



Received: 15 June 2018
Accepted: 15 August 2018
First Published: 20 August 2018

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ENTOMOLOGY | SHORT COMMUNICATION

Cold treatment of “Hass” avocado (*Persea americana* (Lauraceae)) infested by *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) as an additional component of a systems approach

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Abstract: *Thaumatotibia leucotreta* is of phytosanitary importance, and this paper addresses mitigating measures to reduce the risk of its introduction with fresh avocado fruit into importing countries. Applying a cold treatment of 2°C pulp temperature for 20 days resulted in a single *T. leucotreta* survivor from 28,380 individuals treated. When this result is considered as part of a systems approach where the second component is the poor host status, then the probability of accidentally importing live larvae was lowered to levels exceeding established phytosanitary security levels.

Subjects: Environment & Agriculture; Agriculture & Environmental Sciences; Entomology

Keywords: phytosanitary; false codling moth

1. Introduction

Thaumatotibia leucotreta (Meyrick) (Lepidoptera: Tortricidae) is a major pest occurring on fruit trees and crops in sub-Saharan Africa and the Indian Ocean islands situated off the continent (Newton, 1998; Reed, 1974; Van den Berg, 2001). It is present on southern African tropical and sub-tropical cultivated fruit crops such as citrus (Grout & Moore, 2015) and avocado (*Persea americana* (Mill.) Lauraceae) in all production areas (Erichsen & Schoeman, 1994; Grové, 2001; Grové, De Beer, & Joubert, 2010). However, in contrast with citrus (Grout & Moore, 2015; Newton, 1998), *T. leucotreta* is not considered an important economic pest on avocado (Grové et al., 2010). However, the moth species has a large host range of indigenous and cultivated plants (Van den Berg, 2001), and this

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PUBLIC INTEREST STATEMENT

The export of perishable fruit produce is an essential component for growers. However, a barrier to the free movement of these products is the presence of pests and diseases that do not occur in the destination country. In order to overcome this problem the importing country needs to provide assurance that the consignment is free of these. One solution is to cold treat the fruit in such a manner that any insects within are killed but ensuring fruit quality is maintained. This treatment may be considered part of the bouquet of factors (so-called systems approach) that, in combination, will result in the security levels required by the importing countries.

makes the pest a major phytosanitary concern leading Grové et al. (2010) examining a systems approach (Food and Agricultural Organization of the United Nations [FAO], 2017) to prevent accidental importation.

A definition of a systems approach is “the integration of pre- and post-harvest practices, from the production of a commodity to its distribution and commercialization, which cumulatively meet predetermined requirements for quarantine security” (Aluja & Mangan, 2008). Aspects of the life cycle and control of the insect should be considered—including production, pre-harvest, post-harvest, inspection, shipping and distribution of product (FAO, 2017; Jang & Moffitt, 1994). Grové et al. (2010) considered the host acceptability of avocado to *T. leucotreta* and demonstrated that, provided avocado are harvested when hard, then the moth is rarely, if ever, able to complete its life cycle. Packhouse sorting and inspection provided the last element in their systems approach.

One component absent from the proposed systems approach was the effect of the cold disinfestation treatment established for South African avocado for the fruit fly species *Ceratitis capitata* (Wiedemann), *C. rosa* Karsch and *C. cosyra* Walker (Ware & Du Toit, 2017) of 2°C for 29 d and the 18 d treatment for *Bactrocera (invadens) dorsalis* (Hendel) (Ware, Du Toit, Mohamed, Nderitu, & Ekasi, 2012). It has been determined that temperatures above 1°C for 20 days have little detrimental effect on avocado fruit quality (Kok, Bower, & Bertling, 2010). This research examines the effect of this cold treatment on *T. leucotreta* in avocado.

2. Materials and methods

2.1. Cold chambers

The cold rooms (dimensions 3 × 4 × 2.5 m (l × b × h)) were made of Isowall (polystyrene sandwiched between aluminium sheets) and placed on a concrete floor. Danfoss electronic controllers (Danfoss (Pty) Ltd, Rivonia, South Africa) were used to moderate the cold room temperatures. Fans were switched off when defrosting (every 4 h for 10 min). A Grant 2040 Series Squirrel meter/logger (Temperature Controls (Pty) Ltd., Randburg, South Africa) attached to 16 two-wire thermocouples (Type T) (calibrated on melting ice Ware et al., 2012) recorded the hourly temperature of the fruit pulp. The temperature investigated was that of the pulp at 2.0°C. Two probes were used to monitor inlet and outlet temperatures of the cooling unit.

