ASSESSMENT OF A COLD TREATMENT FOR THE DISINFESTATION OF EXPORT CITRUS FROM FALSE CODLING MOTH, *THAUMATOTIBIA LEUCOTRETA* (LEPIDOPTERA: TORTRICIDAE): A REPORT TO THE REPUBLIC OF KOREA

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INTRODUCTION

The false codling moth, *Thaumatotibia leucotreta* (Meyrick), (Lepidoptera: Tortricidae), is considered indigenous to sub-Saharan Africa, the Ethiopian Region and many islands off the African continent. The insect has a wide range of host plants and has adapted to cultivated crops from its original indigenous host plants. It infests citrus, amongst others, in South Africa and is a serious pest of cotton and maize in tropical Africa (Bloem et al. 2003).

In the last 30 years, *T. leucotreta* had been suppressed with a succession of chemical insecticides – in the late nineteen-seventies with larvicidal pyrethroids (Hofmeyr 1977, 1983a&b), followed in the mid-eighties by ovicidal growth inhibitors in the benzoyl urea group (Hofmeyr 1984). The development of resistance by *T. leucotreta* to these insecticides (Hofmeyr & Pringle 1998) necessitated the use of flexible multi-tactic suppression programmes. The tactics included strict orchard sanitation as a prerequisite (picking infested fruit and removing fallen fruit at least once a week), in combination with mating disruption, egg parasitoids or sterile insect releases (Hofmeyr unpublished data). Unfortunately pre-harvest suppression could not guarantee that consignments of exported fruit would be free of *T. leucotreta*. Ever stricter export regulations imposed by a growing list of countries importing fresh citrus from South Africa necessitated that a post-harvest procedure such as a cold disinfestation treatment be assessed for validation as a phytosanitary treatment.

An official protocol for such a study was agreed upon by plant health officials from Korea and South Africa (dated October 1997). However, it was not conducted in the prescribed detail as deviations from the protocol were necessitated by experimental limitations. With mutual agreement by Lee, Kong and Hofmeyr the protocol was slightly revised and the experiment commenced in May 1998. The study is reported on below.

MATERIALS AND METHODS

Origin of experimental fruit

The protocol required the assessment of 3 replicates to be applied concurrently and containing not less than 10 500 larvae each. Six citrus growers in the Citrusdal region were selected to provide navel oranges infested with *T. leucotreta*. A total of 1 226 cartons of oranges, calculated to contain approximately 60 000 oranges, was delivered to the pack house of Goede Hoop Citrus, Citrusdal, within 24 h. On arrival a standard pack house procedure was applied, *viz*. handling of fruit via conveyor belts, outomatic sorting of oranges into various counts etc., as well the application of standard treatments, *viz*. washing in a water bath, treatment with the fungicide thiabendazole, and drying; no wax was applied. The oranges were then packed into standard 15 kg citrus cartons.

Numbers of Experimental Fruit

All fruit mentioned hereafter refer to the infested fruit supplied by the Citrusdal growers.

Fruit for Primary Control: One carton of oranges in each of 4 available fruit counts was collected at

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random from conveyor belts in the packhouse. The oranges were dissected by Hofmeyr and Lee immediately after collection. All larvae, alive or dead, were removed, counted and recorded.

Fruit for Secondary Control: This control was intended to confirm that larvae from the infested oranges could emerge and were physically fit to pupate. A total of 5 600 oranges was collected at random from the combined batches collected for the study. The oranges were divided into 3 replicates that were placed next to each other in a single layer on a 10 mm thick layer of sand on the floor of a derelict building adjacent to the packhouse of Goede Hoop Citrus. The building was not air conditioned and was missing a number of windows. The oranges were removed from the sand up to 3 times per week and the sand sifted to collect all FCM cocoons. The oranges were then replaced on the sand. Decayed oranges were removed and dissected to collect larvae that had not yet left the oranges. The larvae and pupae were counted and recorded.

Fruit for cold treatment: The remaining packed and palletized oranges, 49 872 in total, were transported to Cape Town and placed in a pre-cooling facility at International Harbour Services, Cape Town, for 72 h at a temperature of -1.5°C. At the end of this period the pre-cooled oranges were placed into port-hole containers for cold treatment at an ambient temperature of -1.5°C. Internal fruit temperatures were confirmed to be within the specified, viz, -0.6°C ±0.6°C using Grant Squirrel dataloggers with thermocouples inserted into the oranges for the duration of the cold treatment. The cold-treated oranges were removed 22 d later and transported to Plant Quarantine Services (PQS) in Stellenbosch. The oranges were kept at ambient temperature for 2 d. They were then cut open carefully by a team of workers consisting of the Korean officials, Capespan staff and technicians from PQS to determine the number of dead and surviving larvae. All larva suspected of being alive, but possibly only temporarily immobilized by the cold treatment, were kept in a Petri dish at 26°C until their survival or death could be verified – invariably within 12 h.

RESULTS AND DISCUSSION

Primary Control: A total of 25.3% of the oranges was either uninfested or contained dead larvae (Table 1). A relatively low percentage of smaller larvae were found to be dead due to unknown, non-treatment related reasons. There was no clear infestation trend relating to the various counts of oranges and it was consequently considered unnecessary to assess fruit of the cold treatment by count.

