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Research



# Development of a Postharvest Cold Treatment for *Cryptophlebia peltastica* (Lepidoptera: Tortricidae) for Export of Litchis From South Africa

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## Abstract

The litchi moth, *Cryptophlebia peltastica* (Meyrick) (Lepidoptera: Tortricidae), is endemic to sub-Saharan Africa and certain Indian Ocean islands. It is an important pest of litchis and to a lesser extent macadamias. Litchis are exported to certain markets that consider *C. peltastica* as a phytosanitary pest. Consequently, an effective postharvest phytosanitary treatment is required. This study sought to develop a cold disinfestation treatment for this purpose. First, it was established that the fifth instar was the most cold-tolerant larval stage, as it was the only instar for which there was still some survival after 12 d at 1°C. It was then determined that cold treatment trials could be conducted in artificial diet, as there was no survival of fifth instar *C. peltastica* in litchis after only 9 d at 1°C, whereas it took 15 d at this temperature before no survival of fifth instar *C. peltastica* was recorded in artificial diet. Consequently, cold susceptibility of fifth instar *C. peltastica* and the most cold-tolerant larval stages (fourth and fifth instar) of false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), were compared in artificial diet. There was no survival of *C. peltastica* after 13 d at 1°C, whereas this was only so for *T. leucotreta* after 16 d. Consequently, it can be concluded that any cold treatment that has been proven effective against *T. leucotreta* would be as effective against *C. peltastica*. Finally, it was confirmed that the cold susceptibility of *T. leucotreta* in artificial diet did not overestimate the effect of cold on *T. leucotreta* larvae in litchis.

**Key words:** litchi moth, false codling moth, *Thaumatotibia leucotreta*, phytosanitary, artificial diet

*Cryptophlebia peltastica* (Meyrick) (Lepidoptera: Tortricidae) is broadly distributed in Africa, particularly south of the Sahara (Bradley 1953, Timm et al. 2007, Gilligan and Epstein 2014). It is known as the litchi moth in southern Africa, as it is an important pest of litchis in South Africa, Mauritius, Madagascar, Seychelles, and Réunion Island (Quilici et al. 1988, Mamet and Williams 1993, Van den Berg 2001, Abeeluck et al. 2002, Waite and Hwang 2002, Timm et al. 2007, Manrakhan et al. 2008, Grové et al. 2015), and to a lesser extent macadamias (Schoeman and De Villiers 2015). It can cause significant pre- and postharvest losses to litchi crops (De Villiers 2001) and is also considered a phytosanitary pest by some export markets, due to its endemism to sub-Saharan Africa and the Indian Ocean islands (Manrakhan et al. 2008). Preharvest control options are limited (Grové et al. 2015). These include insect growth regulators (Grové and de Beer 2005) and parasitoid augmentation (Newton and Crause 1990). However, even if these are applied diligently, there is still a chance that infested litchis could be inadvertently exported to quarantine markets. Consequently, an effective postharvest treatment is required for such markets. At present, the only market for which a specific postharvest treatment is applied to South African litchis is the United States (NDA 2018), being irradiation at a minimum absorbed dose of 400 Gy. However, there are logistical and financial challenges associated with this requirement (D. Donkin, personal communication). In addition, litchis are generally exported at a temperature of 1°C, a temperature shown to be able to induce Probit 9 mortality (Couey and Chew 1986) of the closely related *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), commonly known as the false codling moth, if the duration at that temperature is at least 19 d (Moore et al. 2017). Therefore, the aim of this study was to develop a postharvest cold disinfestation protocol for *C. peltastica*, by comparing its cold susceptibility to that of *T. leucotreta*.