2.2. Fruit

Hard physiologically-ready for harvest “Hass” avocado was obtained locally from H.L. Hall and Sons (Pty) Ltd. in Nelspruit (now Mbombela) in the Mpumalanga Province of South Africa. On arrival at the Agri-Biotech Research Consultancies laboratory, the fruit were stored at 5°C until required. Two days before the fruit were used they were removed to a 26°C controlled environment room. On the day the fruit was inoculated, they were dipped in a fungicide (Sporekill—didecyl dimethyl ammonium chloride—1 ml/l water) (Hygrotech South Africa (Pty) Ltd., Pyramid Pretoria, South Africa) for 30 min then air dried at 26°C. Fourteen sound (untreated) fruit were used to measure core pulp temperature. An electric drill was used to make holes in the fruit (depth dependant on the size of the fruit and the pip). The probes were inserted and kept in place with cotton wool. These fruit were randomly arranged within the consignment treated.

2.3. Insects

T. leucotreta eggs on wax paper were obtained from Du Roi IPM (now BioBee) (Letsitele, South Africa). As soon as there was an indication of their hatching (the developing larvae within the eggs turn a red color) the wax paper was divided so that each paper contained approximately 20 eggs. Some 10 pieces of the divided wax paper were placed on groups of 20 fruit that had 50 small holes made in them using minuten pins (depth 3 mm) These were maintained at at 26°C (±2°C). The emerging larvae were allowed to roam and penetrate the fruit naturally.

2.4. Experimental procedure

The most cold-tolerant life stage of the larvae was determined by subjecting different aged life stages (in days) (eggs, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 d) to 2°C for 0, 3, 5, 7 ... up to 19 d. Periods beyond 13 days were not examined as the larvae had begun to pupate. After cold treatment the insects were allowed to develop into 5th instars by placing the fruit at 26°C for 12 d (eggs), 11 d (3-d-old larvae), 10 d (4-d-old larvae), 9 d (5-d-old larvae), 8 d (6-d-old larvae), 7 d (7-d-old larvae), 6 d (8-d-old larvae), 5 d (9-d-old larvae), 4 d (10-d-old larvae), 3 d (11-d-old larvae), 2 d (12-d-old larvae) and 24 h (13-d-old larvae). Thereafter they were dissected and the number of live and dead larvae determined under a stereomicroscope. Insects were deemed to be dead if they did not move unassisted after prodding. A total of 600 fruits were used for each of the larval ages examined.

The most cold-tolerant aged larvae (11-d-old larvae or 4th–5th instar) were then subjected to a large-scale validation trial (three replicates). The eggs were placed on fruit and kept at 26°C until the larvae had developed into the required developmental stage. Fruits were then placed in the cold chamber surrounded by sound fruit and the temperature was gradually dropped to the desired 2.0°C over approximately 3 days (mimic commercial cooling—Defraeye, Verboven, Opara, Nicolai, & Cronje, 2015). Once 50% or more of the 14 probes measuring pulp temperature, randomly placed among the infested fruit, had reached the target temperature or below, the trial was deemed to have commenced. The treatments were terminated after 20 d.

2.5. Statistical analysis

The most tolerant life stage data were subjected to Probit analysis (StatGraphics Plus Version 5.1, Statpoint Technologies Inc. Warrington VA) after correction for natural mortality (Abbott 1925).

3. Results

3.1. Cold room

All three replicates achieved target temperatures within three days of initiating the procedures. The mean temperatures thereafter were replicate 1: 1.87°C (sd = 0.20); replicate 2: 2.05°C (sd = 0.09); and replicate 3: 2.02°C (sd = 0.12).

3.2. Most tolerant life stage

Some 10-d-old larvae survived treatment for 17 d, all were dead at 19 d (Table 1). However, on analysis both 9-d-old and 11-d-old larvae (capsular head measurements indicated that 9-d-old larvae were predominantly 3rd instar and 11-d-old were 4th and 5th instar larvae) were found to be most cold-tolerant (Table 2) with an anticipated 99.9% mortality at 15.4 and 15.8 d, respectively. Both had an upper confidence limit of 16.6 d. Based on these results a 20 d cold treatment was used for the large-scale validation trials using 11-d-old larvae.

3.3. Validation trials

There was a 99.9966% mortality. From a total of 28,380 individuals subjected to the 20 d at 2.0°C cold treatment there was a single survivor (Table 3).

