Biology and Ecology of the False Codling Moth, *Thaumatotibia leucotreta* (Meyrick)

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Zoë Marthalise de Jager

Supervisor: Dr Pia Addison
Co-supervisor: Prof JS Terblanche
Department of Conservation Ecology and Entomology
Faculty of AgriSciences
University of Stellenbosch
South Africa

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ABSTRACT

Thaumatotibia leucotreta, the false codling moth (FCM), is a phytosanitary pest in South Africa posing a substantial threat to many of the country's international export markets. Its pest status is of high importance because it has a wide ecological range and has been reported in all areas where citrus is produced in South Africa. Many methods of control have been implemented, such as chemical and cultural control, mating disruption and sterile insect releases. There was a need to obtain a more accurate understanding of FCM biology on deciduous fruit in South Africa and this then us to pose the questions described in the chapters to follow.

The first aim was focused on the possibility of FCM diapause during winter. If FCM were to undergo diapause this could pose further problems for control methods, but knowledge thereof could also assist in more accurate and timely control methods. Considering past research on other Lepidoptera species, four physiological traits were chosen as indicative of a diapause state. Water loss rate, metabolic rate and the supercooling points should be lower if the individuals were in a diapause state, with a higher fat content expected for these individuals. Diapause induction was attempted through a gradual lowering of the environmental temperature in combination with longer nights to simulate overwintering conditions. Diapause was not observed in these experimental individuals.

The second aim was to better understand the field biology of FCM. This was studied through in-field flight ability studies and damage assessments on four fruit kinds. Six release dates were used to measure the flight ability. The highest recapture rates were at minimum temperatures above 16°C and maximum temperatures averaging above 30°C, although the recapture rates were not significant in relation to the amount released. The recapture rates in the different fruit kinds were not significantly different, with the amount recaptured at the closest distance of 30 m being significantly more than that of the other distances. This was also only for the last release at the warmest temperatures. Fruit damage assessments were conducted and we were able to rear wild FCM from Granny smith apples, Forelle pears, Larry Ann plums and Satsuma and Clementine citrus cultivars. Citrus infestations had the highest count and a prolonged occurrence compared to the other varieties, due to its later harvest period.

The third aim was to study the developmental parameters of FCM in different fruit kinds and an artificial medium. Firstly, FCM did not infest apples, Royal Gala and Pink lady's, under laboratory conditions. Results were obtained using Forelle pears, Clementines and Thompson seedless grapes. On average the grapes had the shortest FCM developmental time from egg to adult stage, followed by oranges and then pears. Pears had the lowest developmental success rate, with that of oranges and grapes being much higher. Infestations took place at the stalk end of the fruit for the grapes and oranges, with the pears being infested at the calyx end.

Future research should include an in-field life cycle, to determine the life cycle of FCM on different economically important fruit kinds under field conditions. The focus could also be shifted to where FCM overwinter, leading to better preventative control leading to lower infestation pressure during harvest periods. This is of utmost importance in an environment where maximum residue levels for pesticides dictate market access.

OPSOMMING

Thaumatotibia leucotreta, die vals kodling mot (VKM) is ‘n fitosanitere pes in Suid Afrika, wat kan lei tot groot finansiele verliese. Die VKM se wye gasheerreeks en die feit dat dit al in al die sitrus verbouings-areas in Suid Afrika opgelet is, maak dit ‘n ernstige pes. Daar word van verskeie beheer metodes gebruik gemaak, insluitend chemiese en kulturele metodes. In sommige areas word daar ook van paaringsontwrigting en steriele insek vrylatings gebruik gemaak en hierdie metodes word gewoonlik met ander gekombineer. Daar is ‘n groot behoefte vir meer inligting omtrent die status van VKM in sagtevrugte in Suid Afrika en het gelei tot die vrae wat in hierdie studie aangespreuk word.

Die eerste doelwit was om te bepaal of die VKM wel diapouse ondergaan. Dit sal verskeie beheermetodes belemmer, maar kennis hiervan kan meer gefokusde en gevolglik meer effektiewe beheermaatreels tot gevolg hê. Daar is gekyk na vier fisiologiese eienskappe wat beduidend tot diapouse van ander Lepidoptera spesies is. Daar word verwag dat VKM wat diapouse ondervind ‘n hoër vetinhoud sal he, terwyl die metabolise tempo, “supercooling’ punte en tempo van waterverlies laer sal wees. Hierdie eienskappe kon egter nie by die individue geidentifiseer word nie. Ons het diapouse probeer induseer deur gebruik te maak van ‘n gesimuleerde oorgang na winterstoestande in die laboratorium. Die toestande het toegelaat vir korter dae en laer gemiddelde temperature gedurende beide die dag en nag.

Die tweede doelwit waarna gekyk is, is die bepaling van VKM se beweging in die boorde en die vrugskade op verskillende vrugsoorte. Daar kon ‘n duidelike tendens geidentifiseer word in die toename van VKM hervangs by temperature bo ‘n minimum van 16°C en gemiddelde maksimum bo 30°C. Daar was 6 vrylatings periodes, met geen betekenisvolle getalle van hervangs nie. Daar was geen betekenisvolle verskille tussen die hervangsgetalle in die verskillende vrugsoorte nie, alhoewel die 30m lokval ‘n betekenisvol hoër gemiddelde hervangs gehad het, in vergelyking met lokvalle by 60m en 90m. Die hoeveelheid vrugskade is ook gemonitor op Granny smith appels, Forelle pere, Larry Ann pruime en Satsuma en Clementine sitrus kultivars. Die vrugte is na die laboratorium geneem waar die VKM tyd gegee is om uit te broei. Al die vrugsoorte het VKM volwassenes opgelewer, maar die eksperiment kon nie op appels in die laboratorium herhaal word tydens die toets van verkillende onwikkeling stadiums nie. Ons glo dus die VKM wat hier vanaf appels uitgebroei

het, is weens sekondere infeksies in die boorde. Die hoogste skadetelling is in die sitrusboord gevind.

Die derde doelwit was om die duur van onderskeie ontwikkeling stadiums te bepaal op vier vrugsoorte, sowel as op ‘n kunsmatige medium. Ons het ondervind dat die VKM nie Royal Gala of Pink lady kultivars kan infesteer onder laboratorium toestande nie. Die vrugsoorte wat dus ontwikkeling kon onderhou was Forelle pere, Clementines en Thompson pitlose druiwe. Die ontwikkeling vanaf eier na volwasse stadium was die kortste op druiwe, gevolg deur lemoene en pere. Die pere het die minste VKM onderhou in vergelyking met die lemoene en druiwe. Al die vrugte is binnegedring naby die aansluiting van die stingel aan die vrugte, behalwe die pere wat nader aan die kelk binnegedring is.

Toekomstige navorsing sal gefokus moet word op die lewenssiklus in die veld, vir die verskillende vrugsoorte. Daar sal ook gekyk moet word na die spesifieke alternatiewe gashere of plekke waar die VKM kan oorwinter sodat beheer meer voorkomend plaas kan vind. Dit sal infestasie vlakke onderdruk, om veral laer druk tydens oesperiodes te verseker. Dit is uiters belangrik om beheer stategieë te kombineer met die hoeveelheid druk vanaf uitvoermarkte oor maksimum residu vlakke van chemiese middels.

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Chapter 1: Literature Review

Taxonomy and classification of false codling moth, *Thaumatotibia leucotreta*

False codling moth (FCM), *Thaumatotibia leucotreta*, was first described by Fuller in 1901 in Natal, South Africa (Catling & Aschenborn 1974). It was named the 'Natal codling moth', falling under the genus *Carpocapsa* (Schwartz 1981), due to its resemblance to the cosmopolitan codling moth of pome fruit (Catling & Aschenborn 1978). In 1913, FCM was described by Meyrick as *Argyroploce* (Brown 2005), in 1958, it was transferred to the genus *Cryptophlebia* by Clarke (Newton 1998). Komai (1999) then placed it under a related genus, *Thaumatotibia*. However, Brown (2005) states that FCM was described as *Thaumatotibia* in 1915 by Zacher. The classification, as used, can be seen in Table 1.1 below.

Table 1.1. Classification of false codling moth (Stibick *et al*. 2010).

Phylum	Arthropoda
Class	Insecta
Order	Lepidoptera
Family	Tortricidae
Tribe	Grapholitini
Genus	*Thaumatotibia* (Meyrick)
Species	*leucotreta* (Meyrick)
Synonym	*Cryptophlebia leucotreta*
Common name	False codling moth

Biology and morphology of the false codling moth

The biology should be discussed by looking at the individual life stages, such as the eggs, the larvae, the pupae, and the adult phases, as well as the life cycle.

The FCM life cycle has no diapause stage, according to Reed (1974). The life cycle may take between 30–174 days to complete, dependent on the prevailing conditions. FCM can remain active throughout the year, if the correct host plant is present. In South Africa, it has been reported to have as many as 5 generations per year on citrus (Venette *et al*. 2003). There can be between 2–10 FCM generations per year, depending on certain external factors, such as temperature, photoperiod, moisture, predators or diseases, and food availability (Venette *et al*. 2003). Heavy rainfall has been found to decrease infestation levels significantly (Gunn 1921). The ratio between wild males and females is 1:2, with females also tending to live longer, on average (Daiber 1980).

Much of the research on the life table of FCM was conducted by CC Daiber between 1978 and 1987. Both laboratory and field trials were undertaken. The available data are best analysed when they are divided into the different life stages of egg, larvae, cocoon, and adult, and into the number of generations per year, as well as in terms of South African FCM specifically.

Egg stage

Biology and morphology

Fertilised females fly at night, depositing their eggs between 17h00 and 23h00. The eggs, which are laid at random over a long period of time, are laid in the depressions of the rind of fruit, on foliage, on fallen fruit, or on smooth, non-pubescent surfaces (Stibick *et al*. 2010). At the optimum temperature of 25°C, the female will lay between 3 and 8 eggs per fruit, while a FCM female can lay up to 800 eggs during her lifetime. If there is a heavy infestation, more than one female will lay her eggs on a fruit. Only a few of these eggs will survive, due to cannibalism (Stibick *et al*. 2010). Eggs take 2 to 22 days to develop, and are sensitive to temperature and humidity. As short a length of time as 2 days spent under freezing point will cause the eggs to die (Daiber 1979a).

It was found that egg hatching will stop at 10.6°C. Furthermore, Daiber (1979a) also found a high mortality rate at a temperature of 13°C or lower, and at a humidity level of 30%, compared to 60% and at 90% relative humidity. The egg stage lasted an average of 14.5, 9.8, and 5.1 days, at temperatures of 15, 20, and 25°C, respectively.

Egg stage life table

The trials conducted during the late 1970s indicated that egg development was closely related to the temperatures to which they were exposed (Table 1.2).

Table 1.2. Developmental patterns of the FCM eggs under variable temperature and humidity conditions (Daiber 1979a).

Temperature	Relative humidity	Developmental time
10	95±5%	-
15	70±10%	14.5
20	60±10%	9.8
25	55±10%	5.1

Daiber further found that the RH also played a major role, with the same temperature (13°C) having a higher mortality rate at 30% RH, compared to at 60% and at 90% RH, respectively (Daiber 1979a). The trials were conducted at a time when FCM was primarily a pest of citrus, and when it had been identified on peaches only in certain areas.

Larval stage

Biology and morphology

Newly hatched larvae enter the fruit through the rind, leaving behind burrows of approximately 1mm in diameter. The site at which they entered becomes obvious due to the frass (fine powdery material) on the surface, and due to the discolouration of the rind (Stibick *et al*. 2010), which is caused by the larva, as seen in Fig. 1.1 below, borrowing into the fruit. The larval developmental period lasts 12 to 33 days in warm conditions, and 35 to 67 days in cool conditions (Daiber 1979b).

Fig. 1.1. Larval instar of the false codling moth.

It is important to note that fruit quality might also affect the length of the developmental period (Stibick *et al*. 2010). The larvae go through five instars. The young larvae feed near the surface of fruit, whereas the mature larvae feed more to the centre of the host (Stibick *et al*. 2010). Generally only one larvae will survive per fruit, but a maximum of up to 3 larvae per fruit have been recorded (Stibick *et al*. 2010). By the time that the larva reaches maturity, the fruit might have fallen to the ground. If the fruit is still intact and on the branch, the larvae use a silken thread to drop to the ground (Stibick *et al*. 2010).

The head capsule width is used as a unit of measure for each larval instar. The head capsule is yellowish brown, with dark pigmentation at the ocellar and postgenal area (Timm *et al.* 2007), which can be seen in Fig. 1.2 below. The first instar larva is generally about 1 mm in length. Daiber (1979b) measured 80 individuals, and found the first instar to have an average head capsule width of 0.21 mm, with each instar showing allometric growth in the head capsule width (see Table 1.3).