## Materials and Methods

### Source of Insects

*C. peltastica* larvae were obtained from River Bioscience (Hermitage, South Africa), where they were reared on artificial diet (Moore et al. 2014) as described by Marsberg (2016). The culture was established from litchi moth larvae collected from infested litchis and pods from Pride of Barbados, *Caesalpinia pulcherrima*, (L.) Sw. (Fabales: Fabaceae) near Nelspruit, Mpumalanga Province, between December 2008 and May 2009 (Hepburn et al. 2009). At the time of the study, the culture has not yet been replenished with any new field-collected material. The culture was maintained in a controlled environment at 27 ± 1°C, 60–80% RH, and a photoperiod of 18:6 (L:D) h. Adult moths were held in oviposition cages, which consisted of an inverted sieve (30 cm diameter) placed over a sheet of wax paper. The wax paper provided a suitable substrate for oviposition and for easy collection of the eggs (egg sheets). Cotton wool moistened with a 10% sugar solution in distilled water was placed around the inside of the cage and used as an adult food source. Egg sheets were collected every second day, divided into segments containing an approximate specified number of eggs. These sheets were surface sterilized by briefly (2–3 s) dipping them in a 9% formaldehyde solution, to prevent microbial contamination.

### Determination of Most Cold-Tolerant *C. peltastica* Instar

Twenty-one jars of predominantly each of the five *C. peltastica* instars, as determined by Marsberg (2016), were obtained from the River Bioscience laboratory culture. Head capsule width ranges for each of the five instars were 0.3–0.5, 0.5–0.7, 0.7–1.2, 1.2–1.6, and 1.5–1.8 mm, for first to fifth instar, respectively. Three jars of each were retained to evaluate immediately as untreated control jars, and the remainder of jars were placed into a custom-built cold treatment chamber at Citrus Research International (CRI) in Port Elizabeth, Eastern Cape (5.0 m × 4.0 m × 2.4 m). The cold room was on a 6-h defrost cycle. A Brainchild data logger (Wika Instruments, South Africa) was used for recording temperatures in the cold room and in jars of diet with larvae, using 16 PT100 probes (Wika Instruments) with a stated accuracy of 0.3°C. Probes were calibrated before each treatment using the freezing point method where the probes were immersed in melting ice and the temperature recorded when they reached equilibrium (Grout et al. 2011, Moore et al. 2016a). A certified thermometer immersed in the melting ice was used to confirm the temperature. Three calibration runs were conducted, and the mean result for each probe was used for correction purposes. Calibration was performed immediately before initiation of trials. Most probes were found to be 100% accurate. Inaccuracy was never measured at more than 0.1°C, and such probes were recalibrated to be fully accurate. Two probes were used to measure air temperatures at each of the inlet and outlet of the cooling coil, and the other 14 probes were inserted into the media in jars to record temperatures at 15-min intervals over the trial period. Cold room temperature was set at 1°C. Three jars of each instar were removed at 3, 6, 9, 12, 15, and 18 d after placement. Jars were then kept at 25°C for 24 h and were thereafter assessed for live and dead larvae. Larvae were determined to be dead when they displayed a brown discoloration and there was no movement after repeated prodding (Moore et al. 2016a). Instars were confirmed by removing samples of approximately 50 larvae from each instar and measuring head capsule width. If any misidentification of instars was recorded, then total numbers recorded for the affected instar were corrected by the appropriate proportion. The trial was replicated three times. Mean mortality for the three replicates was compared between instars after each duration, using a Generalized Linear Model, ANOVA, and the Fisher LSD multiple range test, using Statistica 12.0 (Statsoft Inc., Tulsa, OK, 2013), after correcting treatment mortality for control mortality (Abbott 1925).

### Comparison of Cold Susceptibility of *C. peltastica* Larvae in Litchis and Artificial Diet

Before cold susceptibility of *C. peltastica* could be compared in litchis and artificial diet, the rate of development of *C. peltastica* in litchis first had to be determined in order that the same instar could be compared in the two media. Four neonate *C. peltastica* larvae were placed onto each of 160 sulfonated and 160 unsulfonated litchis using a size 000 paint brush. Fruit were of the Mauritius cultivar and were obtained from a farm in the Tzaneen region of Limpopo Province. Fruit were inoculated within 48 h of being harvested and were kept at ambient temperature until then. Fruit were kept at 27°C to facilitate rapid development of larvae in the litchis. At 8, 11, 14, and 17 d after infestation, 40 sulfonated and 40 unsulfonated litchis were dissected and instars determined through measurement of the head capsule width (Marsberg 2016). As there was no indication that rate of development of larvae in sulfonated and unsulfonated litchis differed meaningfully, they were considered as two replicates within the same trial.