Table 1. Numbers of *T. leucotreta* infested oranges and natural larval mortality in a control sample of navel oranges

Carton no.	Fruit count (no. of fruit/carton)	No. of infested fruit with larvae		%	
		alive	dead	fruit infested*	dead larvae
1	40	29	2	77.5	6.5
2	48	45	0	93.8	0.0
3	56	42	5	83.9	10.6
4	56	40	2	75.0	4.8
5	72	53	4	79.2	7.0
6	72	48	3	70.8	5.9
Total	**344**	**257**	**16**	**79.4**	**5.9**

*% of fruit infested with alive or dead larvae.

Secondary control: The 49.8% of *T. leucotreta* collected from the sand in this control (Table 2) differed substantially from the 79.4% of fruit in the primary control from which larvae were recovered. As the oranges from the 2 controls were collected from the same batch of fruit the degree of infestation should have been comparable (Table 2). It is quite possible that fruit decay was mainly responsible for this difference. When infested oranges drop from the trees under normal circumstances, fruit decay is often delayed due to lower and/or inconsistent ambient temperatures. This provides ample time for larvae to leave the fruit and pupate in the soil. When infested oranges are stored indoors, temperatures are more conducive to rapid decay development. Sour rot *Geotrichum candidum* in particular is usually fatal to larvae. The decayed oranges

were not examined for infestation as it is often extremely difficult to detect dead larvae in decayed fruit – the cadavers usually decompose much faster in decayed than sound fruit. Conversely, the oranges in the primary control were cut open within 6 h of harvest; the larval mortality was appreciably lower as the fruit had no opportunity to decay. This control therefore better reflected non-treatment related mortality. The secondary control was consequently of less value and yielded little data of value.

Table 2. Numbers of live *T. leucotreta* larvae and pupae collected from oranges of the secondary control.

	Replicate		
	1	**2**	**3**
No. of oranges used	1 983	1 833	1 784
Calculated no. of infested oranges*	1 575	1 455	1 416
No. of live larvae and pupae recovered	797	725	710

*Numbers of infested oranges reduced by 20.6% to compensate for non-treatment related mortality established in primary control (Table 1).

Cold treated Larvae: More than 34 000 dead larvae were dissected from the cold-treated oranges (Table 3). However, not all of these larvae would have died due to the cold treatment as it was clear from the primary control that a number of the larvae would have died from unknown, non-treatment related factors. The number of retrieved larvae were therefore reduced by 5.9% to compensate for this factor. A total of 32 782 larvae remained that were subjected to the cold treatment, a few short of the number required at a probit 8.7 level. However, although the numbers were adjusted, they still exceeded the 10 500 test insects per replicate required according to the Korean protocol. A few larvae that contrasted with obviously dead larvae (flaccid bodies mostly black or variegated in color), *i.e*. having a more natural, turgid body shape and evenly reddish color were put aside as there was doubt about their mortality. None of these larvae showed any movement at any stage and turned black within 24 h. No live larvae were therefore found in the infested fruit.

Table 3. Mortality of *T. leucotreta* larvae in navel oranges treated with -0,6°C for 22 days.

	Replicate		
	1	**2**	**3**
No. of oranges dissected	16 814	16 716	16 342
No. of larvae recorded in dissected oranges	11 427	12 285	11 125
No. of larvae adjusted for natural mortality*	10 753	11 560	10 469
Number of live larvae	**0**	**0**	**0**

*Numbers of larvae in oranges reduced by 5.9% to compensate for non-treatment related mortality established in primary control (Table 1).

CONCLUSION

It was demonstrated at a probit 8.7 level that a temperature of -0.6°C ±0.6°C maintained for 22 d would be lethal to *T. leucotreta* larvae in packed navel oranges. The result should be sufficient to validate the treatment for the phytosanitary disinfestation of export citrus.

ADDITIONAL EXPERIMENT CONDUCTED BY CAPESPAN TO ESTABLISH THE EFFICACY OF -0,6°C FOR 16 DAYS TO KILL FALSE CODLING MOTH LARVAE IN PACKED NAVEL ORANGES

An additional unofficial experiment was conducted to establish whether 22 d cold treatment at -0,6°C is necessary to kill FCM larvae in fruit, or whether the cold sterilization period could be reduced to 16 d. This experiment was conducted concurrently with the secondary control treatment of the above official study. It was in all respects similar with regard to fruit origin, method used, cold treatment specifications (except for treatment duration) and efficacy evaluation. Only one replicate was assessed. No control specific to this experiment was used. As it was conducted at the same time as the official study, data from the secondary

control from that experiment were applicable to both studies.

No live larvae were found in the cold-stored oranges (Table 4). The experiment was conducted with a limited number of larvae and it is possible that there could be survivors when larger numbers are exposed. However, the assessment demonstrated that 16 d exposure to -0.6°C ±0.6 was lethal to larvae and that the more severe 22 d treatment at the same temperature might include a certain safety margin.

Table 4. Efficacy of -0,6°C for 16 days to kill false codling moth larvae in packed navel oranges.

No. of oranges dissected	16 387
No. of larvae recorded in dissected oranges	11 097
No. of larvae adjusted for natural mortality*	10 442
Number of live larvae	**0**

*Numbers of larvae in oranges reduced by 5.9% to compensate for non-treatment mortality established in primary control (Table 1).

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