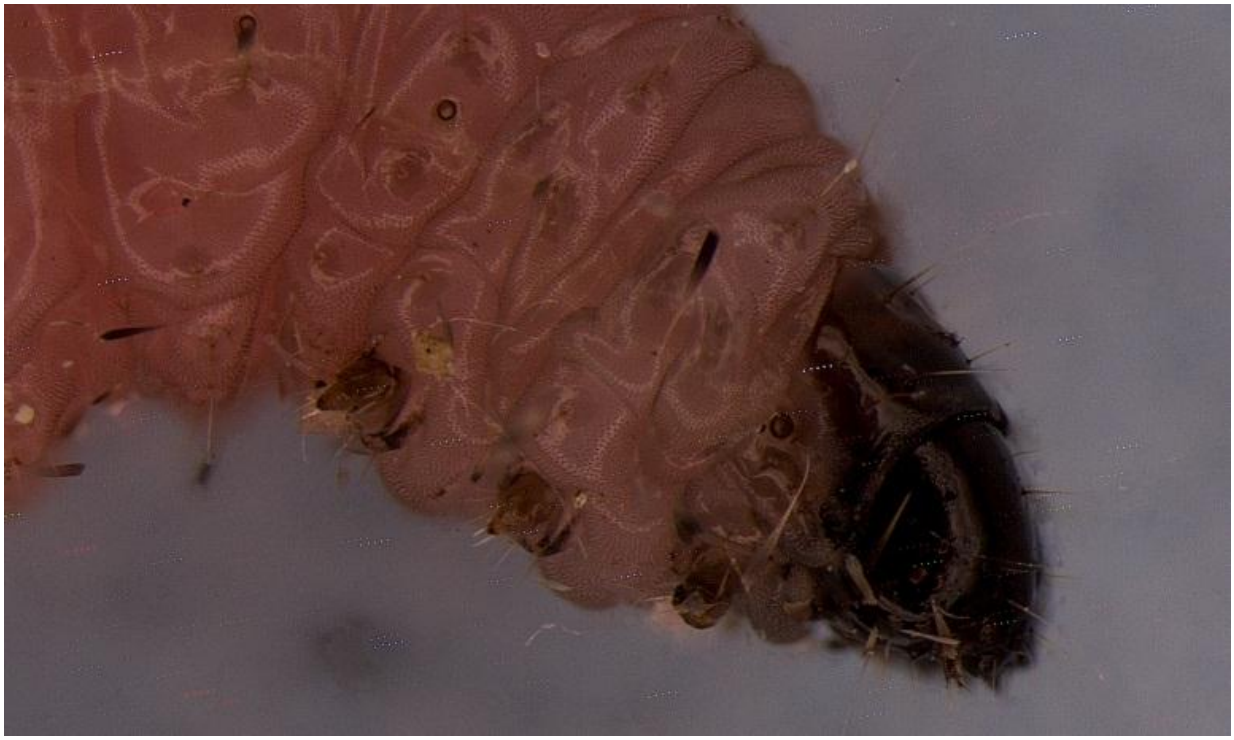

Fig. 1.2. Distinct dark head capsule of false codling moth larva instar.

Table 1.3. The width of the head capsule of FCM larval instars, and the proportional increase from instar to instar (Daiber 1979b).

Larval instar	Width in mm		
	Average	Variation of 95%	Proportional increase
1	0.21	0.17–0.25	
2	0.37	0.32–0.43	1.75
3	0.61	0.50–0.72	1.63
4	0.94	0.82–1.07	1.55
5	1.37	1.25–1.49	1.45

Daiber (1979b) also found that the development tempo of the larvae correlated with temperature, although they were not retarded by temperatures in excess of 20°C. Development will be influenced by lower temperatures, for instance when larvae enter a chill coma at temperatures between 3°C and 7°C (Boardman *et al.*, 2011). The upper lethal temperature (resulting in 50% mortality) has been found to be 38 to 45^{0}C for 2 to 2.5h (Johnson & Neven 2010). Daiber also found a correlation between food quality and the duration of the larval stage, with poor food quality having a negative effect on development (Daiber 1979b).

Larval stage life table

Trials to establish the developmental pattern, and the duration, of the FCM larval stage were also conducted by Daiber in the late 1970s. Observing five larval stages, he also concluded a correlation between the developmental times and the observed temperature. The results of this trial can be seen in Table 1.4 below, with the developmental time declining significantly as the temperature was increased. The research showed that the developmental stages were shorter during the first four instars and the last instar before the prepupal phase was found to be significantly longer. Daiber further observed that the developmental times of the larvae

were very much dependant on the quality of the food available. Daiber (1979b) concluded that the last six weeks before harvest were the most suitable for heavy infestations.

Table 1.4. Developmental patterns of the FCM larvae, as derived by Daiber (1979b).

Temperature	Developmental time
	Total
15	46.6
20	18.8
25	11.6

Cocoon and pupal stage

Stage biology and morphology

The fifth instar larva of FCM forms a cocoon from soil particles and silky body substances, as can be seen in Fig. 1.3 below. At this stage it goes through the prepupal phase, later moulting into a pupa. The pupae were found to have a sex ratio of 1:1 between males and females (Daiber 1979c), with the ratio concerned being found to be independent of the ambient temperatures to which the cocoons were exposed. Daiber (1979c) found that the duration of the cocoon stage was closely inversely correlated to the ambient temperatures, when it was measured at 15, 20, and 25°C. If the cocoons where exposed to low relative humidities, or to frequently irrigated soils, the number of adults emerging was significantly decreased. Daiber (1979c) also found that a higher mortality rate occurred during the cocoon stage, at a temperature of 10.5°C or less (Daiber 1979c).

The new cocoon is covered by sand. The prepupae may form a new cocoon at the soil surface. Prepupae form an inactive stage that lasts between 2 and 27 days (Daiber 1979c).

The males take between 13 and 49 days to emerge, while the females take between 11 and 39 days to do so (Daiber 1979c). Pupation may occur on the soil surface, in the soil, on fallen fruit, or in debris. The pupae first start to emerge from the cocoon, before the adult emerges (see Fig. 1.4 below).

Fig. 1.3. False codling moth pupa, before adult emergence.

Fig. 1.4. False codling moth exuvia, after adult emergence.

Cocoon life table

Daiber (1979c) found the cocoon stage to be the most sensitive phase in the developmental cycle of FCM. The development can be influenced by RH, temperature, soil cover, and rainfall.

Table 1.5. Developmental patterns of FCM cocoon (Daiber 1979c).

Item	15°C		20°C		25°C	
First prepupae observed (days)	60		28		14	
Last pupae observed (days)	160		62		34	
Period during which cocoons were observed (days)	100		34		20	
Average duration of cocoon stage (days)	♀	♂	♀	♂	♀	♂
	50.7	55.8	22.3	23.8	12.9	13.9

As can be seen in Table 1.5 above, Daiber found a strong inverse relationship to exist between the developmental time, at average temperatures of 15°C, 20°C, and 25°C, respectively. He also noted higher mortality rates at temperatures of 10.5°C and lower for both the prepupal and pupal phases. The same pattern was noted when the prepupae and pupae were exposed to low humidity levels, or to frequent irrigation intervals. The sex ratio of the pupae was observed as being 1:1 between the males and the females, and this relationship was described as being independent of temperature (Daiber 1979c). This relationship was contrary to the sex ratio of 1:2 that Daiber described for wild males and females (Daiber 1980). The ratio might differ with bigger sample sizes.

The data clearly indicated that the intensity and the frequency of rainfall will have a significant influence on the FCM population size, resulting in the severe reduction of emergence in heavily soaked soils (Daiber 1979c).

Adult stage

Adult emergence

FCM adults fly during the night and rest in the shade during the day. The females live longer than do the males, with the former living 16 to 70 days, compared to the 14 to 57 days lived by the latter. The adults can disperse over several hundred meters, with the numbers being controlled by the temperature, and by the availability of hosts (Stibick *et al*. 2010). The newly emerged FCM adult can be seen in figures 1.5a and 1.5b below.

Fig. 1.5a. Newly emerged false codling moth adult (top view).

Fig. 1.5b. Newly emerged false codling moth adult (side view).

Females attract the males after dark by means of pheromones. The pheromone release peaks 5 hours after dark, and decreases until sunrise, as reported by Bestmann *et al*. (1988 in Stibick *et al*. 2010). The sequence of the complete life cycle can be seen in Fig. 1.6 below.

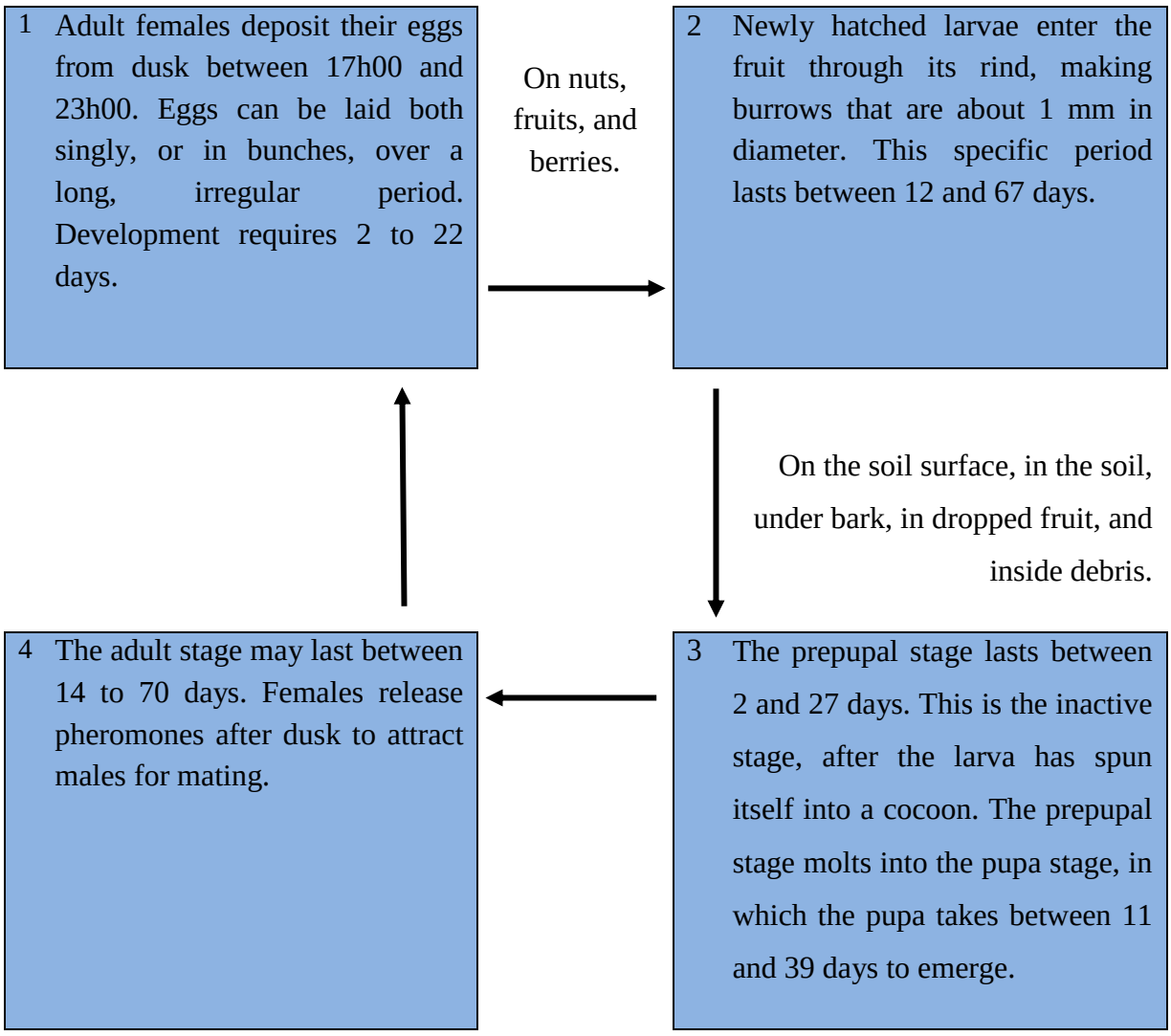

Fig. 1.6. FCM life cycle (derived from Stibick *et al*. 2010).

Adult life table

FCM life span and egg laying was observed at constant temperatures of 10°C, 15°C, 20°C, and 25°C in laboratory conditions (Daiber 1980). Daiber found the average lifespan of FCM to be longest at 20°C, whereas most of the eggs were laid at a temperature of 25°C. Very few eggs were laid at 15°C. It was found that the life cycle is the longest at lower temperatures, with up to six generations per annum being induced on an artificial medium (Daiber 1980).

Geographic distribution of false codling moth

The Western Cape is outside the natural distribution range of FCM. FCM was introduced into the Western Cape, where it was first observed in the Citrusdal area in 1974 (Honiball 2004). It has previously been stated that FCM is present in all citrus-producing areas in South Africa, although it seems to be moving to stone fruit agricultural areas, where up to 28% of late peach cultivar damage occurs as a result of FCM (Stibick 2006).

FCM is endemic to Southern Africa, particularly in the tropical and subtropical areas (Schwartz 1981). Further research should be conducted to determine its pest status in the Western Cape, with areas such as Citrusdal, De Doorns and Riebeeck Kasteel reporting FCM damage.

Host plants in the Western Cape for false codling moth

FCM has been known to attack numerous host species. The following lists are of recorded cultivated and natural host species, according to Catling and Aschenborn (1974), Gunn (1921), Newton & Crause (1990), Schwartz (1981), and Stotter (2009).