As it was determined that the fifth instar was the most cold-tolerant larval stage, litchis infested with predominantly fifth instar *C. peltastica* and jars of artificial diet containing predominantly fifth instar *C. peltastica* were used to compare cold susceptibility of *C. peltastica* in litchis and in artificial diet. Twenty-five jars of artificial diet with fifth instar larvae and approximately 700 litchis that had been inoculated with neonate *C. peltastica* larvae (four per litchi) 14 d previously and kept at 27°C for 14 d to develop to fifth instar were placed into the cold room. The cold room was set at 1°C, and the temperature was adjusted, so that probes inserted into both litchis and artificial diet recorded 1°C. At 3, 6, 9, 12, and 15 d, five jars of artificial diet and between 30 and 102 litchis were removed from the cold room, placed at 25°C for 24 h to warm up and live and dead fifth instars determined, enumerated, and compared. On the day that the trial was initiated, four control jars with fifth instars and 100 *C. peltastica*-inoculated litchis were dissected and evaluated to determine instars present, but, more importantly, natural mortality without cold treatment. Two replicates of the trial were conducted.

### Comparison of Cold Susceptibility of *C. peltastica* and *T. leucotreta*

Due to the findings of the previous trial, 60 jars of artificial diet with fifth instar *C. peltastica* and 60 jars of artificial diet with fourth and fifth instar *T. leucotreta*, the most cold-tolerant life stages of *T. leucotreta* (Myburgh 1965, Moore et al. 2016b), were placed into a cold room set at 1°C. An additional 10 jars of each were used as untreated control jars and evaluated when the other jars were placed into the cold room. Ten jars of each species of those that were placed into the cold room were removed at periodic intervals, i.e., 4, 7, 10, 13, 16, and 19 d. Although the previous trial indicated that all *C. peltastica* larvae should be dead after 15 d at this temperature, the trial was run for 19 d as this was shown to be the time required at 1°C to achieve Probit 9 efficacy of *T. leucotreta* (Moore et al. 2017). After removal from cold, the jars were kept at 25°C for 24 h and thereafter assessed for live and dead larvae and instars confirmed by measuring head capsule width of a sample of approximately 50 larvae randomly collected from assessments of both species. Mortality was again corrected for control mortality (Abbott 1925) and total

numbers of larvae reduced by the proportion of larvae determined to be smaller than the most cold-tolerant life stage per second. The trial was replicated three times.

### Comparison of Cold Susceptibility of *T. leucotreta* in Litchis and Artificial Diet

The cold treatment of 1°C for 19 d for *T. leucotreta* was determined in artificial diet, after showing its equivalence to oranges (Myburgh 1965, Moore et al. 2016a). Therefore, this same question was addressed for litchis by comparing cold susceptibility of *T. leucotreta* larvae in artificial diet and in litchis. This was determined simultaneously to the above trial, by infesting litchis with *T. leucotreta* larvae. Approximately 700 litchis from each of three orchards, each being a replicate, were obtained after being sulfonated. All litchis were Mauritius cultivar from a farm in the Tzaneen region of Limpopo Province. Four neonate *T. leucotreta* larvae were placed onto each litchi using a size 000 paint brush. Fruit were kept at 27°C to facilitate rapid development of larvae in the litchis. At 8, 11, 14, and 17 d after infestation, 20 litchis were dissected and instars determined through measurement of the head capsule width (Marsberg 2016). It was determined that the majority of larvae were at fourth and fifth instar 13 d after inoculation. Consequently, litchis were placed into the cold room set at 1°C at this stage. Preparation of each replicate was coordinated to be placed into the cold room simultaneously with the artificial diet replicates used in the above trial. Approximately 90 litchis were removed from the cold room at 4, 7, 10, 13, 16, and 19 d, as with the previously described trial. After removal from cold, the litchis were kept at 25°C for 24 h and thereafter assessed for live and dead larvae and instars confirmed by measuring head capsule width of a sample of approximately 50 larvae randomly collected from assessments. Mortality was corrected for control mortality (Abbott 1925) and total numbers of larvae reduced by the proportion of larvae smaller than the fourth instar because these smaller instars were determined to be the most cold-susceptible life stages. This was done for all three replicates. Mean mortality values were obtained for the three replicates of each treatment and were compared using a Generalized Linear Model ANOVA and the Fisher LSD multiple range test, using Statistica 12.0 (Statsoft Inc., 2013).

