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

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ABSTRACT

False codling moth *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) is a pest of many agricultural products in sub-Saharan Africa. As such it impeded the export of southern African citrus to certain international markets. A study was conducted in 2005 in conjunction with the People's Republic of China to validate a post-harvest cold treatment consisting of storing export oranges at -0.6°C ±0.6°C for 22 days. Two consecutive experiments were conducted. The primary experiment consisted of cold treating nearly 33 000 late instar larvae in rearing jars, as well as more than 1 000 larvae in naturally-infested navel oranges. The secondary experiment entailed cold treating more than 1 000 larvae in naturally-infested navel oranges and 35 000 eggs on wax paper sheets. All larvae and eggs were killed by the treatment. The experiments were monitored by Chinese plant health officials who shared the work load and the results were approved.

INTRODUCTION

False Codling Moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is a pest in Africa, infesting, *inter alia*, certain citrus cultivars in southern Africa. It was thus a pest of phytosanitary concern and impeded the export of citrus to certain countries. In 1998 an experiment was conducted for the Republic of Korea to validate cold storage of citrus fruit as a post-harvest treatment (Hofmeyr *et al*. 1998). This study was successfully concluded and facilitated the export of citrus fruit to Korea using an in transit treatment of -0.6°C ±0.6°C for 22 d. The assessment reported on in this publication was conducted in 2005 to demonstrate to a delegation of plant health officials from the People's Republic of China that a similar cold disinfestation treatment would kill all *T. leucotreta* larvae infesting such fruit.

It is very seldom possible to collect large numbers of citrus fruit infested with *T. leucotreta* larvae within a reasonable period of time. This is because the type of crop damage caused by *T. leucotreta* that would permit rapid collection of large numbers of infested fruit usually occurs over weeks rather than days. It was therefore proposed that the primary experiment should consist of treating larvae in artificial diet. A secondary experiment, consisting of navel oranges that were naturally infested with larvae in the orchard, would be conducted on a smaller scale to confirm the results.

MATERIALS AND METHODS

PRIMARY EXPERIMENT

The primary experiment was initiated before arrival of the Chinese Delegation (ChiDel) in South Africa but was concluded in their presence. According to an experimental protocol agreed to in advance by the Chinese and South African plant health authorities, the primary investigation was to be conducted on 10 000 larvae, reared in an insectary on artificial diet in rearing jars. For comparative purposes, sufficient naturally-infested navel orange fruit to produce 1 000 *T. leucotreta* larvae, would be collected from an orchard.

1 Larvae in Rearing Jars

One hundred and twenty five glass rearing jars were prepared with a diet consisting mainly of maize meal (Ripley et al. 1939) in Ceder Biocontrol Insectary, Citrusdal, South Africa. Each jar was inoculated with 400-

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500 *T. leucotreta* eggs and closed with a cotton wool stopper. The jars were incubated at 26°C for larval development. They were removed 15 d later when many larvae had developed to the 5th (final) instar. One rearing jar was collected at random from the batch of 125 jars, the diet removed and 70 larvae were collected at random. They were heat-treated in boiling water and placed into 70% ethyl alcohol for preservation. The larvae were subsequently sent to Citrus Research International (CRI), Nelspruit, Mpumalanga, for head capsule measurements to determine the age distribution of the larvae (Daiber 1979).

Untreated Control: Ten rearing jars with mature larvae were collected at random from the batch of 125 jars. The cotton wool stoppers were removed the same day, replaced with rolled-up strips of corrugated single face cardboard, 30 mm wide, as a pupation substrate (JHH, unpublished) and incubated at 26°C. The cardboard tops were replaced 5 times at 3 d intervals. The cardboard strips were pulled apart to collect the pupae for counting purposes. Some larvae had pupated on the surface of the diet in the rearing jars. The moths developing from these larvae were counted upon eclosion. The total number of pupae and moths were recorded (i) to determine the mean larval production per jar to enable calculation of the relative number of larvae used for the cold treatment and (ii) to establish the time to be allowed for any surviving larvae in the cold-treated rearing jars to pupate.

Cold Treatment: One hundred and fourteen rearing jars were packed into 3 standard 15 kg citrus cartons, each containing 38 jars (19 jars in each of 2 layers).