4. Discussion

The 10-d-old larvae were marginally more cold susceptible than the 9- or 11-d-old larvae. A possible explanation is that during moulting the insect is less cold tolerant. Contrary to the views of Boardman, Grout, and Terblanche (2012), Ware et al. (2016) and Moore, Kirkman, and Hattingh (2016a, 2016b), Myburgh (1965) did not determine the most cold tolerant life stage of *T. leucotreta* and a comparison with the Myburgh study is difficult. Ware and Du Toit (2011) demonstrated that a 2°C treatment over 22 d was sufficient to sterilize 101,133 10-d-old larvae. Slight difference in rearing temperature may account for the difference in cold tolerance of the 10-d-old larvae to the current research. Moore et al. (2016c), using insects reared on artificial diet,

Table 1. Corrected percentage mortality (Abbott 1925) of immature *Thaumatotibia leucotreta* in “Hass” avocado overtime. Each replicate consisted of 25 fruit that were naturally infested by the first instar larvae

Life stage (days)	Rep	0*	Number of days at 2°C															
			3	5	7	9	11	13	15	17	19							
0 (eggs)	1	69.0 (280)	5.1	38.4	81.0	100	100	-	-	-	-	-	-	-	-	-	-	
	2	71.5 (329)	0	56.3	82.6	100	100	-	-	-	-	-	-	-	-	-	-	
3	1	0 (150)	2.3	16.3	28.3	90.9	100	-	-	-	-	-	-	-	-	-	-	
	2	15.9 (147)	6.4	20.4	62.6	97.1	100	-	-	-	-	-	-	-	-	-	-	
7	1	1.8 (39)	1.8	5.6	14.1	68.2	88.2	100	-	-	-	-	-	-	-	-	-	
	2	3.6 (103)	1.6	2.5	14.1	91.2	100	-	-	-	-	-	-	-	-	-	-	
8	1	2.9 (79)	0	2.4	21.5	80.3	100	100	-	-	-	-	-	-	-	-	-	
	2	2.7 (71)	2.7	11.3	21.2	61.9	96.2	100	-	-	-	-	-	-	-	-	-	
9	1	1.9 (70)	2.9	11.3	19.4	-	80.9	100	-	-	-	-	-	-	-	-	-	
	2	0 (67)	3.3	14.9	23.6	71.4	95.2	100	-	-	-	-	-	-	-	-	-	
10	1	2.1 (175)	0	19.3	38.2	85.5	94.7	98.5	100	-	-	-	-	-	-	-	-	
	2	0 (122)	0	7.5	33.3	73.9	76.5	97.7	99.3	100	-	-	-	-	-	-	-	
11	1	1.0 (94)	0	18.4	54.3	85.3	96.5	100	-	-	-	-	-	-	-	-	-	
	2	1.7 (147)	1.7	4.1	24.1	65.1	94.3	97.9	99.4	99.3	100	-	-	-	-	-	-	
12	1	1.2 (85)	4.9	22.9	41.5	73.2	100	95.3	100	-	-	-	-	-	-	-	-	
	2	0 (195)	0	15.5	45.9	73.4	87.7	99.7	99.3	100	-	-	-	-	-	-	-	
13	1	0 (90)	0	1.7	35.6	61.3	92.1	65.7	100	-	-	-	-	-	-	-	-	
	2	0 (109)	0	9.4	22.0	25.6	70.8	68.7	100	-	-	-	-	-	-	-	-	

*Actual mortality (%); numbers in parenthesis are the average number of larvae in replicate.

Table 2. Probit analysis detailing the number of days various aged immature *Thaumatotibia leucotreta* in “Hass” avocado were expected to survive after cold treatment of 2°C. Data were corrected for natural mortality (Abbott 1925)

Life stage (days)	Mortality (days (CL))		Regression	Percentage of deviance
	90%	99.9%		
0 (eggs)	5.7 (5.4–6.0)	14.1 (13.2–15.1)	$y = 0.2152x + 0.0642$	79.04
3	9.0 (8.8–9.4)	12.3 (11.8–13.8)	$y = 0.5521x - 3.69839$	93.31
7	10.3 (10.0–10.3)	13.4 (12.9–14.0)	$y = 0.58325x - 4.72783$	92.53
8	10.2 (10.0–10.8)	13.4 (12.9–14.1)	$y = 0.56475x - 4.58187$	96.12
9	11.1 (10.5–11.4)	15.4 (14.7–16.6)	$y = 0.42316x - 3.41685$	95.58
10	10.7 (10.5–11.2)	15.2 (14.6–15.8)	$y = 0.408712x - 3.10615$	95.92
11	11.0 (10.7–11.4)	15.8 (15.2–16.6)	$y = 0.379298x - 2.90266$	92.90
12	10.7 (10.4–11.0)	15.3 (14.8–16.0)	$y = 0.392626x - 2.29218$	98.08
13	10.9 (10.5–11.3)	15.3 (14.7–16.0)	$y = 0.409750x - 1.37259$	94.41

Table 3. Survival of 11 d old *Thaumatotibia leucotreta* in “Hass” avocado after 20 d cold treatment of 2°C

Replicate	Control			Treated		
	Live larvae	Number of fruit	Average number of larvae /fruit	Number of fruit	Estimated number of larvae treated	Number of live larvae
1	2,971	400	7.43	989	7,348	1
2	4,224	320	13.75	1,031	14,176	0
3	2,896	309	9.37	873	8,180	0
Total	10,091	1,029	9.81	2,893	28,380	1

demonstrated that there was no significant difference in the mortality for 3rd, 4th or 5th instars treated at 2°C for 18 d. These results are in general agreement with this study.