## Results

### Determination of Most Cold-Tolerant *C. peltastica* Instar

The mean hourly temperature, mean hourly maxima, and mean hourly minima (all ± SE) for the first and second replicates were 1.09 ± 0.01, 1.18 ± 0.01, and 0.99 ± 0.01, respectively. For the third replicate, they were 1.09 ± 0.01, 1.19 ± 0.01, and 0.98 ± 0.01, respectively.

Average numbers of larvae for each instar per replicate per treatment were 49.1, 120.3, 112.7, 79.7, and 74.3 for first to fifth instars, respectively. Control mortality for second instars for one of the replicates and for third instars for two of the replicates was higher than mortality after 3 d at 1°C. Consequently, no correction for mortality was possible in these three cases. There was a relatively high degree of variation in results between replicates, resulting in a fairly high SE in some cases. Consequently, none of the differences in mortality between the instars after the different time period were statistically significant (Fisher LSD multiple range test; $\alpha = 0.05$). Nevertheless, the fifth instar was confirmed to be the most cold-tolerant instar, as it experienced the highest level of survival after 9 d at 1°C and was the only instar with any survival after 12 d (Table 1). Consequently, all further work was conducted with this instar.

### Comparison of Cold Susceptibility of *C. peltastica* Larvae in Litchis and Artificial Diet

After 8, 11, 14, and 17 d at 27°C, 44, 61, 53, and 23 live *C. peltastica* larvae (or pupae) were recorded, respectively, from the 80 inoculated litchis assessed on each occasion. The proportion of larvae in each instar of the total larvae recorded after each of the periods was calculated. No first instar larvae were recorded at any stage. It was determined that the occurrence of fifth instars peaked at 14 d (Fig. 1). At 14 d, in sulfonated litchis, fifth instars were 66.7% of all larvae and in unsulfonated litchis, 65.0%, thus justifying pooling the data for sulfonated and unsulfonated litchis as two replicates. Consequently, for subsequent trials comparing cold susceptibility of *C. peltastica* larvae in litchis with that of *C. peltastica* larvae in artificial diet, 14-d infested litchis (at 27°C) were used.

In these trials, the mean hourly temperature, mean hourly maxima, and mean hourly minima (all ± SE) in the litchis were 1.06 ± 0.02, 1.30 ± 0.03, and 0.84 ± 0.02, respectively. In the artificial diet, they were 1.08 ± 0.03, 1.28 ± 0.03, and 0.91 ± 0.02, respectively. These values were identical for both replicates in each medium. After only 9 d at 1°C, no survival of fifth instar *C. peltastica* was recorded in litchis (Table 2). However, in artificial diet, no survival was only recorded after 15 d. Sample sizes for litchis were a lot smaller than those for artificial diet. This was unavoidable, due to the limited supply of litchis and the fact that infestation in litchis only averaged around 0.5 larvae per litchi (540 larvae in 1,102 litchis inoculated), despite litchis being inoculated with four neonate larvae each. Nevertheless, litchi sample sizes were considered adequate. For example, the 94.71% mortality recorded in artificial diet after 9 d would have translated into a survival of five