Table 1.6. Cultivated hosts of false codling moth, *Thaumatotibia leucotreta.*

Cultivated hosts		
Family	**Common Name**	***Genus & Species***
Annonaceae	Custard apple	*Annona cherimoya*
Cactaceae	Prickly pear	*Opuntia ficus-indica*
Ebenaceae	Persimmon	*Diospyros virginiana*
Fabaceae	Bean	*Phaseolus* spp.
Fabaceae	Common oak	*Quercus robur*
Lythraceae	Pomegranate	*Punica granatum*
Malvaceae	Cotton	*Gossypium hirsutum*
Malvaceae	Okra	*Abelmoschus esculentus*
Myrtaceae	Guava	*Psidium guajava*
Oleaceae	Olive	*Olea europeae*
Poaceae	Maize	*Zea mays*
Poaceae	Sorghum	*Sorghum halepense*
Rosaceae	Apricot	*Prunophora armeniaca*
Rosaceae	Nectarine	*Prunus persica* variety *nectarina*
Rosaceae	Peach	*Amygdalus persica*
Rosaceae	Pear	*Pyrus* spp.
Rosaceae	Plum	*Prunophora domestica*
Rutaceae	Mandarin	*Citrus reticulata*
Rutaceae	Orange	*Citrus sinensis*
Rutaceae	Tangelo	*Citrus reticulata* (Hybrid)
Rutaceae	Tangerine	*Citrus reticulata*
Sapindaceae	Litchi	*Litchi chinensis*
Solanaceae	Peppers	*Capsicum* spp.
Theaceae	Tea	*Camellia sinensis*
Vitaceae	Grape	*Vitis* spp.

Table 1.7. Wild hosts of false codling moth, *Thaumatotibia leucotreta.*

Wild host plants		
Family	**Common name**	**Genus & species**
Anacardiaceae	Marula	*Sclerocarya caffra*
Anacardiaceae	Wild plum	*Harpephyllum caffrum*
Annonaceae	Wild custard apple	*Annona senegalensis*
Asparagaceae	*Alubuca* sp.	*Alubuca* sp.
Asparagaceae	*Asparagus crassicladus*	*Asparagus crassicladus*
Combretaceae	Red bush willow	*Combretum apiculatum*
Crassulaceae	Jade plant	*Crassula ovate*
Ebenaceae	African ebony	*Diospyros mespiliformis*
Ebenaceae	Jakkalsbessie	*Diospyros lycioides*
Euphorbiaceae	Castor oil plant	*Ricinus communis*
Euphorbiaceae	Kudu berry	*Pseudolachnostylis maprouneifolia*
Fabaceae	African walnut	*Schotia beachypetala*
Fabaceae	Karoo boer-bean	*Schotia afra*
Fabaceae	Port Jackson willow	*Acacia saligna*
Moraceae	Wild fig	*Ficus capensis*
Myrtaceae	Waterbessie	*Syzygium cordatum*
Oleaceae	Red sour plum	*Ximenia caffra*
Oleaceae	Wild olive	*Olea europea* subsp. *Africana*
Passifloraceae	Passion flower	*Passiflora* sp.
Podocarpaceae	Real yellowwood	*Podocarpus latifolius*
Rhamnaceae	Buffalo thorn	*Ziziphus mucronata*
Salicaceae	Kei apple	*Dovyalis caffra*
Sapotaceae	Red milkweed	*Mumisops zeyheri*
Solanaceae	Snake apple	*Solanum tomentosum*

Pest status and economic significance of false codling moth

The pest status of FCM has previously been described as being dependent on a combination of such factors as: ecological suitability; host suitability/availability; survey methodology; taxonomic recognition; entry potential into a country; the destination of already infested material; and the potential economic impact that goes hand in hand with establishment potential (Venette *et al*. 2003).

Infestation by FCM can result in a major fruit drop from December to April. In very extreme cases, up to 80% of the fruit can be destroyed by this pest (Hofmeyr 1998), where, in the past, the percentage of FCM destruction was reported as being only 20% (Newton, Anderson & Verceil 1986). FCM also results in significant yield losses (≥30%) of macadamia crops in both South Africa and Israel (La Croix & Thindwa 1986). FCM is described as being present in all areas where citrus is produced in South Africa (Schwartz 1981).

The fact that FCM may be confused on a taxonomic level was found to be a less important factor than the damage that FCM causes. FCM may, for instance, be confused with *Cydia pomonella* (codling moth) on a visual, as well as on a damage symptom basis. Various comparisons have been drawn between its life stages and habitats and those of codling moth, although their host ranges differ significantly (Gunn 1921). C*ydia pomonella* is a major pest, which is more specific to apples and pears (Addison 2005). Both pests may be present on such crops as macadamias and litchis (Newton & Crause 1990), but certain morphological characters, as described by Timm *et al.* (2007) can be relied on. A more important factor is the survey methodology used, as dissection of the fruit is necessary to identify the larvae that are near the pulp of the fruit, while a survey is being undertaken for eggs and adults.

Factors that prove to be of high importance for FCM, a phytosanitary pest, is its ecological suitability and its host specificity.

Damage done to fruit

Penetration by larvae can only be detected at an early stage through the careful inspection of the fruit. The colour of the young green peel eventually assumes a yellow colour, where penetration took place. Penetration marks on ripe fruit appear decayed, with the orange peel becoming sunken and brown (Hofmeyr 1998).

The penetration hole is enlarged as the mature larvae attempt to pupate and to leave the fruit. Frass will then be found on the damaged surface. Penetrated fruit take up to three to five weeks before they fall from the tree, while newly penetrated fruit pose a serious threat in the form of post-harvest decay, with the damage not easily being detected. Damage done to the fruit increases its vulnerability to scavengers and fungal infections (Hofmeyr 1998).

Temperature tolerance of false codling moth

The research that was conducted on thermal tolerance in FCM was aimed at its significance in terms of pest population levels. Tests were also conducted to test the rapidity of cold hardening, where limited evidence was found. The testing of the temperature, including the duration of the exposed temperature, revealed a significant effect on the adult FCM. A survival rate of 50% was found at 2 h of exposure to -4.5°C, while 10 h exposure to -0.5°C also revealed a survival rate of 50%. The adult's age and its gender had no significant effect on the low temperature tolerance (Stotter & Terblanche 2009). Fasting, humidity and inoculative freezing (through direct contact with water), can influence the supercooling temperatures of FCM larvae (Boardman *et al*., 2011). This is important in post-harvest pest control, where temperature regulation is a tool commonly used for pest sterilization on exported crops.

Diapause in Lepidoptera

Diapause in Lepidoptera can be compared to hibernation in mammals, in that it is a manner of reserving energy through the minimisation of water loss, and a drop in the metabolic rate. During winter, the low temperature and the unsuitable weather conditions may result in

limited food resources being available (Denlinger 1986), as well as environmental stresses. Diapause is, therefore, an adaptation to conditions that are incompatible with active development (Denlinger 2000). Such adaptation is ideal, as insects in Southern Africa, are still not always able to development and reproduction during the colder part of the year, although little compared to northern hemisphere countries. Development can, however, be resumed as soon as the favourable conditions return (Denlinger 2008).

Diapause is often restricted to a specific developmental stage. Some Lepidoptera undergo diapause due to genetic programming, regardless of the prevailing environmental conditions, although such a state is mostly present in insects that undergo one generation per year. Obligatory diapause can be affected neither by environmental cues, nor by length of day and temperature variations (Denlinger 2000).

Facultative diapause is not predetermined, and may be determined by certain environmental cues, such as by temperature and by variations in the length of day. The use of such diapause leads to a more flexible life cycle. The diapause state is often initiated in the early developmental phases, known as the photosensitive phase. The common cues that are observed by these insects ensure that their diapause, and subsequent emergence therefrom, are synchronised, which is of importance for adults seeking mates. The earlier initiation of diapause allows for the accumulation of additional reserves to ensure successful diapause (Denlinger 2000).

The three major phases of diapause, and their subphases, are indicated in Table 1.8 below. The whole process of diapause is very dynamic, and the numerous successive phases create great variation in physiological adaptations (Kostal 2006).

Table 1.8. The phases of diapause subdivided into three (Kostal 2006).

Pre-diapause	Diapause	Post-diapause
1. Induction Environmental cues are transduced for the token stimuli to reach critical phase. This sensitive period allows for a switch to take place in the ontogenetic pathways.	1. Initiation Development ceases, and metabolic suppression follows. Physiological preparations for diapause are initiated, and the intensity of diapause may increase.	1. Quiescence Following the termination of diapause, the development remains inhibited by unfavourable conditions.
2. Preparation When the induction and the initiation phase of diapause are separated by a developmental phase, it allows for the insect to be programmed for the later expression of diapause. This allows for both behavioural and physiological preparations for diapause to take place.	2. Maintenance The developmental arrest will continue, although conditions are favourable for direct development. Specific token stimuli may prevent the termination of diapause. The intensity of diapause may gradually decrease, while increased sensitivity to its termination will follow.	
	3. Termination Diapause intensity is decreased to a level where the potential for development is restored, and the individuals within a population are synchronised.	

Regulation of diapause

Environmental regulation

The photoperiod is the most precise measure of seasonal changes in nature. The insects relying on facultative diapause often use shortened day length as a cue for the initiation of diapause. The critical photoperiod is the point at which the day length is shortened enough to cause the switch between a non-diapause and a diapause state. The insect is unable to measure the actual shortening of the day, but instead interprets it as being either long or short (Denlinger 2000).

If the photoperiod were to be regarded as being the primary cue for diapause initiation, temperature could be incorporated as a factor influencing critical photoperiod. Temperature is often able to influence the incidence of diapause under already present diapause-inducing day lengths (Denlinger 2000).

Saunders (1971) proposes a model in which the insect requires both a measure of day length (short vs. long), and a measure of the number of short days experienced. The more regulated the individuals' diapause initiation is to become, the more synchronised this phase will be within a population (Denlinger 2000).

Food sources may also be a cue to initiate diapause. Protein, carbohydrates and water content may be the only cue in areas near to the equator, where the seasonal indicators are inadequate (Denlinger 1986), with this mostly being within 5° of the equator (Denlinger 2000).

Hormonal regulation

Diapause can be initiated either by the presence, or by the absence, of certain hormones. The most important hormonal groups influencing the regulation of diapause are directly involved in insect development. The two most important groups are juvenile hormones (JH) and ecdysteroids.

Management of FCM

FCM is currently being suppressed by a combination of cultural, chemical, biological, and microbial control methods. Biocontrol makes use of the egg parasitoid *Trichogrammatoidea cryptophlebiae* Nagaraja. The egg parasitoid should be released numerous times, while the fruit are susceptible to FCM, ie. when fruit is ripe. Up to 125 000 parasitoids are necessary per ha in the Western Cape (Hofmeyr 1998). *Trichogrammatoidea cryptophlebiae* has been considered as the most important parasitoid for FCM since 1974 by Catling & Aschenborn (1978), who also state that up to 90% egg parasitism was found in field trials (Van den Berg *et al.* 1987).

Chemical control

In the 1950s, Hepburn & Bishop (1954) reported that pyrethroids could decrease FCM infestations by 66 to 75%. They also found that infestation levels of less than 5% would be uneconomical to treat using chemicals. Chitin synthesis inhibitors is a group of chemical control products that disrupts the embryonic development of the larvae in the eggs. Good product coverage is important, as it will only be effective if the eggs are laid on the residue. A typical treatment can provide light protection (Hofmeyr 1998).

Chemical control has also been compromised in the Western Cape, due to resistance reported in this regard (Hofmeyr & Pringle 1998; Carpenter, Bloem & Hofmeyr 2007). Rainfall means that the chemical has to be reapplied, which has serious economic implications (Hepburn & Bishop 1954).

Monitoring

FCM eggs are transparent and very small, complicating the inspection of fruit. The Lorelei monitoring system, which may be used to determine whether a spray is necessary, has proven to be very effective (Hofmeyr 1998). Using a pheromone trap is an effective long-term monitoring system for FCM.

Traps should be checked weekly, from November until harvest, with the treatment threshold for FCM being 10 catches per trap per week (Grout *et al.* 1998). Such a treatment threshold would economically justify chemical control (Hofmeyr 1998).

Cultural control

Cultural control suggestions are based on the use of manual labour practices to combat infestation levels, mostly in addition to using other methods as part of an effective IPM programme. The surroundings should be cleared of all native host species. Heavy irrigation should be undertaken to kill the pupae in the soil, or cultivation should be done so as to destroy hibernating insects. Infested fruit should be destroyed. The fruit on the ground should be picked up, with those that are still on the tree being picked and removed as well (Hofmeyr 1998). It should be standard weekly practice to pick up the fruit that has dropped to the ground. The success of such control practices is dependent on whether they are adopted on an area-wide basis, as the overall infestation levels require suppression (Hepburn & Bishop 1954).