2 Larvae in Naturally-Infested Fruit

One thousand three hundred and sixty six navel orange fruit with symptoms of *T. leucotreta* infestation were picked from trees in an orchard on the farm Kweekkraal, Citrusdal. One hundred and fourteen oranges showing symptoms of *T. leucotreta* infestation were cut open on the same day and the larvae removed to determine (i) non-treatment related mortality and (ii) age distribution. The larvae were heat-treated in boiling water and placed into 70% ethyl alcohol for preservation. As above, the larvae were sent to CRI, Nelspruit, for head capsule measurements.

Untreated Control: One hundred oranges with symptoms of *T. leucotreta* infestation were incubated at 26°C in a laboratory at the research facility of CRI, Citrusdal, to determine non-treatment related mortality. The fruit were individually placed onto vermiculite in 500 ml plastic containers to facilitate pupation of larvae escaping the fruit. To confine the larvae, each container was closed with a mesh lid. The containers were inspected every third day to remove cocoons and decayed fruit. When a cocoon was found in a container, the fruit and vermiculite were removed and discarded and the cocoon placed back into the container for pupal eclosion.

Cold Treatment: One thousand one hundred and sixty six oranges were packed into 22 standard 15 kg citrus cartons.

3 Cold Storage

The 3 cartons with rearing jars and 22 cartons with naturally-infested oranges were transported to a cold room complex at the Post-harvest Research Department of the Infruitec Research Institute (Agricultural Research Council), Stellenbosch. The cartons were placed into a cold room at -0.6°C for a pre-cooling period of 72 hours, which was ample time to reduce the internal temperatures of oranges and rearing jars to the required -0.6°C ±0.6°C. The cartons with rearing jars were stacked pyramidically (2 at the bottom and one at the top). The cartons with oranges were loosely stacked in 3 rows of 6-7 cartons each.

4 Temperature management and data recording

Temperatures were monitored as follows:

Ambient Temperature in Cold Room: The air temperature in the cold room was controlled and monitored from a main computer in a centrally located control room in the cold room complex. Control instruments in the cold room were calibrated before the experiment commenced. Temperature data were recorded every 30 minutes and the data were automatically saved to file every 24 hours.

Ambient Temperature in Citrus Cartons: Two Hobo dataloggers were used to monitor the air temperature inside each of 2 cartons containing rearing jars and fruit. Each Hobo was placed next to the jar or fruit containing the second (middle) Squirrel thermocouple in the middle of the particular citrus carton. They were removed from the cartons when the experiment was concluded 22 d later and the temperature data downloaded to a computer at the CRI laboratory, Citrusdal.

Internal rearing jar and fruit temperature: Three thermocouples from a Grant Squirrel datalogger SQ800 were inserted into the diet of a jar in the centre of each carton. Similarly, 3 thermocouples from a similar Grant Squirrel datalogger were inserted into a fruit in the middle of a carton at the top, middle and bottom of each row of cartons respectively.

The cold room was momentarily opened at the end of the pre-cooling period to confirm the temperature in the rearing jars and fruit with a glass, pencil-type thermometer. The cold room was then locked and remained so for 21 d, when the cold room was opened for a few minutes to introduce the fruit from the secondary experiment for a 72 h pre-cooling period. The 22 d primary cold treatment was concluded the next day.

The thermocouples from the first Squirrel datalogger were removed from the rearing jars and transferred to the oranges of the secondary experiment 24 h before termination of the primary experiment. The second Squirrel datalogger in the oranges was left *in situ* until the primary treatment was concluded 24 hours later and was then removed.

5 Evaluation of treatment efficacy

The rearing jars and fruit were removed from the cold room after 22 d and placed into a temperature controlled laboratory at the Entomology Department, Infruitec, for incubation at 26°C to allow surviving larvae to revive and pupate. The cotton wool stoppers from the rearing jars were replaced with cardboard tops on the same day to simplify the search for pupae from any surviving larvae. The rearing jars and fruit were incubated for 26 d and 2 d respectively before evaluation.