**Table 1.** Mean mortality (± SE) of each of the five *Cryptophlebia peltastica* instars after exposure for various periods of time at 1°C from three replicates

| | Instar | | | | |
|---|---|---|---|---|---|
| Duration at 1°C (d) | 1 | 2 | 3 | 4 | 5 |
| 0 (control) | 9.46 ± 2.02 | 11.84 ± 3.72 | 21.26 ± 4.22 | 1.55 ± 0.88 | 1.69 ± 1.69 |
| 3 | 14.62 ± 8.76 | 5.05 ± 2.78 | 1.49 ± 1.49 | 15.70 ± 2.59 | 7.67 ± 3.24 |
| 6 | 55.71 ± 15.88 | 61.57 ± 20.28 | 60.60 ± 25.16 | 57.11 ± 12.27 | 68.32 ± 17.40 |
| 9 | 100 | 97.59 ± 2.41 | 97.68 ± 2.32 | 97.00 ± 2.74 | 94.71 ± 1.84 |
| 12 | 100 | 100 | 100 | 100 | 99.13 ± 0.87 |
| 15 | 100 | 100 | 100 | 100 | 100 |
| 18 | 100 | 100 | 100 | 100 | 100 |

Mortalities for treatments (days 3–15) have been corrected for control mortality (Abbott 1925).

larvae in the litchi sample of 95 fruits after that period of time. It can therefore be concluded that further cold treatment trials can be conducted with *C. peltastica* larvae in artificial diet, without any risk of overestimating the efficacy of the cold treatment in litchis. A similar study and conclusion was reached by Myburgh (1965) and Moore et al. (2016a) when comparing the cold susceptibility of *T. leucotreta* larvae in oranges and artificial diet.

### Comparison of Cold Susceptibility of *C. peltastica* and *T. leucotreta* and Comparison of Cold Susceptibility of *T. leucotreta* in Litchis and Artificial Diet

Mean hourly temperatures, mean hourly minima, and mean hourly maxima were calculated for jars and litchis for each of the three replicates for the full duration of each replicate (Table 3).

Larger numbers of *T. leucotreta* larvae in artificial diet were used than *T. leucotreta* larvae in fruit and *C. peltastica* larvae in artificial diet (Table 4). This was unavoidable, as the size of the *C. peltastica* laboratory culture did not permit the use of more larvae. In addition, the same number of rearing jars of larvae was used for *T. leucotreta* and *C. peltastica*. However, *C. peltastica* larvae are larger than *T. leucotreta* larvae, and consequently, the number of larvae reared per unit of diet is lower for *C. peltastica* than for *T. leucotreta* (approximately one third; Moore et al. 2014, C. Chambers, personal communication). Although several hundred litchis were obtained from three different orchards, and each was inoculated with four neonate *T. leucotreta* larvae, many of the litchis were not successfully infested and those that were, usually were not infested with more than one larva. Nevertheless, the numbers are considered adequately large for the findings to be considered reliable.

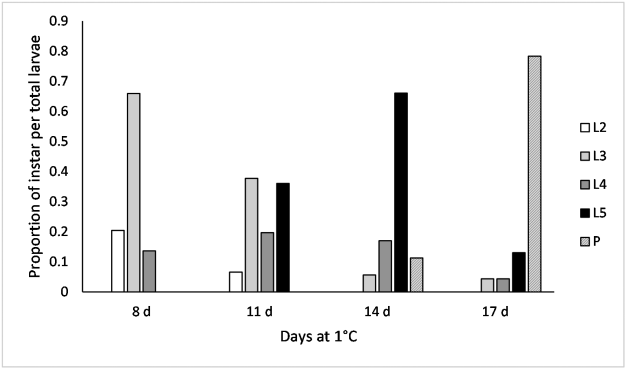


**Fig. 1.** Proportion of each *Cryptophlebia peltastica* instar in litchis at 27°C, after various periods after inoculation of litchis with neonate larvae (L2 = second instar, L3 = third instar, L4 = fourth instar, L5 = fifth instar, P = pupae).