'Attract and kill' and mating disruptants (MD)

'Attract and kill' and MD are two supplementary treatments that are used for suppressing FCM population levels using pheromones. The former treatment lures and kills the males, thus resulting in reduced mating, and, consequently, in reduced population levels. As this method is not as effective as other mating disruption products are, it is only recommended under light infestation levels (Stotter 2009). MD confuses or repels males, causing a reduced population level, due to less mating taking place than might otherwise occur (Hofmeyr 1998). Using high-dose pheromone point sources tends to confuse wild male FCM, preventing them from finding females for mating (Carde & Minks 1995), which also makes pheromone-baited traps ineffective and more difficult to interpret (Stotter 2009).

Sterile insect release

Sterile insect technique (SIT) is being incorporated into the IPM programme. Preliminary studies have shown a 94.4% reduction in fruit drop in navel orange orchards in Citrusdal (Stotter 2009). SIT relies on an over-flooding effect, with the ratio of released males: wild males being 10:1 for SIT to be effective (Hofmeyr & Hofmeyr 2004).

SIT is an environmentally-friendly and host-specific control measure that is compatible with biocontrol. To combine SIT with biocontrol, a thorough knowledge of biocontrol agents is required. The parasitoid may not adversely affect the sterile insect in its effectivity, just as the

sterile insect may not interfere with the effectivity of the biocontrol agent. SIT can easily be integrated into area-wide integrated pest management programmes. Using such control tactics, together with improved infrastructure, will ensure that FCM can be adequately eradicated from a geographical area (Carpenter *et al.* 2007). The efficiency of FCM rearing has been much improved by increasing the efficiency of mass rearing techniques, thereby increasing the durability of the rearing techniques, and protecting the workers involved from the effect of moth scales, which can cause severe allergies (Du Toit & Schwartz 1990).

Dose selection is very important in order to ensure that current SIT programmes are effective. This is mainly because it is practically impossible to separate the insects by gender on a large scale, hence requiring that both males and females be irradiated (Bloem & Bloem 2000). While it is important that the released females are sterilised, it is just as important that the sterilising radiation be kept as low as possible, in order to maintain the mating competitiveness. As female Lepidoptera are sterilised at a lower radiation dose than are the males, it is more practical for females to be treated to ensure 100% sterility, while the males produce a limited number of sterile F1 progeny (Bloem *et al.* 2003).

A gamma radiation dose of 150–200 Gy is necessary to assure 100% sterility (Bloem *et al.* 2003). Carpenter *et al.* (2004) tested the compatibility of FCM SIT with release of *T. cryptophlebiae* parasitoids. They found that *T. cryptophlebiae* would successfully develop, and emerge from, the possible crosses made during a SIT programme. It was further established that *T. cryptophlebiae* prefer non-irradiated FCM as a host. It should be more economical to rely on the synergism of a combined treatment of SIT and parasitoids. During trials it was confirmed that FCM treated with 200 and 150 Gy underwent a reduction of 32% and 25% in parasitism, respectively (Carpenter *et al.* 2004).

Aim

The aim of this study was to assess basic biological parameters of FCM on deciduous fruit in South Africa, which are needed to refine and improve management methods, in particular the timing of chemical control and more effective application of SIT.

Objectives

- Attempt to initiate diapause, if present in FCM. This would allow a better understanding of the overwintering period that can allow for more focused control measures.
- Study in-field flight ability of FCM males and the coverage of the movement between orchards, and subsequent fruit damage. This would clarify whether insects are able to move out of an area fast enough to avoid density-dependant restrictions.
- Examine developmental parameters of FCM on different fruit kinds. Calculate intrinsic rate of natural increase on artificial diet. This would allow for a better insight into FCM's ability to survive and reproduce on specific alternate hosts.References

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Chapter 2: An experimental test of diapause induction in False Codling Moth, *Thaumatotibia leucotreta* (Meyrick), (Lepidoptera: Tortricidae)

Introduction

Insects inhabiting variable environments possess several strategies for coping with periods of low temperature or limited resource availability (Lee 2010). One such physiological adaptation is diapause, or the ability to undergo metabolic arrest or reproductive dormancy, and is characteristic of a range of pest insects, especially in the Lepidoptera (e.g. Noctuidae (Phillips & Newsom 1966; Chen *et al*. 2013), Pyralidae (Yao & Fukaya 1974) and Tortricidae (Sieber & Benz 1977; Lyon *et al*. 1972)). In Lepidoptera, diapause is generally induced by shortening day length and decreasing ambient temperatures, or possibly increasing variability in temperatures, likely reflecting a cue for seasonal shifts in abiotic conditions (Danks 2002; Denlinger 2002; Tauber & Tauber 1976; Bradshaw & Holzapfel 2010). Understanding diapause induction and termination responses is particularly significant in a population dynamics context as the ability to enter diapause allows some species to survive for several years and have complex life-cycles (Denlinger 2008). Moreover, diapause is typically accompanied by a marked improvement in low temperature tolerance (Storey and Storey 1991; e.g. Andreadis et al. 2005; Khani & Moharramipour 2010), thereby potentially allowing a pest species to survive what would have otherwise been a lethal post-harvest cold sterilisation for the non-diapausing individual (Bell 1994).

One major pest of economic concern in southern Africa is the false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick), which infests mainly citrus and some deciduous fruits in this region, but also is a pest of cotton, macadamia nuts and maize in other parts of Africa (e.g. Reed 1974). FCM is multivoltine and polyphagous, with cryptic life-stages and a typical lepidopteran life-cycle in several respects, requiring approximately 800 degree-days to complete development (Venette et al. 2003). FCM is indigenous to southern Africa, the

Ethiopian region, and many islands on the African continent (Stofberg 1954; Catling & Aschenborn 1974; CIBS 1984)

According to Reed (1974), 'no diapause or resting stage has been recorded previously'. The taxonomic identification of FCM is however particularly problematic, and molecular markers combined with morphological features are required to suitably distinguish larvae, especially the earlier instars, from other closely-related tortricid pests in the region (e.g. codling moth, *Cydia pomonella*) (Timm *et al*. 2008).

Apart from an early field assessment (Reed 1974) which therefore may have been compromised by taxonomic uncertainty surrounding cryptic developing life-stages, no studies have investigated diapause induction within an experimental framework for FCM, and none to date have used a suite of physiological traits potentially indicative of the diapause state. Here we therefore examined, in an experimental physiology approach, the potential for diapause induction using larvae that were subjected to diapausing-inducing conditions that were broadly similar to those used for diapause induction in other tortricids (Gangavalli & Aliniazee 1985; Bell 1994), and measured a range of physiological responses that could be potentially indicative of entry into the diapause state. Physiological traits that were determined included i) resting metabolic rate with the expectation of a reduction in metabolic rate upon entry into diapause (Papanastasiou *et al*. 2011; Denlinger 1986), ii) the supercooling point (SCP), which is the freezing temperature of body fluids and in the case of FCM is equivalent to the low temperature mortality threshold since the species is classified as chill susceptible and dies upon freezing (Boardman et al. 2012). We also determined iii) body size and condition with the expectation that FCM larvae preparing for diapause would sequester body lipid reserves and body water content (Wipking *et al.* 1994), and show iv) lower water loss rates compared to non-diapausing individuals (e.g. Yoder *et al*. 1994).

Material and Methods

Early instar larvae were obtained from XSIT rearing facility in Citrusdal, Western Cape, South Africa where they are mass-reared under controlled conditions and the culture is regularly supplemented with wild-collected individuals from the local region to ensure

genetic homogeneity with wild-type FCM (for further details see Stotter & Terblanche 2009; Carpenter et al. 2007). For each physiological trait a new cohort of individuals was obtained and reared through the treatment and control conditions. The larvae were divided approximately evenly between the two treatment groups (Control [CON] and Diapause Treatment [DT]), *n* = approximately 800 larvae in each container. The larvae were fed an artificial diet provided by XSIT (Citrusdal, Western Cape). Larvae were then acclimated for at least twelve days in climate chambers prior to starting the diapause induction treatments (YIH DER growth chamber, model LE-539, SCILAB instrument CO Ltd., Taiwan). In all treatments, the temperature and humidity were verified with temperature/relative humidity Thermochron iButtons (0.5°C accuracy; Hygrochron DS1923-F5, accuracy ±0.6%, Maxim/Dallas Semiconductor, Sunnyvale, CA, USA).

Diapause Treatment

In order to induce diapause, individuals in the diapause treatment (DT) group were exposed to conditions of gradually varying daily fluctuations in temperature and photoperiod in an environment chamber. The DT was designed to simulate conditions of autumn and the entry into winter in locations where FCM are known to occur (see e.g. Stotter & Terblanche 2009). The temperature regime fluctuated between 28°C during the day and 16°C at night (mean temperature of 22.98°C) and decreased over four days systematically to a maximum of 16°C during the day and 4°C at night (mean of 11.136°C). The minimum and maximum daily temperatures were then kept constant at these temperatures and mean conditions for the remaining 3 days. Photoperiod changed at the same time as temperature and in a similar manner. Initially, the light cycle was 12:12 [L:D] and daylight decreased at constant hourly intervals daily to reach 8:16 [L:D] after four days. This was then kept constant for the following three days. The control group from the same cohort of larvae was exposed to treatment temperatures that were kept constant at the initial temperatures and daylength (mean temperature of 22.79°C; 12:12 [L:D]), as described for the DT experimental group. The trial for both DT and CON continued for fourteen days where upon larvae were assayed for physiological traits.

Mansingh (1971) proposed that the classification of diapause be based on the evolution of biochemical, physiological and phenological adjustments due to ecological adversity (Bell

1994). Therefore a series of physiological tests, listed in Table 2.1, were completed to establish whether these adjustments were made and diapause is present.

Table 2.1. The expected reaction to four physiological tests for false codling moth larvae that are exhibiting diapause traits, compared to the control group of laboratory reared larvae.

Traits	**If diapause**
Water loss rate	↓
Fat content	↑
Metabolic rate	↓
Supercooling points	↓

Physiological assays

Supercooling points

Sixteen larvae from the control and treatment groups (total n = 32) were used to determine the supercooling (i.e. freezing) point. A programmable circulating and refrigeration bath filled with ethanol (CC410wl, Huber, Berching, Germany) was programmed to maintain 15°C for 30 min before decreasing the temperature from 15°C to -15°C at a rate of 0.25°C/min. Larval body temperatures were recorded using thermocouples (T-type, 36 standard wire gauge, Omega Engineering, Inc.) and a thermocouple datalogger (USB TC-08, Pico Technology, Cambridgeshire, UK; see Boardman *et al*., 2012 for additional details).

Water loss rate

Twenty one, fifth instar, larvae from both the treatment and control groups (total n = 42) were used to determine water loss rate (WLR) under either low <5% or high >94% relative humidity at average 25.4°C. Larvae were weighed using a Mettler Todelo Analytical Ax504 balance to 1 mg (Mettler Toledo Products, Greffensee, Switzerland) and placed in ventilated

5ml microtubes. Control and DT larvae were randomly distributed between relative humidity experiments. One group were placed in a container filled with distilled water (average 94.2% RH), while the second group's container was filled with silica gel averaged 3.3% RH). The lid of each container was fitted with a temperature/relative humidity iButton to ensure desired conditions were achieved. The experiment continued for 4 days after which each larva was weighed and WLR was calculated as the amount of mass loss divided by exposure time.

Body composition

After WLR estimation, larvae (n = 42) from the WLR experiment were dried at 60°C for 24 h in order to determine dry body mass, before total lipids were extracted using chloroform:methanol (1:1 v/v) solution washed three times (once per day) and baked dry, and lipid-free mass was estimated following previously established methods (see Boardman *et al*. 2013 for details). Body water content (BWC) was calculated as the difference between dry mass and the mass at the end of the WLR experiment, while body lipid content (BLC) is assumed to be the difference between the dry mass and lipid-free dry content (Naidu and Hattingh 1988).

Metabolic rate

Multiplexed respirometry was used to determine the metabolic rate of CON and DT larvae following the method outlined in Basson and Terblanche 2010 (and see further details in e.g. Boardman *et al*., 2013). Air from an aquarium pump was passed through scrubber columns containing soda lime and 50:50 silica gel:Drierite (WA Hammond Drierite Company Ltd., Ohio, USA) to remove CO_2 and H_2O. A mass flow control valve (Sidetrak, Sierra International, USA), connected to a mass flow control box (Sable Systems, Las Vagas, Nevada, USA), was used to maintain a 200 ml/min (at standard temperature pressure dry= STPD) flow rate. Data were recorded using a Li-7000 infra-red gas analyser and LiCor software (LiCor, Lincoln, Nebraska, USA). Activity was monitored electronically in one individual per run (AD-2, Sable Systems). Each multiplexed respirometry run included six individuals (3 CON larvae and 3 DT larvae). Larvae were weighed before and after

respirometry on a microbalance (accuracy ± 0.1 mg; AB104-S/Fact, Mettler Toledo International, Inc.) and placed in individual custom-made cuvettes maintained at 25°C using the programmable bath. Larvae were randomly assigned to a cuvette. After a 60 min baseline recording of an empty cuvette, the VCO_2 from larvae in each cuvette was measured for 40 min, before a final baseline was recorded in order to allow correction for potential analyser drift.