Avocado is considered a poor host for *T. leucotreta* (Grové et al., 2010) and fruit flies (Diptera: Tephritidae) (De Graaf, 2009; Ware et al., 2016). Among the reasons for this is that avocado fruit does not ripen on the tree (De Villiers, 2001) and that the hard, pre-harvest fruit do not harbor immature stages (although the moth and flies can complete their larval life cycle within soft fruit) (Grové et al., 2010). Other attributes are its hard exocarp (Oi & Mau, 1989), its ability to regenerate tissue and encapsulate fruit fly eggs (Kay & Schroeder, 1963; Ware et al., 2016) and antibiosis (Mwatawala, De Meyer, Makundi, & Maerere, 2006). Egg laying in avocado normally peaks in January, approximately 4 months before the “Hass” harvest commences (Grové et al., 2010). The reduced oviposition activity and egg viability (Catling & Aeschenborn, 1974) at harvest will probably result in a low level of infestation.

Although *T. leucotreta* is often commonly trapped in avocado orchards their presence in the fruit is uncommon (Grové et al., 2010). No mature fifth instar *T. leucotreta* were found in fruit that had been artificially infested with eggs six weeks previously (Grové et al., 2010). Hard fruit that is attacked by early instar larvae show a characteristic white exudate with granular excreta that is easily visible in the field and packhouse (Grové et al., 2010). It was noted that fruit that were penetrated by *T. leucotreta* larvae tend to abort (Grové et al., 2010). Grové et al. (2010) proposed

that a knowledge of the relationship between the presence of these lesions and live larvae can be used to determine the risk imposed by the pest.

Fruit fly life history differs considerably from *T. leucotreta* in that multiple fruit fly may colonize in a single fruit whereas there is generally only one *T. leucotreta* larva per fruit. This has implications when considering the probability of there being a mating pair within any shipment (Follett & McQuate, 2001). Furthermore, there is a high within-fruit mortality (95.95%) (Grové et al., 2010). This finding is considered an underestimate as it was obtained after large numbers of eggs (up to 100) were placed on single fruits—the moth normally oviposits a single egg on a fruit.

Avocado's poor host status for *T. leucotreta* suggests that the traditional Probit 9 (no survivors in 93,613 tested at a 95% CL (Couey & Chew, 1986) standard is excessive (Follett & McQuate, 2001). A cold treatment of 20 d at 2°C is an effective phytosanitary security measure for fruit fly (Ware & Du Toit, 2017). Using this fruit fly treatment protocol, it was determined that the single *T. leucotreta* (from 28,380 individuals treated) surviving this treatment translates to a very low probability of importing a living larva within a consignment. If one adds further population reductions occurring after pre-harvest agrochemical treatments and inspections (Grové et al., 2010), the level of security will far exceed the generally accepted Probit 9 level of the stand-alone cold treatment of less than 3 survivors in 100,000 (95% CL) (Follett & Neven, 2006). Furthermore, Moore et al. (2016c) demonstrated that of the few larvae that survived a partial cold treatment, a further 70% died thereafter. Of those that did develop to adulthood, only a small minority managed to produce viable offspring.

Acknowledgements

The authors would like to thank Derek Donkin, Johan Graaf, Vaughan Hattingh and Elsje Joubert for their constructive comments on an earlier draft. Funding was provided by the South African Avocado Growers' Association.

Funding

This work was supported by the South Africa Avocado Growers Association i.e. Growers [Contract].

Competing Interest

The authors declare no competing interest.

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Citation information

Cite this article as: Cold treatment of "Hass" avocado (*Persea americana* (Lauraceae)) infested by *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) as an additional component of a systems approach, Anthony Brian Ware & C. L. Neethling du Toit, *Cogent Food & Agriculture* (2018), 4: 1513314.

Key research activities

Agri-Biotech Research Consultancies conducts research, primarily entomological, for the fruit and vegetable industries. A major activity in this sphere is phytosanitary research on fruit fly and false codling moth for the avocado, pome and stone fruit and grape industries - an example is the research recorded in this paper. Research is also done on behalf of major chemical companies and includes efficacy testing, non-target effects, residue

testing and toxicity testing. Much of this research is directed towards the registration of agrochemicals.

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