Head capsule measurements of larval samples from each replicate indicated that all *T. leucotreta* larvae were fourth or fifth instars. It was therefore not necessary to adjust numbers, as it was previously shown that fourth and fifth instars are similarly and the most cold-tolerant *T. leucotreta* larval life stages. However, 3.33% of *C. peltastica* larvae in replicates 1 and 2 were fourth instar, and as fifth instar had been shown to be the most cold-tolerant instar, *C. peltastica* numbers for these two replicates were reduced by 3.33% each (Table 4).

Control mortality of *C. peltastica* larvae in artificial diet for the three replicates was 0.17 ± 0.09% (mean ± SE) and for *T. leucotreta* in litchis was 1.15 ± 1.15%, whereas no control mortality was recorded for *T. leucotreta* larvae in artificial diet. After 13 d at 1°C, no more survival of *C. peltastica* was recorded, whereas this was only so for *T. leucotreta* in artificial diet after 16 d (Table 5). This confirmed that *C. peltastica* is not more cold tolerant than *T. leucotreta*, and any cold treatment that is effective against *T. leucotreta* will be effective against *C. peltastica*. In addition, no survival of *T. leucotreta* in litchis was recorded after 13 d, indicating that cold treatment trials with *T. leucotreta* in artificial diet would not overestimate the effect of cold on *T. leucotreta* larvae in litchi fruit. This was despite temperatures being slightly higher in litchis than in artificial diet (Table 3).

## Discussion

Execution of cold treatment trials with *C. peltastica* would not have been possible a few years ago, as it is only in the last few years that a laboratory culture of *C. peltastica* has been successfully established (Hepburn et al. 2009, Marsberg 2016) despite previous attempts

**Table 2.** Mortality of fifth instar *Cryptophlebia peltastica* in artificial diet and litchis after various durations at 1°C from two replicates combined

| | | Mortality after various durations | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Medium | Fifth instars | 0 d | 3 d | 6 d | 9 d | 12 d | 15 d | 18 d |
| Diet | Total | 902 | 643 | 199 | 567 | 611 | 626 | 675 |
| | Dead (%) | 0.10 | 19.79 | 47.87 | 94.71 | 99.86 | 100 | 100 |
| Fruit | Total | 83 | 54 | 53 | 95 | 51 | 21 | 93 |
| | Dead (%) | 4.60 | 43.62 | 95.65 | 100 | 100 | 100 | 100 |

**Table 3.** Mean (± SE) hourly temperature in artificial diet and litchis for each of the three replicates

| | Mean hourly temperatures (± SE) | | | | | |
|---|---|---|---|---|---|---|
| | Artificial diet | | | Litchis | | |
| Rep | Mean | Minima | Maxima | Mean | Minima | Maxima |
| 1 | 1.02 ± 0.01 | 0.76 ± 0.01 | 1.29 ± 0.04 | 1.09 ± 0.02 | 0.76 ± 0.01 | 1.40 ± 0.04 |
| 2 | 1.00 ± 0.01 | 0.75 ± 0.00 | 1.23 ± 0.03 | 1.07 ± 0.01 | 0.75 ± 0.01 | 1.35 ± 0.04 |
| 3 | 0.99 ± 0.00 | 0.76 ± 0.01 | 1.18 ± 0.00 | 1.05 ± 0.01 | 0.75 ± 0.01 | 1.29 ± 0.01 |