ExpeData (Version 1.1.25 software, Sable Systems) was used to baseline-correct respirometry data to account for analyser drift and transform data: $\dot{V}CO_2$ recorded in ppm was transformed to µl/h and $\dot{V}H_2O$ recorded in ppth was transformed to mg/h. Resting metabolic rate (RMR) was extracted (Terblanche *et al*. 2004) together with mean $\dot{V}CO_2$ and $\dot{V}H_2O$. Mean $\dot{V}CO_2$ and mean $\dot{V}H_2O$ were obtained from the central 30 min of each individual's recording. Where excretion events took place, data were carefully selected to exclude these portions. In both runs, the $\dot{V}H_2O$ from the first individual was deleted as the water channel took too long to stabilise and provide a reliable reading despite using strongly hydrophobic Bev-A-Line tubing throughout the plumbing of the system.

Statistics

Data were checked for normality and equal variance (using a Shapiro-Wilks and Levene's test respectively) and where these assumptions were violated, non-parametric tests were used. Resting metabolic rate, mean $\dot{V}CO_2$ and mean $\dot{V}H_2O$ were not correlated with start mass ($P > 0.24$ in all cases). Differences between CON and DT larvae were analysed with a t-test for RMR and $\dot{V}CO_2$, and a Mann-Whitney U-test for $\dot{V}H_2O$. The SCP of CON and DT larvae were analysed using a t-test as SCP were not correlated with start mass ($r = -0.11$, $P = 0.54$). A generalized linear model (GLZ, normal distribution and an identity link function) was used to investigate the effects of treatment (CON vs DT) and relative humidity on WLR, BWC and BLC. In all GLZ analyses, start mass was used as an independent variable. Using a Type 3 model GLZ, treatment had no effect on any of the variables (Table 2.2). The GLZ analyses were thus rerun using a Type 1 model with start mass and relative humidity as the variables (in this order). In all cases, analyses were run using STATISTICA 11.0 (Statsoft, Oklahoma, USA).

Table 2.2. Summary of generalised linear model (GLZ, Type 3 likelihood with a normal distribution and identity link function) results for water loss rate, body water content and body lipid content testing for an effect of experimental treatment (DT or CON). Each GLZ was run separately and significant effects are highlighted in bold.

Variable	Parameter estimate ± SE	Wald stat	P-value
Water loss rate			
Intercept	0.000001 ± 0.00002	0.0005	0.98
Start mass	**0.001 ± 0.0004**	**11.66**	**<0.001**
Treatment	-0.000002 ± 0.000005	0.18	0.68
Relative humidity	**0.00005 ± 0.000005**	**71.87**	**<0.000001**
Body water content			
Intercept	-0.0004 ± 0.002	0.02	0.88
Start mass	**0.58 ± 0.04**	**170.58**	**<0.000001**
Treatment	0.0004 ± 0.0006	0.50	0.48
Relative humidity	**-0.005 ± 0.0006**	**77.70**	**<0.000001**
Body lipid content			
Intercept	-0.0005 ± 0.001	0.18	0.67
Start mass	**0.14 ± 0.02**	**47.64**	**<0.000001**
Treatment	-0.0003 ± 0.0003	1.80	0.18
Relative humidity	0.0002 ± 0.0003	0.84	0.36

Results and discussion

The RMR and mean $\dot{V}CO_2$ (i.e. including potential activity periods) of CON larvae was significantly lower than DT larvae (t_{10} = -2.85, P = 0.02 and t_{10} = -2.41, P = 0.04; Fig. 2.1). Although mean $\dot{V}H_2O$ was lower in CON than DT larvae, there was no significant difference (n = 5 per group; Z = -0.84, P = 0.40). The SCP were not significantly different between CON and DT larvae (t_{30} = -0.98, P = 0.33, Fig. 2.2). There were no differences between treatment groups in WLR, BWC or BLC (P = 0.68, P = 0.48 and P = 0.18). WLR was

significantly higher in larvae exposed to low relative humidity, and these larvae lost significantly more water (i.e. had lower BWC at the end of the experiment; Figure 2.3a, b). BLC was unaffected by dehydration tolerance experiment (Table 2.3, Figure 2.3c). Although body mass played a significant role in determining WLR, BWC and BLC owing to the generally positive correlations with size, relative humidity was still a factor in WLR and BWC, but not BLC (Table 2.3).

Table 2.3. Summary of generalised linear model (GLZ, Type 1 likelihood with a normal distribution and identity link function) results for water loss rate, body water content and body lipid content. Treatment (CON or DT experimental groups) was not included as a factor as it had no effect on WLR, BWC or BLC (see Table 2.2). GLZ were run separately and significant effects are highlighted in bold.

Variable	Parameter estimate ± SE	Wald stat	P-value
Water loss rate			
Intercept	0.000001 ± 0.00002	0.001	0.97
Start mass	**0.001 ± 0.0004**	**11.52**	**<0.001**
Relative humidity	**0.00005 ± 0.000005**	**71.55**	**<0.000001**
Body water content			
Intercept	-0.0004 ± 0.002	0.03	0.86
Start mass	**0.58 ± 0.04**	**169.54**	**<0.000001**
Relative humidity	**-0.005 ± 0.0006**	**76.74**	**<0.000001**
Body lipid content			
Intercept	-0.0004 ± 0.001	0.14	0.71
Start mass	**0.14 ± 0.02**	**45.07**	**< 0.000001**
Relative humidity	0.0002 ± 0.0003	0.79	0.37

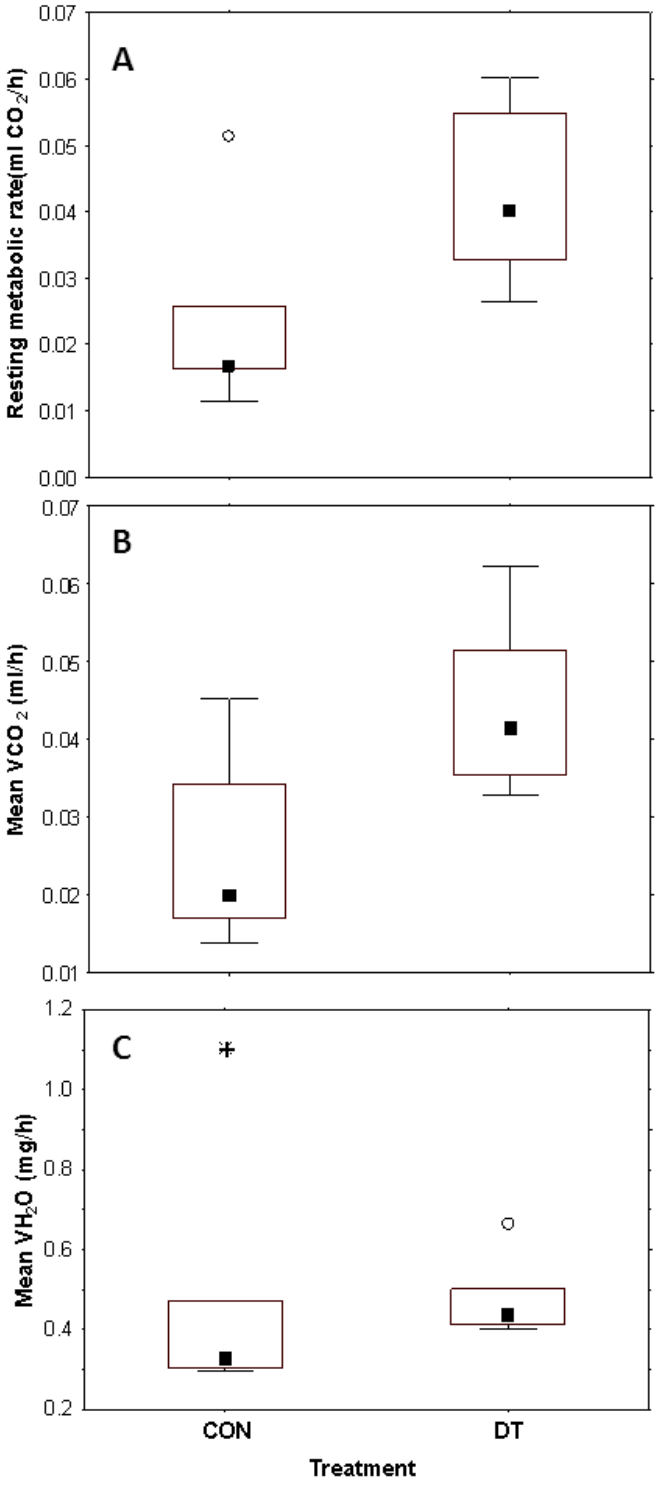

Figure 2.1. Box plots for resting metabolic rate (A), Mean $\dot{V}CO_2$ (B) and mean $\dot{V}H_2O$ (C) for larvae from control (CON) and diapause treatment (DT) groups. Black squares indicate the median, boxes the 25-75% percentiles, whiskers the non-outlier range, open circles the outliers and stars the extremes.

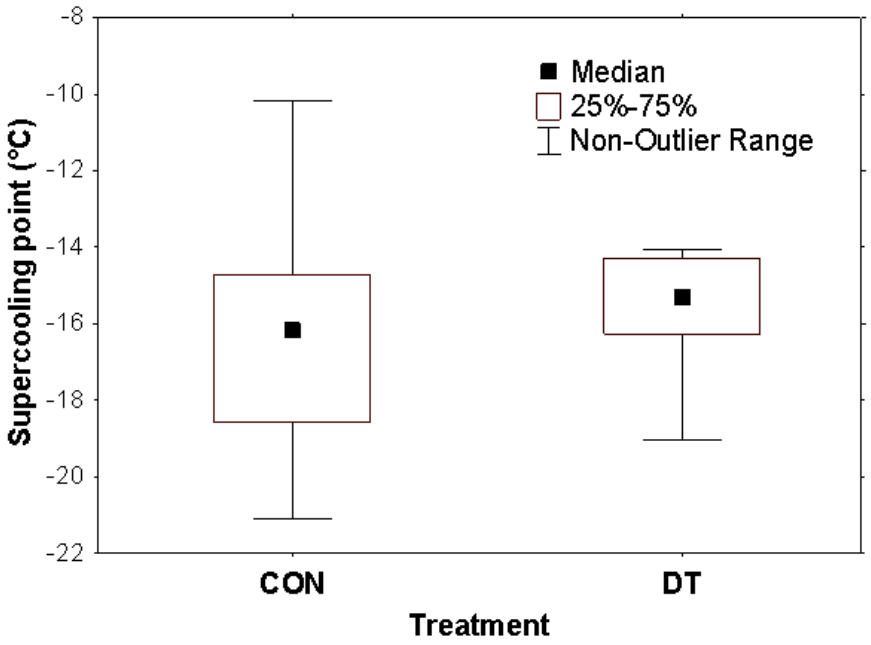

Figure 2.2. Box plots for supercooling points for larvae from control (CON) and diapause treatment (DT) groups. Black squares indicate the median, boxes the 25-75% percentiles and whiskers the non-outlier range.

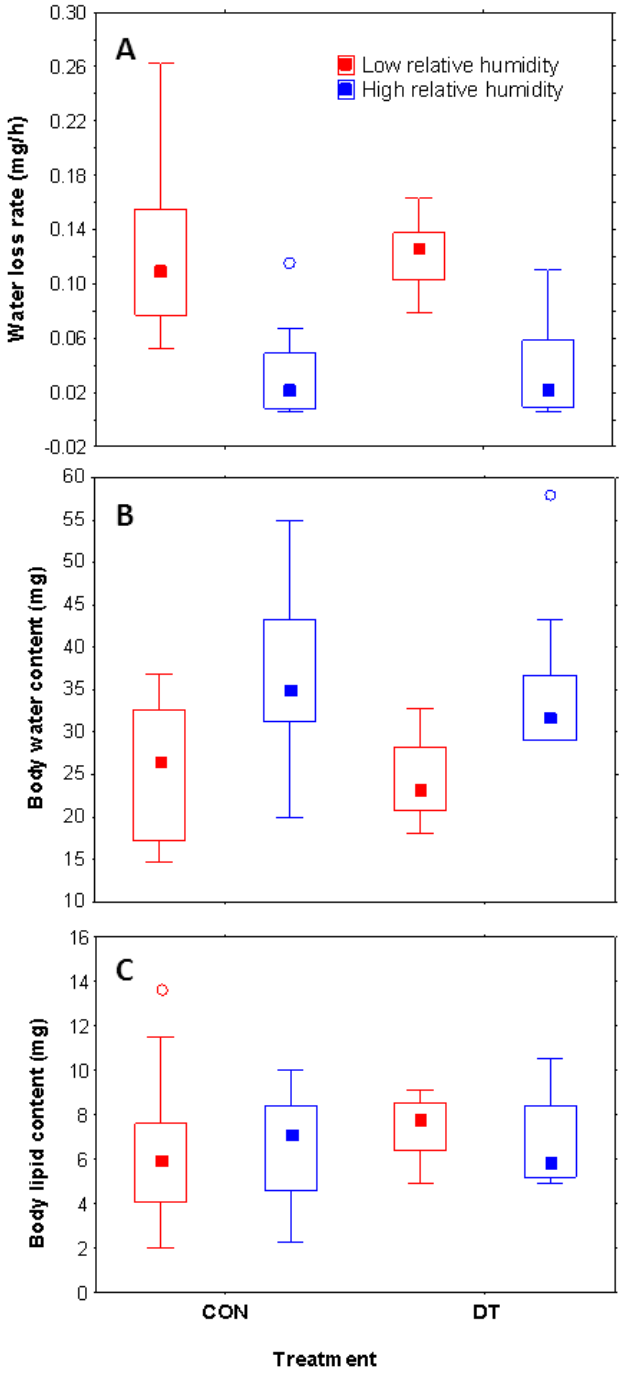

Figure 2.3. Box plots for water loss rate (A), body water content (B) and body lipid content (C) for larvae from control (CON) and diapause treatment (DT) groups. Larvae were exposed to either low (average 3.3%, red) or high (average 94.2%, blue) relative humidity. Black squares indicate the median, boxes the 25-75% percentiles, whiskers the non-outlier range, open circles the outliers and stars the extremes.