Larvae in rearing jars: It was impractical to search for surviving larvae in the rearing jars as the diet could not be removed from the jars without damaging any surviving larvae. Treatment failure was therefore considered to be the ability of a surviving larva to pupate. The cardboard tops were removed, closely inspected at 15 d after treatment and the tops replaced. At 26 d the tops were removed again and the 2 layers of each cardboard top were pulled apart and examined for pupae. The diet surface of the rearing jars was also inspected for any larvae that may have survived the cold treatment to pupate.

Larvae in naturally-infested fruit: After incubation the oranges were cut open individually and inspected for larvae. The cutting procedure was conducted by JHH and MH (CRI), the 3 ChiDel representatives, and François Moller (South African Agricultural Food Quarantine Inspection Service). All larvae were carefully removed with a forceps and placed into Petri dishes. These larvae were inspected for any sign of movement by one or more members of the ChiDel. Any larvae that the ChiDel could not immediately confirm were obviously dead were placed into a rearing jar with fresh diet and incubated at 26°C. The larval cadavers were discarded.

SECONDARY EXPERIMENT

The secondary experiment was conducted in the presence of the ChiDel. According to the protocol, the experiment was to be conducted in naturally-infested fruit only, involving 1 000 test insects. It was also requested at that time that *T. leucotreta* eggs were included in the assessment.

1 Temperature Management and Data Recording

Ambient Temperature in Cold Room: The same cold room and integrated temperature control equipment from ARC Infruitec used for the primary experiment was also used for the secondary experiment. The 2 experiments overlapped by 24 h and data recording continued uninterrupted.

Ambient Temperature in Citrus Cartons: The 2 Hobo dataloggers from the primary experiment were transferred from the primary to the secondary experiment and placed inside 2 cartons of oranges at the beginning of the pre-cooling period.

Internal Fruit Temperature: The thermocouples from the first Squirrel datalogger used in the primary experiment were removed from the rearing jars and transferred into the fruit from the secondary experiment at the start of their 72 h pre-cooling period. The calibration of the 2 Squirrel dataloggers was questioned at this time by the ChiDel and the datalogger was removed at the end of the pre-cooling period. It was replaced with a Honeywell Q11 datalogger, supplied by Bernard Hennings (Perishable Products Export Control Board, Cape Town). This instrument was calibrated twice and then installed in fruit in the cold room. Six thermocouples were evenly distributed in the stack of 28 cartons. Both Squirrel dataloggers were sent to the agents, Monitoring and Control Laboratories (MCL, Johannesburg) for downloading of the data, recalibration and certification.

2 Larvae in Naturally-Infested Fruit as well as Eggs

A total of 1 298 navel oranges with symptoms of *T. leucotreta* infestation was picked from an orchard on the same farm as before (Kweekkraal, Citrusdal) one day before conclusion of the primary experiment. Fifty six fruit showing symptoms of *T. leucotreta* infestation were cut open; 50 larvae were collected to determine natural mortality and age distribution. Six fruit were uninfested and were discarded. As in the primary experiment the larvae were treated, preserved and sent to CRI, Nelspruit, for head capsule measurements.

Untreated Control: Fifty fruit with symptoms of *T. leucotreta* infestation were incubated at 26°C in the laboratory at ARC Infruitec, Stellenbosch, to determine non-treatment related mortality. The fruit were again individually placed in plastic containers as before and incubated. The containers were inspected on several occasions to remove decayed fruit.

Cold Treatment of Larvae and Eggs

(i) ***Larvae in oranges***: One thousand one hundred and ninety two fruit were packed into 28 standard 15 kg citrus cartons. They were placed into the same cold room used for the primary experiment at ARC Infruitec and stacked pyramidically in one row. As mentioned above, treatment of the primary and secondary experiments overlapped by 24 h. The cold room, still at -0,6°C, consequently had to opened for a few minutes to introduce the fruit from the secondary experiment.

The oranges were removed from the cold room 22 d later and placed into the laboratory at ARC Infruitec for incubation at 26°C for 2 d to allow surviving larvae to revive. The oranges were then individually cut open and inspected the same day. The exact cutting procedure as in the primary experiment was conducted by the same persons from CRI and ChiDel, as well as Welma Pieterse (Plant Protection Research Institute, National Department of Agriculture, Stellenbosch) and François Moller.