**Table 4.** Numbers of fifth instar *Cryptophlebia peltastica* in artificial diet (Cp diet), fourth and fifth instar *Thaumatotibia leucotreta* in artificial diet (Tl diet), and fourth and fifth instar *T. leucotreta* in litchis (Tl fruit) exposed to 1°C for various durations for three replicates

| | Control | | | 4 d | | | 7 d | | | 10 d | | | 13 d | | | 16 d | | | 19 d | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replicate | Cp diet | Tl diet | Tl fruit | Cp diet | Tl diet | Tl fruit | Cp diet | Tl diet | Tl fruit | Cp diet | Tl diet | Tl fruit | Cp diet | Tl diet | Tl fruit | Cp diet | Tl diet | Tl fruit | Cp diet | Tl diet | Tl fruit |
| 1 | 878 | 2,978 | 29 | 594 | 2,088 | 113 | 636 | 1,812 | 77 | 463 | 1,171 | 133 | 507 | 2,442 | 124 | 525 | 2,366 | 56 | 573 | 1,394 | 68 |
| 2 | 637 | 1,961 | 25 | 497 | 1,474 | 58 | 643 | 2,379 | 59 | 459 | 2,011 | 55 | 799 | 1,534 | 45 | 711 | 1,888 | 83 | 512 | 2,110 | 80 |
| 3 | 962 | 1,601 | 28 | 518 | 2,329 | 171 | 425 | 2,358 | 136 | 994 | 2,006 | 188 | 474 | 1,367 | 169 | 546 | 1,476 | 69 | 505 | 1,961 | 49 |
| Total | 2,477 | 6,540 | 82 | 1,608 | 5,891 | 104 | 1,704 | 6,549 | 272 | 1,916 | 5,008 | 376 | 1,780 | 5,343 | 338 | 1,781 | 5,730 | 208 | 1,591 | 5,465 | 197 |

Numbers are corrected for head capsule audits, which reduced only *C. peltastica* larvae in replicates 1 and 2 by 3.33%.

**Table 5.** Mortality (%) of fifth instar *Cryptophlebia peltastica* in artificial diet (Cp diet), fourth and fifth instar *Thaumatotibia leucotreta* in artificial diet (Tl diet), and fourth and fifth instar *T. leucotreta* in litchis (Tl fruit) after various durations at 1°C, including the mean and SE for the three replicates

| Replicate | 4 d | | | 7 d | | | 10 d | | | 13 d | | | 16 d | | | 19 d | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cp diet | Tl diet | Tl fruit | Cp diet | Tl diet | Tl fruit | Cp diet | Tl diet | Tl fruit | Cp diet | Tl diet | Tl fruit | Cp diet | Tl diet | Tl fruit | Cp diet | Tl diet | Tl fruit |
| 1 | 25.73 | 1.01 | 26.92 | 99.39 | 95.36 | 87.75 | 99.79 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 2 | 72.29 | 0 | 31.03 | 99.70 | 71.92 | 83.72 | 100 | 98.91 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 3 | 62.16 | 18.25 | 38.88 | 99.76 | 93.00 | 69.40 | 100 | 99.05 | 98.12 | 100 | 99.93 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Mean | 53.39 | 6.42 | 32.28 | 99.62 | 86.76 | 80.29 | 99.93 | 99.32 | 99.37 | 100 | 99.98 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| SE | 14.14 | 5.92 | 3.51 | 0.11 | 7.45 | 5.57 | 0.07 | 0.34 | 0.63 | 0 | 0.02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| * | a | b | ab | a | ab | b | a | a | a | a | a | a | a | a | a | a | a | a |

Mortality for the treatments has been corrected for control mortality (Abbott 1925).

*Mean values for treatments followed by the same letter are not significantly different (Fisher LSD multiple range test; $\alpha = 0.05$).

(Grové et al. 2004, Steyn and Grové 2005). A laboratory culture is necessary for such trials, as it would not be possible to collect a sufficiently large sample of infested litchis in the field; farmers ensure control of the pest in the field and even where inadequate control is applied, infestation would not reach the requisite level for such trials. In addition, naturally infested fruit will often rot too rapidly to be able to complete the study, as was reported by Myburgh (1965) and Moore et al. (2016a) for *T. leucotreta* in citrus and was experienced in this study with litchis that were evaluated several days after infestation.