The results of this study are significant since they provide evidence from several physiological traits supporting the assumption of a limited diapause response in a major agricultural crop pest in Africa, the false codling moth *Thaumatotibia leucotreta*. To date, the species' inability to enter diapause has been largely based on a few early field observations (Reed 1974), which may have been confounded by taxonomic uncertainty in early life-stages (Timm *et al*. 2008; see Introduction). No studies have previously attempted to induce diapause in this species using a controlled change in photoperiod and temperature. To this end, the present work is both novel and important, particularly for pest management of the species in the region. For example, uncertainty around factors that should be incorporated into predictive modelling (e.g. day-degrees) can be reduced to improve forecasting efforts (Tobin *et al.* 2003).

Although some physiological traits varied in response to the different treatment conditions, these were not consistent with the direction of our a priori predictions for diapause induction. Given the limited responses of the suite of traits we measured in FCM, it is perhaps surprising that we did not find evidence for either diapause induction or an acclimation response to lower temperatures and longer nights. Laboratory acclimation and seasonal acclimatization responses in e.g. SCP have been documented previously in other Lepidoptera, including e.g. Argyrotaenia franciscana, Choristoneura fumiferana, Cydia pomonella, Grapholita molesta,; Lobesia botrana (Milonas & Savopoulou-Soultani 1999; Knight & Croft 1986; Han & Bauce 1993; Yokoyama & Miller 1989; Neven 1999, 2004; Khani & Moharramipour 2010; Chidawanyika & Terblanche 2011; Hansen 2002; Andreadis et al. 2005). Metabolic rates and water loss rates estimated at rest here are similar to a previous study (Boardman et al., 2013). The increase in MR in the DT group may perhaps be an upregulation of metabolic activity in preparation for lowering environmental temperatures, given that activity was carefully excluded, or perhaps be a consequence of repair from damage occurring during the low night-time temperatures experienced by the larvae. Clearly the relative humidity level larvae were exposed to influenced the water loss rate, as expected based on the physics of saturation deficit (Hadley 1994), with drier conditions eliciting higher loss rates in resting larvae. These rates, and the associated storage of body water and body lipid content, did not however appear to be influenced significantly in any direction in the DT relative to the control group. This suggests limited capacity to alter water loss rates and energy consumption rates through acclimation responses (i.e. physiological adjustments) during this developmental stage, unlike many other insect species examined to date (e.g.

Terblanche et al. 2010). This also suggests limited responses of these traits to variation in temperature and photoperiod more generally under field conditions, as has been shown for FCM in other studies of larvae and the adult stage (e.g. acclimation or hardening, see Stotter and Terblanche 2009; Boardman et al. 2012), with potential implications for understanding field population responses. Indeed, if this species has an overall limited plasticity in these traits it would support the suggestion that the species has sufficient basal tolerance to environmental low temperatures to not require rapid physiological adjustments, and perhaps such pronounced low temperature tolerance partly explains the lack of, or any apparent need for, a diapause response. Further work comparing acclimation responses in thermal traits across stages (e.g. Marais et al. 2009), coupled with field estimates of physiological rates during different seasons, would add strength to this conclusion.

Conclusion

A limited ability to induce diapause under the conditions examined here should however be interpreted with some caution as it is possible FCM may nevertheless enter diapause in the wild, but our experimental methodology did not reproduce the precise set of abiotic conditions which trigger diapause under natural conditions (Tauber & Tauber 1976; Hunter & McNeil 1997). Another potential methodological limitation is that in some Tortricidae species the parental generation must experience a specific set of altered seasonal conditions for the F1 generation to be able to enter diapause (Hunter & McNeil 2000; Mousseau & Dingle 1991; Dixon 1971). Thus, this work could be further improved by expanding the range of conditions and generations assayed for their potential ability to induce diapause in FCM. Regardless, this is an important first step towards rectifying a major knowledge gap on diapause responses, and thermal acclimation responses during development in the biology of FCM more generally. The results of the present study are essential to understanding field population dynamics and developing integrated pest management strategies for FCM in southern Africa.

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Chapter 3: Distribution of the false codling moth, *Thaumatotibia leucotreta*, in stone, pome and citrus orchards

Introduction

A major gap exists in research regarding the in-field ecology of the false codling moth (FCM), *Thaumatotibia leucotreta,* particularly on deciduous fruit. The highly polyphagous nature of FCM (Hepburn & Bishop 1954) leads to a constant utilisation of suitable habitat and food resources. The in-field flight ability has, so far, only been studied thoroughly in citrus orchards in the Citrusdal area (Stotter 2009).

The harvest times of the different citrus cultivars result in a host availability period of approximately 48 weeks of the year, while the host availability of grapes and stone fruit is much shorter, lasting from 22 to 24 weeks of the year for all cultivars, respectively. Apples are harvested for 14 weeks of the year, while pears are harvested for 12 weeks of the year in South Africa. This leads to long periods of up to 6 months without there being a suitable host among some commercial crops. The question remains as to whether the FCM population moves to its natural hosts for extended periods from the deciduous fruit orchards, or between orchards, to supplement its host and resource needs (Grové *et al.* 1999). Stotter (2009) no evidence of shuttling between host resources patches in the Citrusdal area.

A list of more than 50 host species of both cultivated and natural host species were recorded for FCM (Gunn 1921; Catling & Aschenborn 1974; Schwartz 1981; Newton & Crause 1990; Stotter 2009). These are outlined in more detail in the general introduction (Chapter 1). Damage assessments conducted throughout the year in orchards with different cultivated hosts planted in close proximity would be indicative of possible host preference, and of distribution ability. Other key questions include where FCM overwinter, how far they travel between hosts, and to what extent they utilise these hosts.

The sterile insect technique has been used against a wide range of pests, including Tephritidae and Lepidoptera (Klassen & Curtis 2005; Tyson *et al*. 2008). SIT has also been used as a management method on FCM, so further questions include whether sterile males distribute and locate hosts effectively at different times of the year, and whether they selectively distribute into different fruit crops. The males are assumed to be indicative of the female population, as the wild population has a sex ratio of 1:2 (male:female) (Daiber 1980). The female distribution is of importance, as their progeny result in the damage-causing larval stage (Hofmeyr 1998). SIT depends on the production of quality sterile males being released in high enough numbers into the targeted wild population (Calkins & Parker 2005). The quality should be high enough to ensure field competitiveness with the wild counterparts (Bloem *et al.* 2003) and, therefore, good distribution ability. At present, SIT on FCM is only being implemented in Citrusdal, Olifants river valley in the Western Cape, South Africa and Sunday river valley area, Eastern Cape, South Africa (www.xsit.co.za). Ideally this method should be applied on a regional, area-wide scale (Kogan 1998). Therefore it is desirable to expand this method to include deciduous fruit crops in future. For this reason, it is important to determine how sterile insects will react when released in deciduous fruit orchards, in terms of flight ability and host preference, as areas with deciduous fruits typically have a more diverse arrangement of potential hosts for FCM and could therefore impact on how the method is applied.

The aim of the study was 1) to determine what the degree of movement of sterile, male FCM was with regard to different commercial deciduous hosts between orchards at different times of the year; and 2) to determine the host preference in four fruit kinds at different times of the year. This information will provide insight into the field mobility and the adaptability of FCM to secure its survival and would be of value in optimising FCM control strategies in past seasons.

Materials and methods

Flight ability studies of sterile males

Flight ability studies were conducted at the Welgevallen Experimental Farm (S 33º 56' 55.1", E 18º 52' 16.6"), Stellenbosch University Campus, during the 2011/2012 season. The left side of the orchard (Fig. 3.1) was adjacent to a natural forest, while the right side was adjacent to

citrus orchards. Pear blocks seven and eight were nearest to a newly established vineyard, while blocks one and two bordered on an open field.

Twenty yellow delta traps were laid out at equal distances from four release points (Fig. 3.1). The traps were placed vertically, diagonally and horizontally in reference to each release point. The distances of the traps were 30, 60 and 90 m from the centralised release point. The chosen geographic area included four fruit kinds, namely oranges, pears, apples, and plums. This design would provide an accurate estimation of the flight distribution, and of the dispersion ability, across orchard blocks.

Six thousand sterile FCM was used for each release, which was received from the XSIT rearing facility in Citrusdal, Western Cape, South Africa, were divided evenly by weight, between the four release points.The individuals had a presumed sex ratio of (1:1). Each individual orchard group was marked with a different flourescent micronised dust (Day GLO Colour Corporation, Cleveland, OH), to distinguish them in traps once recaptured. It was found in a previous study on codling moth (*Cydia pomonella*) that there was no negative effect on flight ability of moths treated with such dyes (Keil *et al*. 2001). Yellow delta traps (Chempac, Paarl, South Africa) were baited with a pheromone lure, namely Chempac FCM lure (Z-8-dodecenyl acetate (62.5 g/kg), E-8-dodecenyl acetate (62.5 g/kg), E-7-dodecenyl acetate (62.5 g/kg)) (Chempac, Paarl, South Africa) to recapture the FCM. The moths were released at 12:00h, which is within 5 hours after receiving them from XSIT, to ensure that a high quality of sample and competitive individuals were obtained.

The temperature and other environmental conditions were monitored for the six-day duration of each of the six repetitions of the trial. The traps were checked daily for the presence of FCM, and the numbers were recorded (from August 2011 to February 2012), until there were no more daily catches.

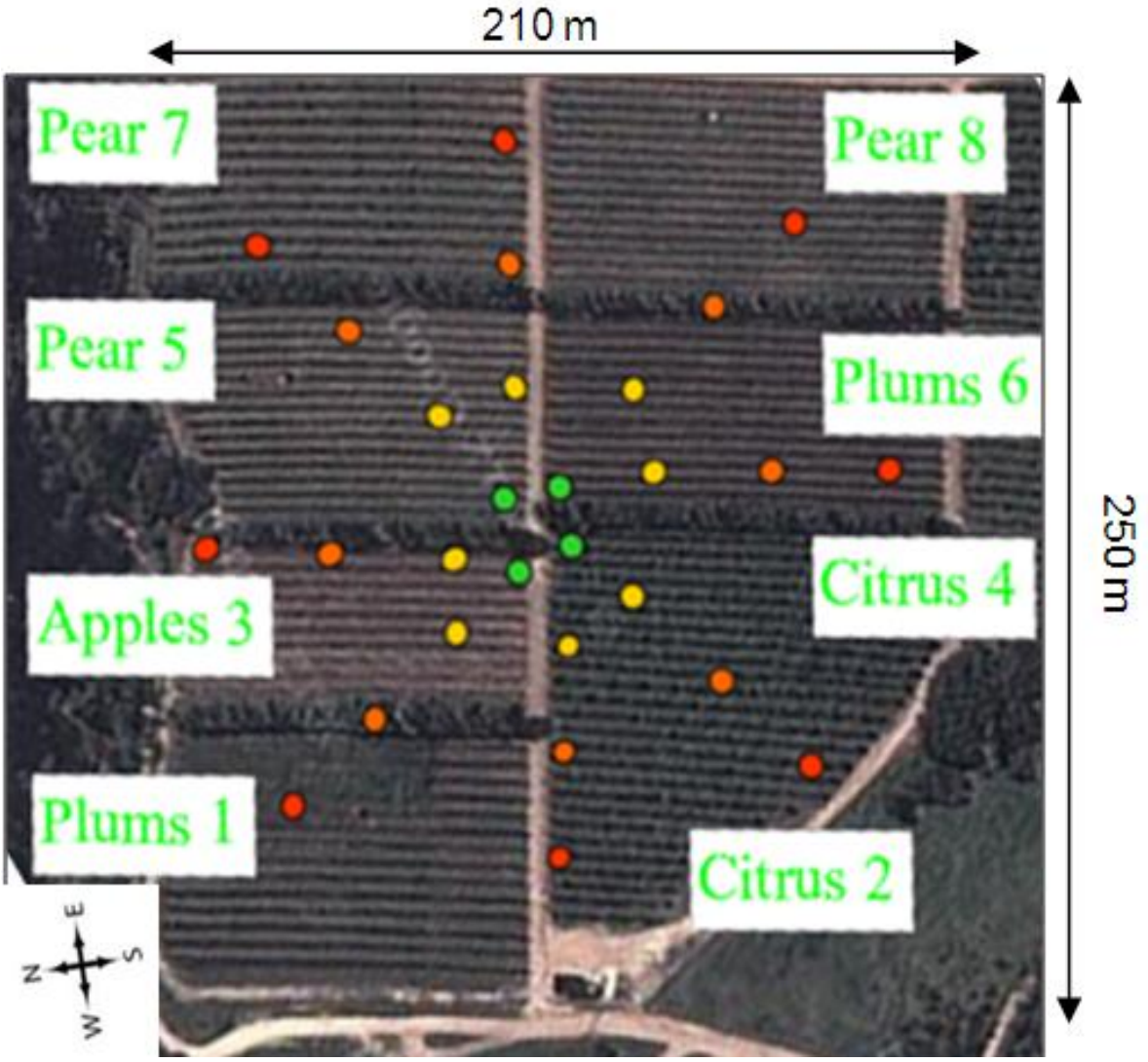

Fig 3.1. Experimental layout to test the flight ability of false codling moth in deciduous and citrus fruit orchards at Welgevallen experimental farm, Stellenbosch. Central release points (green dots) were used in four of the orchards. Different coloured dots indicate the respective distances from the release points. Yellow = 30 m trap; orange = 60 m trap; and red = 90 m trap (www.maps.google.co.za)