(ii) ***T. leucotreta Eggs*:** Two circular wax paper sheets with 24-hour old *T. leucotreta* eggs ("white" stage *i.e*. undeveloped), and 2 similar sheets with 48-hour old eggs ("red" stage *i.e*. partially developed) were obtained from Ceder Biocontrol Insectary in Citrusdal. Each sheet, 200 mm in diameter, contained approximately 10 000 eggs. A quarter sheet with eggs was cut from a sheet of each egg age for control purposes. The eggs sheets were placed into a plastic container 220 mm x 220 mm and placed into the cold room together with the oranges. The control sheets were placed into Petri dishes and incubated at 26ºC in the CRI laboratory, Citrusdal. These eggs hatched approximately 6 d later and were examined 3-4 days later for hatching by the ChiDel using a stereo microscope. The eggs were removed together with the oranges 22 d post-treatment (see (i) above) and incubated at 26°C for 2 d. They were then examined in a similar way to the control eggs.

RESULTS

PRIMARY EXPERIMENT

1 Temperature Management and Data Recording

Data recorded by ARC Infruitec in the cold room showed that the air temperature in the cold room during treatment of the primary experiment was, with one exception, well within the required range of -0.6ºC ±0.6ºC. Six days into the cold treatment the main cooling compressor at the cold room complex broke down and all operations were shut down. The compressor was fixed within the next 13 hours, but during that time the cold room and internal fruit temperatures increased to more than 10ºC. However, after cooling resumed, the internal fruit temperatures were reduced to below 0ºC within the next approximately 12 hours.

An internal fruit temperature of -0.3ºC was recorded with the pencil-type thermometer at the end of the pre-cooling period. This confirms in general the readings recorded by the Squirrel dataloggers at that time.

Calibration of the Squirrel dataloggers by MCL showed that both Squirrel dataloggers were accurate, with a maximum deviation of 0.2ºC from 0ºC. The recorded temperature data for oranges and rearing jars was therefore acceptable.

It was not possible to calibrate the Hobo dataloggers, as they did not possess external thermocouples. However, their readings corresponded closely with the reading of ambient temperature by ARC Infruitec, as well as the Squirrel dataloggers, and their readings were therefore also acceptable for both replicates.

During treatment all temperatures recorded from the rearing jars and oranges with the integrated ARC equipment, the Squirrel dataloggers and the Hobo dataloggers, were well within the range of -0.6°C ±0.6°C.

2 Larvae in Rearing Jars

Measurements of the larval head capsules showed that 98.6% of the larvae from the randomly selected control rearing jar had already developed to the 4^{th} and 5^{th} instars when the 112 rearing jars were introduced into the cold room. Almost all of the test insects were therefore most tolerant to cold compared to younger larvae (Myburgh 1963).

Untreated Control: A mean of 289 pupae and moths were recovered per rearing jar from the 10 jars selected at random from the 125 jars used for this experiment. The last moths emerged 28 d after incubation.

Cold-treated Larvae: It is calculated from the number of larvae recovered per jar from the untreated control that a mean of 32 946 larvae, i.e. conforming to approximately Probit 8.7, had potentially been exposed to the cold treatment, 23 000 more than the protocol required. None of the larvae showed any sign of life during the inspections (15 d and 26 d after incubation) and none had survived to pupate in either the rearing jars or the cardboard tops.

3 **Larvae in Naturally-Infested Fruit**

Head capsule measurements showed that 74.6% of the larvae in the fruit had developed to the final instar when cold treatment of the bulk of the fruit started. Of the 114 larvae cut from the fruit, only one was dead. Non-treatment related mortality was therefore 0.9%.

Untreated Control: One hundred and four pupae were collected from 96 of the 100 data fruit incubated in the plastic containers. The remaining 4 oranges were found to be uninfested upon dissection. One hundred and 2 moths developed from the pupae. These data confirm that larvae from the cold-treated fruit would have been quite fit to reproduce in the absence of cold treatment and that the experimental material was of good quality.

