30 May 2006.

Barry, G.H. Cultivar Innovation: Effective sourcing and commercialisation of citrus cultivars in Southern Africa. AllFresh Conference and Expo, Sun City, 30-31 August 2006.

Barry, G.H. Panel discussion on production and markets in the region. 2006 Conference of the International Federation of Essential Oils and Aroma Trades, Cape Town, 28-29 November 2006.

Cronjé, P.J.R., Barry, G.H. & Huyshamer, M. Factors affecting rind breakdown of Nules Clementine mandarin. Southern African Society for Horticultural Science Conference, Stellenbosch, 29-30 May 2006.

Le Roux, H.F., van Vuuren, S.P. & Manicom, B.Q. Huanglongbing in South Africa. Huanglongbing (greening) International Workshop, Ribeirão Preto, SP, Brazil, 16-20 July, 2006.

Le Roux, H.F., van Vuuren, S.P., Pretorius, M.C. & Buitendag, C.H. Management of Huanglongbing in South Africa. Huanglongbing (greening) International Workshop, Ribeirão Preto, SP, Brazil, 16-20 July, 2006.

Moore, S.D. 2006. River Bioscience. In: 1st International Biocontrol Manufacturers Association Meeting, Lucerne, Switzerland, 23-24 October 2006.

Moore, S.D., Malan, A. & Kirkman, W. 2006. A phenologically based programme for season-long control of false codling moth on citrus, with particular use of a granulovirus and entomopathogenic nematodes. In: Society for Invertebrate Pathology 9th International Colloquium on Invertebrate Pathology and Microbial Control and 39th Annual Meeting, Wuhan, China, 26-31 August 2006.

Pretorius, M.C., van Vuuren, S.P. & Pietersen, G. The current status and management strategies of Huanglongbing (citrus greening disease) in the Western Cape Province of South Africa. SASPP Congress, Kopanong, Benoni, 21-24 January 2007.

Verreynne, J.S. The role of hormones in alternate bearing in citrus. Southern African Society for Horticultural Science Conference, Stellenbosch, 29-30 May 2006.

citrus research international

Citrus Research International (Pty) Ltd Reg. No 2001/007745/07
PO Box 28 Nelspruit 1200 South Africa *** 2 Baker Street Nelspruit 1200 South Africa
Tel: +27 13 759 8000 Fax: +27 13 755 0578 E-mail: cri1@cri.co.za Website: www.cri.co.za / www.citrusres.com