Damage assessments in various fruit kinds

Damage assessments were conducted biweekly for the 2011/2012 fruit-growing season (from November 2011 to May 2012). Each of the eight blocks described in Fig. 3.1 was monitored. Ten fruit, or fruit clusters, were monitored per tree. This was repeated on twenty-five evenly spaced trees within each orchard block (Brown & Pringle 2006). After the damaged fruit were removed from the orchard, they were placed in emergence boxes (Fig. 3.2). Each such box consisted of a black plastic 30 × 24 × 24 cm perspex box, with an opening on one side, and with a replaceable jar (1000 ml in diameter) covering the opening. The jar was clear, to

allow the penetrating light to attract the adult moths into the jar for collection. The emergence boxes were then placed inside a rearing room, which was kept at a constant temperature of 25°C, with a constant 12:12 (L:D) photoperiod. Each emergence box contained a diet medium as described by Guennelon *et al*. (1981), supplied by Entomon Pty (Ltd), to ensure optimum emergence. This method was chosen instead of a destructive method using larval identification, to ensure that no larval misidentification would occur. The adults were then identified using keys developed by Timm *et al*. (2008).

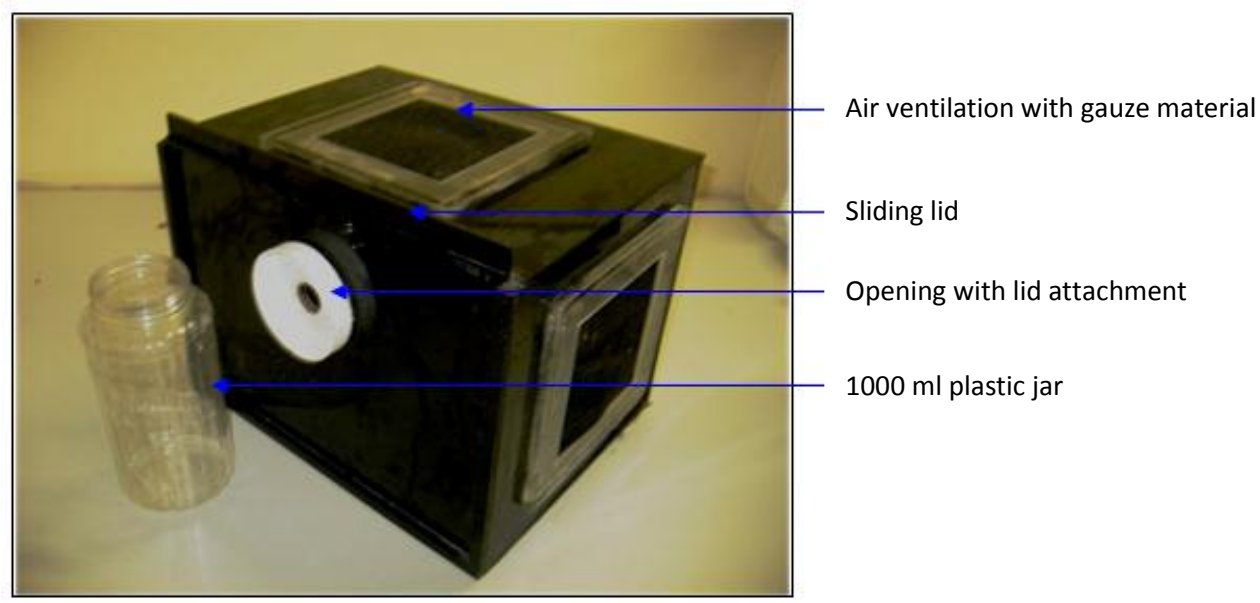

Fig. 2.2. Emergence box used for false codling moth emergence, with 1000 ml plastic jar to cover opening.

Results and Discussion

Flight study of sterile males

For the six release dates the total number of recaptured moths can be seen in Table 3.1 below. The release dates were chosen to coincide with a decrease in the number of rainfall days , and an increase in the average temperature (Appendix to this chapter). Temperature data are important, as preliminary studies found the minimum temperature for flight in wild FCM to be in the region of 10-15^{0}C, while that for laboratory-reared moths was slightly higher (Stotter & Terblanche 2009).

Table 3.1. Recaptured numbers of sterile false codling moth adults released on various dates throughout summer in mixed fruit orchards on Welgevallen experimental farm, Stellenbosch.

Release	Total recapture
24 Aug 2011	0
17 Oct 2011	0
03 Jan 2012	0
5 Jan 2012	10
26 Jan 2012	76
06 Feb 2012	73

Adults recaptured during the January and February release dates are indicated in Fig. 3.3. During the second recapture date in January, there was almost no movement of the moths from the fruit kind in which they were released to another fruit kind (Fig. 3.3A), while the moths became more mobile, moving between fruit kinds, as the season progressed (Fig. 3.3B & C). Pears caught the highest number of moths (42) in pheromone traps, during the 26 January releases, followed by plums (16), apples (11), and citrus (7). On 6 February the highest trap catches were in plums (25), followed by citrus (21), apples (16) and pears (11).

The pears also showed a fair distribution between the different trap distances. There was a general tendency to more horizontal movement between the blocks, compared to vertical movement. Regardless of whether the vertical movement was uphill or downhill, it only amounted to 6 cross-boundary movements, with the horizontal movement adding up to a total of 19 catches. This might be because of the 2+ m high windbreaks between the vertically aligned orchards. The windbreaks may, further, have harboured insectivorous birds, which would have prevented an upsurge in the population (Forman 1995). Windbreaks influence distribution through wind reduction, microclimate modification and vegetative diversity (Pasek 1988).

Pearson (1958) agrees that FCM would prefer citrus during the ripening period, compared to during the periods that are earlier on in the season. We would therefor expect the increased damage during the ripening period in citrus. The recapture rates were generally low compared

to the great number of FCM that were released, which could have been due to the virus infestation, or other quality parameters.

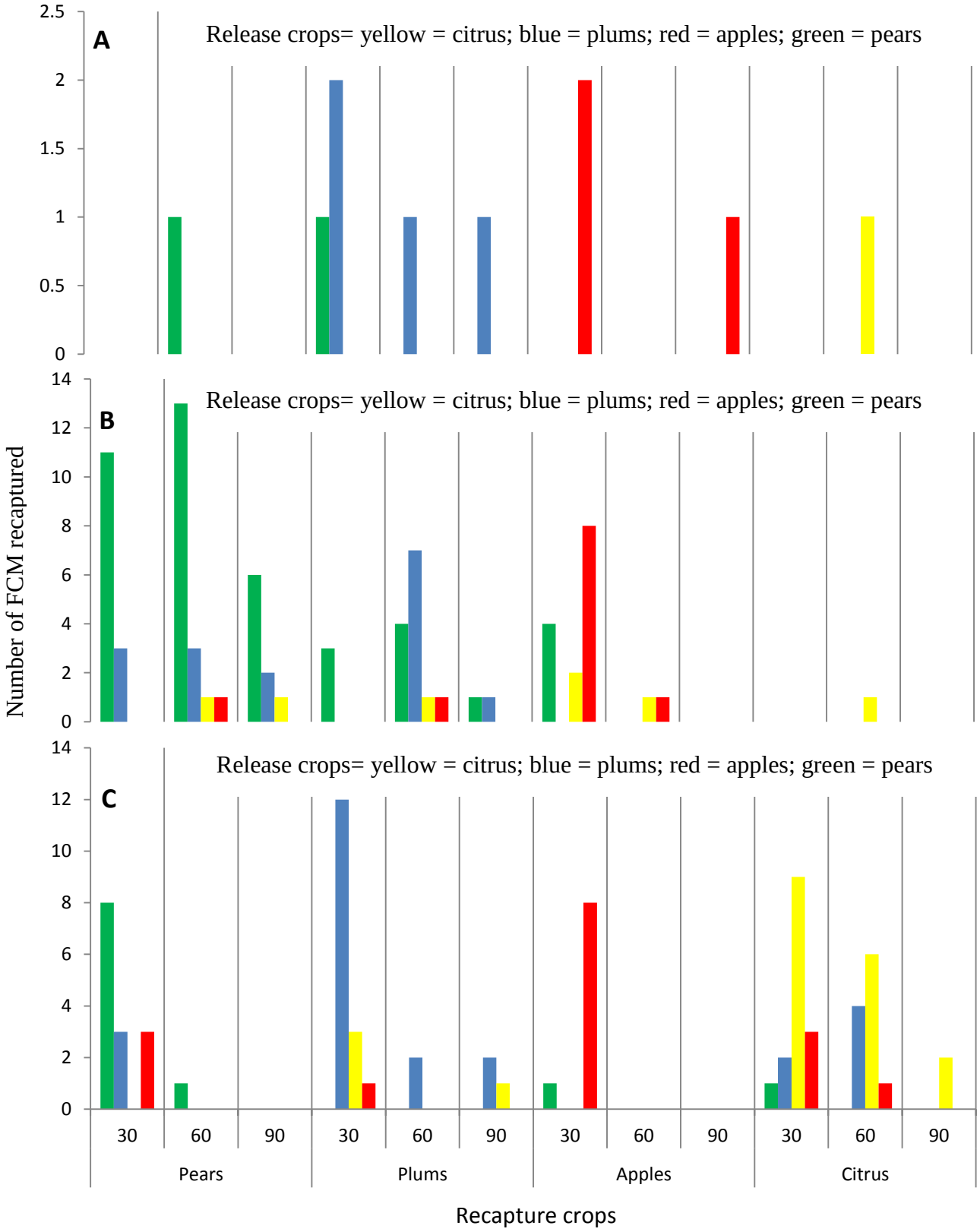

Fig. 3.3. Total recapture of sterile false codling moth males on Welgevallen experimental farm per fruit type, at distances of 30, 60 and 90 m. The release dates were as follows:
A = 5/01/2012; B = 26/01/2012; C = 6/02/2012.

The interaction between fruit kind and distance was found not to be significant (ANCOVA $F_{6;24}$ = 0.26701; ρ>0.9) (Fig. 3.4). The main effects could, therefore, be evaluated individually. Neither distance (ANCOVA $F_{2;24}$ = 2.2026; ρ>0.1) (Fig. 3.5), nor kind of fruit (ANCOVA $F_{3;24}$ = 0.42024; ρ>0.7) influenced the recapture rate (Fig. 3.6). This could be due to the very low trap counts, and to the variation between the recapture dates.

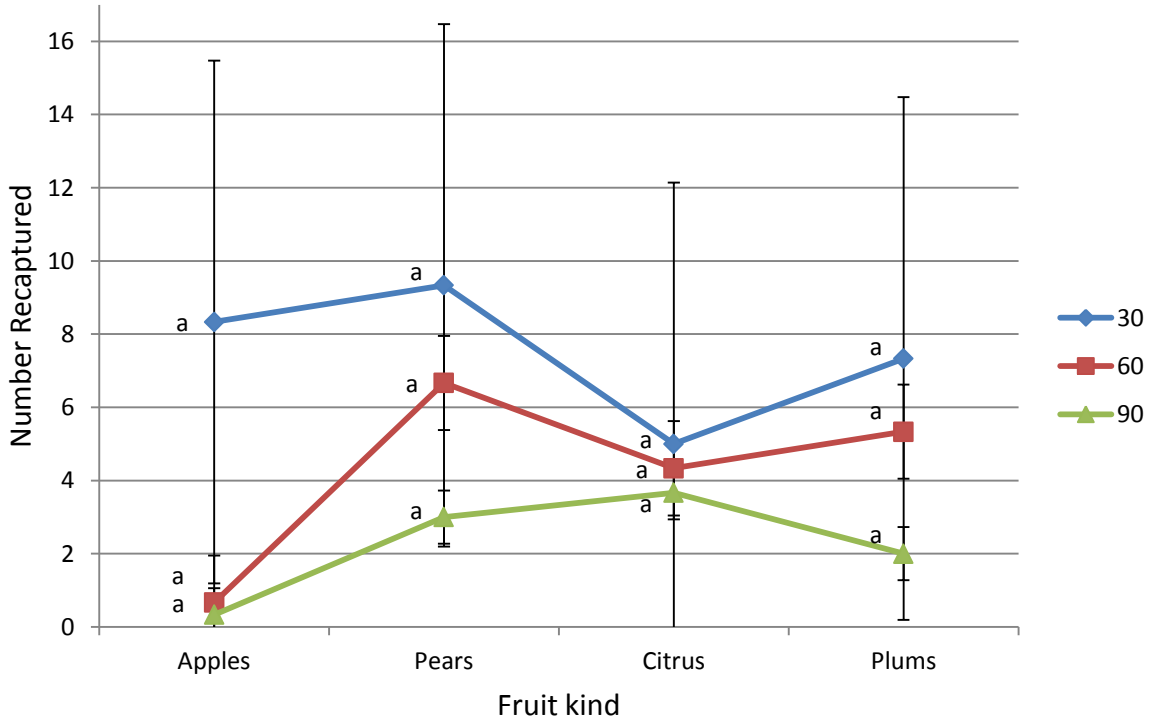

Fig. 3.4. The mean recapture rate of sterile false codling moth males for four kinds of fruit (apples, pears, plums, and oranges), trapped at distances of 30 m, 60 m and 90 m from each central release point, with 95% confidence intervals. Bars with the same letter show that the differences were not significant ($\rho \leq 0.05$).