Cold-Treated Larvae: A total of 1 036 larvae were removed from the 1 166 cold-treated fruit. Due to 0.9% natural mortality (see Section 3 above) a total of 1 027 live larvae (1 000 larvae required as per protocol) would therefore have been potentially exposed in the 1 166 cold-treated fruit. On body colour alone, *viz*. larvae with a pinkish body colour in contrast to obviously dead larvae with variegated to black body colour, the ChiDel isolated 9 larvae from the total number of insects removed from the fruit. They were put onto diet in 2 rearing jars, to allow more time for possible larval revival. None of these larvae showed any signs of movement after being removed from the fruit. The 8 larvae turned completely black within 24 hours. The remaining larva, although obviously dead and showing no signs of life, had not yet turned completely black 16 d later and although largely desiccated, was still reddish in colour. This demonstrated that colour alone was not a criterion that should be used to determine whether a *T. leucotreta* larva had survived the cold treatment. Not one live larva was found in the oranges during inspection in spite of the increased temperatures temporarily caused by the faulty cooling equipment.

No live larvae were found in both the rearing jars and oranges notwithstanding the brief increase in internal fruit temperatures due to the malfunctioning cooling equipment. This demonstrates that the cold treatment was still completely effective despite the temporarily higher temperatures.

SECONDARY EXPERIMENT

1 **Temperature Management and Data Recording**

During treatment the air temperature in the cold room recorded with the integrated ARC Infruitec equipment were, with one exception, within the range of -0.6°C ±0.6°C. Eleven days into the cold treatment a solenoid valve in the cooling equipment of the cold room became faulty and temperature management had to be conducted manually during the next 72 hours. This caused air temperatures in the cold room to fluctuate abnormally in the range 1.2ºC to -1.4ºC. Deviations to below the lower required temperature of -1.2ºC occurred, although not once for longer than one successive reading. However, internal fruit temperatures were not detrimentally influenced and were at all times within the required range of -0.6ºC ±0.6ºC.

Ambient fruit carton temperatures recorded with the 2 Hobo dataloggers confirmed the readings from the ARC Infruitec instruments. Internal fruit temperatures recorded with the Honeywell datalogger were well within the range of -0.6°C ±0.6°C.

2 **Larvae in Naturally-Infested Fruit**

Head capsule measurements showed that 86% of the larvae from the fruit had developed to the 4^{th} (28%) and 5^{th} (58%) instars when cold treatment of the bulk of the fruit started. Two dead larvae were recovered from the sample of 50 infested fruit. Non-treatment related mortality was therefore 4%.

Untreated Control: Forty nine pupae were collected from 43 of the 50 fruit in the containers. Seven oranges were uninfested and no dead or live larvae were found when inspected. Forty nine moths emerged

from the pupae. These data prove, as in the primary experiment, that larvae from the cold-treated fruit would have been viable and able to develop to the next life stage in the absence of cold treatment. The experimental material was thus of good quality.

Cold-Treated Larvae: A total of 1 047 larvae were cut from the 1 192 cold-treated fruit. Due to 4% non-treatment related mortality (see Untreated Control above) a total of 1 005 live larvae would therefore have been potentially exposed in the 1 192 cold-treated fruit (1 000 larvae were required as per protocol). As in the primary experiment, 12 larvae were collected that had maintained a fairly natural looking body colour (whitish for younger larvae and reddish for older larvae), but showing no sign of movement. To confirm that they were dead they were placed into a jar with rearing medium by the ChiDel for further investigation. They were inspected daily for 5 d consecutively and were then pronounced dead. No live larvae were found in the oranges during inspection.

3 ***T. leucotreta* Eggs**

Eighty three percent of the untreated control eggs hatched. The apparently high percentage egg mortality was normal for eggs deposited on wax paper (Hofmeyr, unpublished data). No development to the black stage (shortly before hatching) was observed in both ages of cold-treated eggs. All eggs were unhatched, and were therefore killed by the cold treatment.

CONCLUSION

No live larvae were discovered following the cold treatment of navel orange fruit containing approximately 2 000 *T. leucotreta* larvae, as well as nearly 33 000 *T. leucotreta* larvae reared in diet. The experiment also included 35 000 eggs which did not develop and were killed by the cold treatment.

The cold treatment consisted of a 22 d cold storage period at -0.6ºC ±0.6ºC. This procedure can therefore be considered a successful cold disinfestation treatment to kill *T. leucotreta* eggs, as well as larvae in naturally-infested citrus fruit.

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