To conduct the trials, it firstly had to be established how many larval stages *C. peltastica* has, how to differentiate these, and which is the most cold-tolerant instar. Using Dyar's law, Marsberg (2016) determined that *C. peltastica* has five instars, with the average increment in head capsule width between each instar being 0.2835 mm, determined using Dyar's ratio (Dyar 1890, Hsia and Kao 1987, Broughton 1999, Francisco and do Prado 2001). It was then determined that at 1°C, the fifth and final instar was the most cold tolerant, as it was the only instar for which there was still some survival after 12 d. However, all larvae were recorded as dead after 15 d.

It was then determined that there was no survival of fifth instar *C. peltastica* larvae in litchis after only 9 d at 1°C, whereas it took 15 d at this temperature before no survival of fifth instar *C. peltastica* larvae was recorded in artificial diet. It was therefore demonstrated that it was justifiable to conduct cold treatment trials with *C. peltastica* larvae in artificial diet without risk of over estimating the effect of cold on larvae in litchi fruit.

Consequently, a larger-scale replicated trial was conducted, comparing mortality of *C. peltastica* larvae (fifth instar) and *T. leucotreta* larvae (fourth and fifth instar), both in artificial diet. Results confirmed the findings in the previous trial conducted with smaller numbers that *C. peltastica* is more cold-susceptible than *T. leucotreta*. In this trial, there was no more survival of *C. peltastica* after 13 d at 1°C, whereas this was only so for *T. leucotreta* after 16 d. Consequently, it can be concluded that any cold treatment that has been proven effective against *T. leucotreta*, would be at least as effective against *C. peltastica*. Although the cold treatment that has been commercially applied for *T. leucotreta*, specifically to citrus, for markets that have required such a treatment, such as United States, China, and South Korea, has been a subzero temperature for 22 d, Moore et al. (2017) subsequently determined that the cold treatment could be improved, with 19 d at ≤1.2°C or 16 d at ≤ −0.1°C still achieving Probit 9 level efficacy. Such treatments would therefore also be adequate to provide Probit 9 efficacy against *C. peltastica*. Although Probit 9 is a rather rigid and arguably excessive standard for phytosanitary risk mitigation (FAO 2007), particularly for an insect that solitarily infests fruit (Newton 1998, Grové and de Beer 2017), if such a treatment is accepted by an export market for *T. leucotreta*, then there should be no hurdle to the same treatment being accepted for *C. peltastica*.

In final mitigation of any counterargument to this conclusion, the cold susceptibility of *T. leucotreta* was compared in litchis and artificial diet to determine whether there was any risk of over estimating the effect of cold on *T. leucotreta* larvae in litchis, by conducting the trials in artificial diet. This was confirmed to not be the case, as has been shown for *T. leucotreta* infestation in citrus fruit versus artificial diet (Myburgh 1965, Moore et al. 2016a). In addition, there is no reason to expect that cold susceptibility of an insect will differ in different fruit types. For example, Ware and Du Toit (2016) recorded 100% mortality of more than 35,000 fourth and fifth instar *T. leucotreta* after 20 d at 0.8°C (mean daily maximum of 1.2°C) in grapes, a result very similar to that achieved by Moore et al. (2017) with the same species and life stages in citrus. There is a vast body of work on cold treatment of Mediterranean fruit fly in various fruit types, which will reveal the same lack of differences in cold susceptibility of fruit fly larvae in the different fruit types, particularly as the lethal time value increases (e.g., Jessup et al. 1993, Santaballa et al. 1995, Hashem et al. 2004).

In summary, any cold treatment demonstrated to be an effective postharvest phytosanitary treatment against *T. leucotreta* would be at least as effective against *C. peltastica*. This would therefore include those recently demonstrated to provide Probit 9 efficacy against *T. leucotreta*, the most useful of which convert into 16 d at or below −0.1°C and 19 d at or below 1.2°C, commencing once all probe readings are at or below −0.2°C and 1.0°C, respectively (Moore et al. 2017). This would provide an alternative to postharvest irradiation of litchis for *C. peltastica* as a phytosanitary treatment and potentially open up export opportunities for litchis from southern Africa to new markets.

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