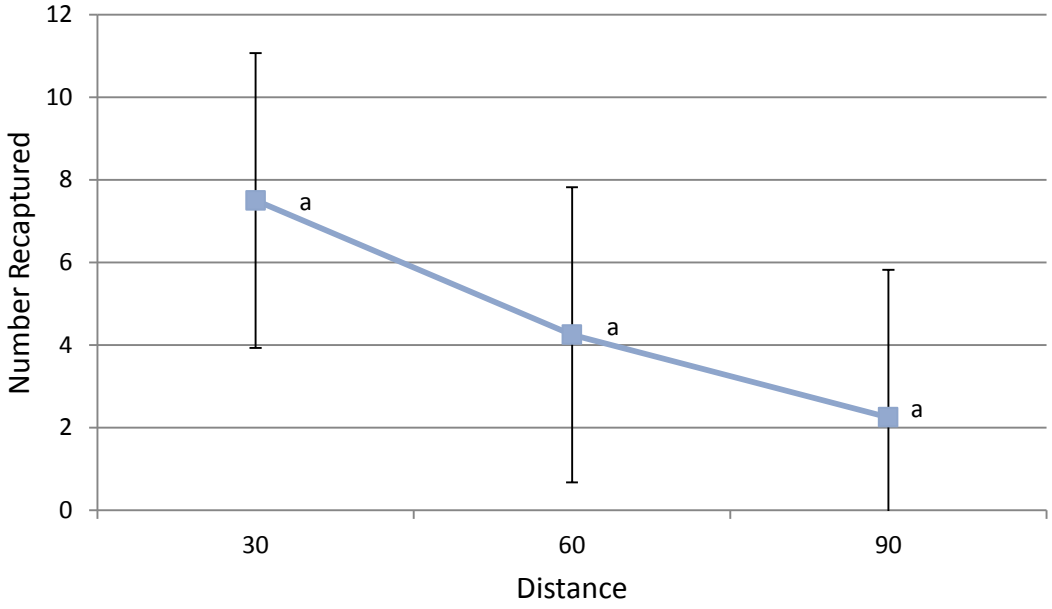

Fig. 3.5. The mean recapture rate of sterile false codling moth males at distances of 30 m, 60 m and 90 m from a central release point, located in each of four orchards (apples, pears, plums and oranges), with 95% confidence intervals. Bars with the same letter show that the differences were not significant ($\rho \leq 0.05$).

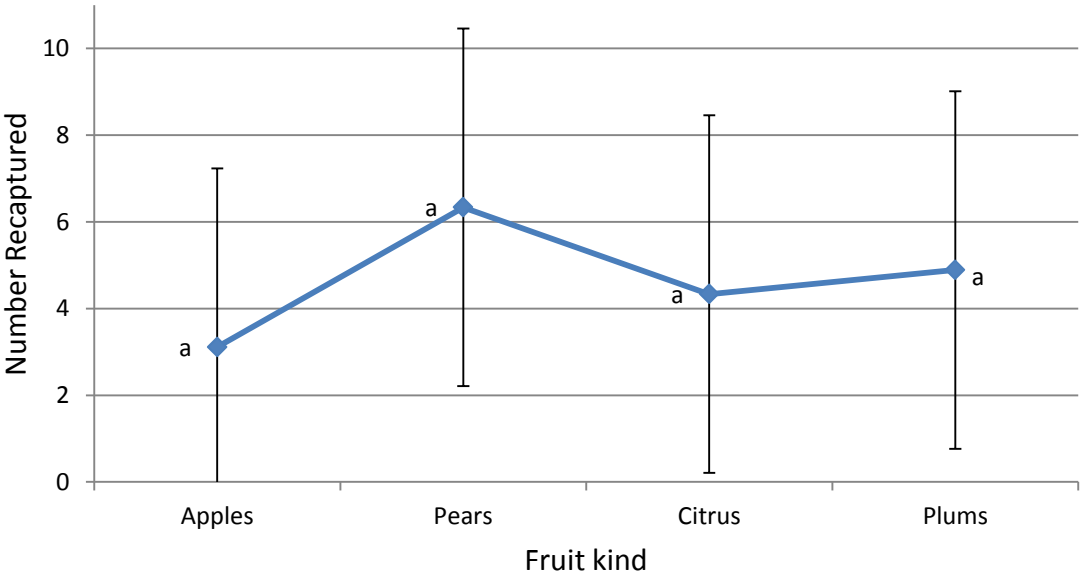

Fig. 3.6. The mean recapture rate of sterile false codling moth males for four kinds of fruit (apples, pears, plums, and oranges) for three release dates, with 95% confidence intervals. Bars with the same letter show that the differences were not significant ($\rho \leq 0.05$).

The interaction between the distance from the release point and the release date can be seen in Fig. 3.7 below. As this was significant (ANOVA $F_{4;27}$ = 2.9203; ρ>0.03), the main effects could not be evaluated individually. Bonferroni's test confirmed that the recapture rate at 30 m distance on 6 February 2012 was significantly higher (df = 27; ρ = 0.0367) than were the other captures, at all distances on the other release dates. The higher average day and night temperatures experienced during this release period would have encouraged a better flight ability compared to the previous release dates.

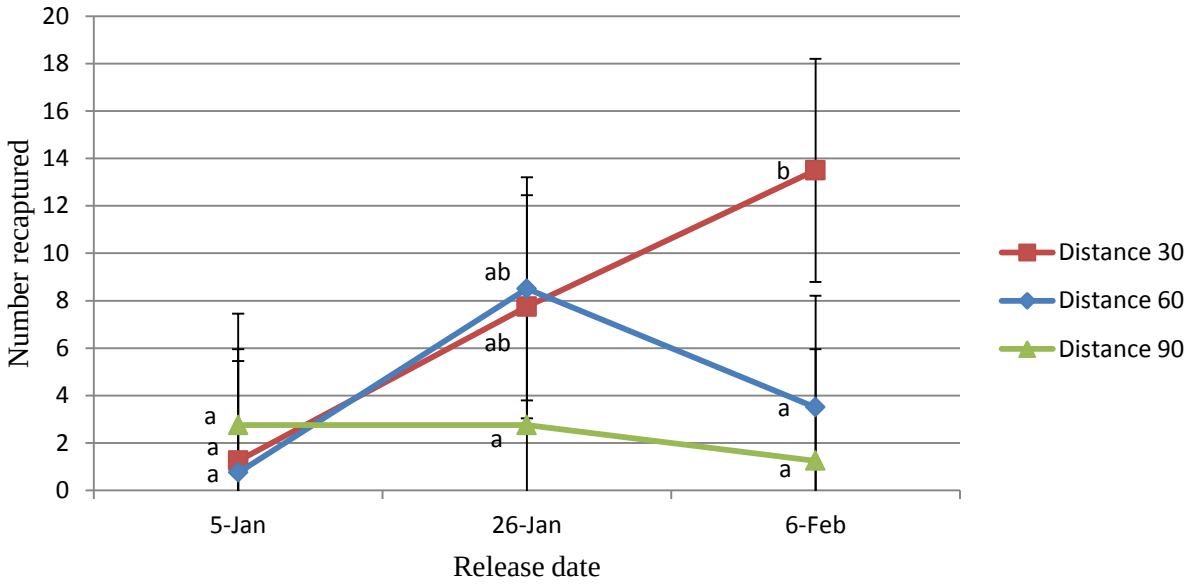

Fig. 3.7. The average recapture rate of sterile false codling moth males for three release dates, at distances of 30 m, 60 m and 90 m from each central release point, with 95% confidence intervals. The releases were made on four kinds of fruit (apples, pears, plums, and oranges). Bars with the same letter show that the differences were not significant (ρ≤0.05).

Damage assessments conducted on various kinds of fruit

Fig. 3.8 below illustrates the average number of FCM reared from seven orchard blocks, containing pears, citrus, and plums. A strong preference for citrus as the fruit ripened was noted, with plums and pears being preferred for the first half of the 15-week monitoring period. This indicates that FCM, due to its polyphagous nature, is present from relatively early on in the season, but that it is more likely to cause fruit damage during the period of increased ripening.

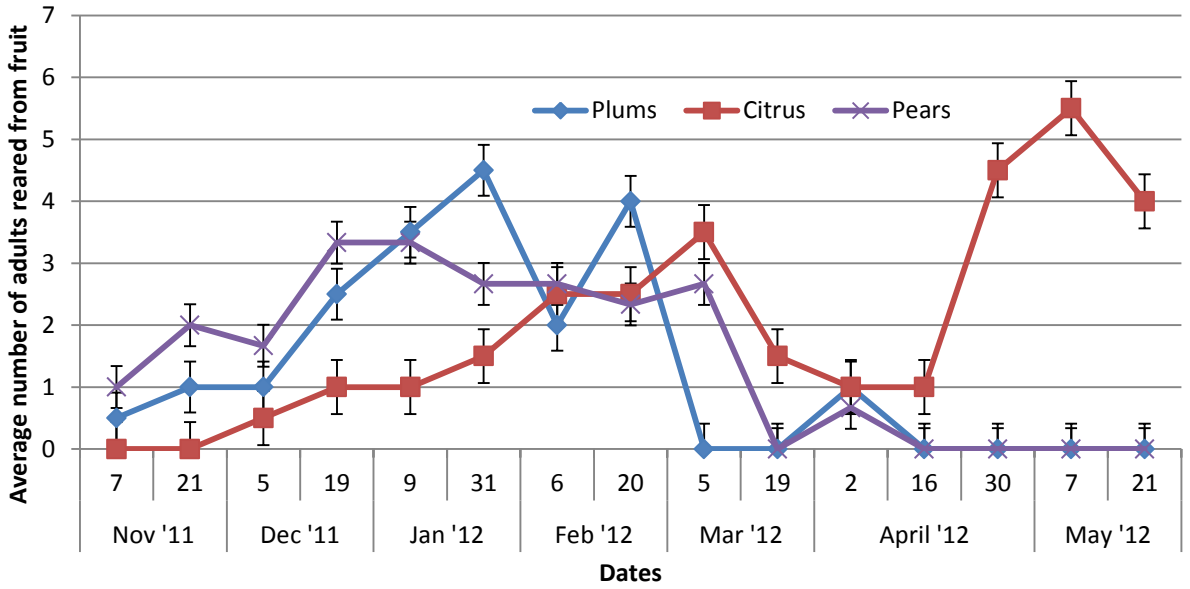

Fig. 3.8. The average number (±SE) of false codling moth adults reared from damaged fruit in seven orchard blocks containing plums (= blue), citrus (= red), and pears (= purple) at Welgevallen experimental farm, Stellenbosch, over the course of one growing season. Ten fruit, or fruit clusters, were monitored per tree. This was repeated on twenty-five evenly spaced trees within each orchard block.

FCM and codling moth (CM), *Cydia pomonella* (Lepidopetra: Tortricidae) were both reared from one apple orchard (Fig. 3.9). The FCM and the codling moth adults that were reared from the fruit were distinguished through a clear dark band on the hindwing of the CM adults, as can be seen in Fig. 3.10 below. The external damage on the fruit, known as frass, could not be used as a definitive identification method, as CM also deposits frass around the entry hole (Annecke & Moran 1982). FCM counts were lower when compared to the codling moth counts. CM has a high preference for apples (Azizyan *et al.* 2002), and is the primary pest of economic importance in pome fruit production areas in the Western Cape (Giliomee & Riedl 1998).

Since both moths appeared from the same damaged fruit, it is possible that FCM entered as a secondary contaminant of the damaged fruit. They could then have exited from the fruit, and have developed on the diet medium in the emergence boxes. It was, therefore, not possible to determine whether the apples were utilised as hosts by the FCM (see Chapter 4 for further analysis in this regard).

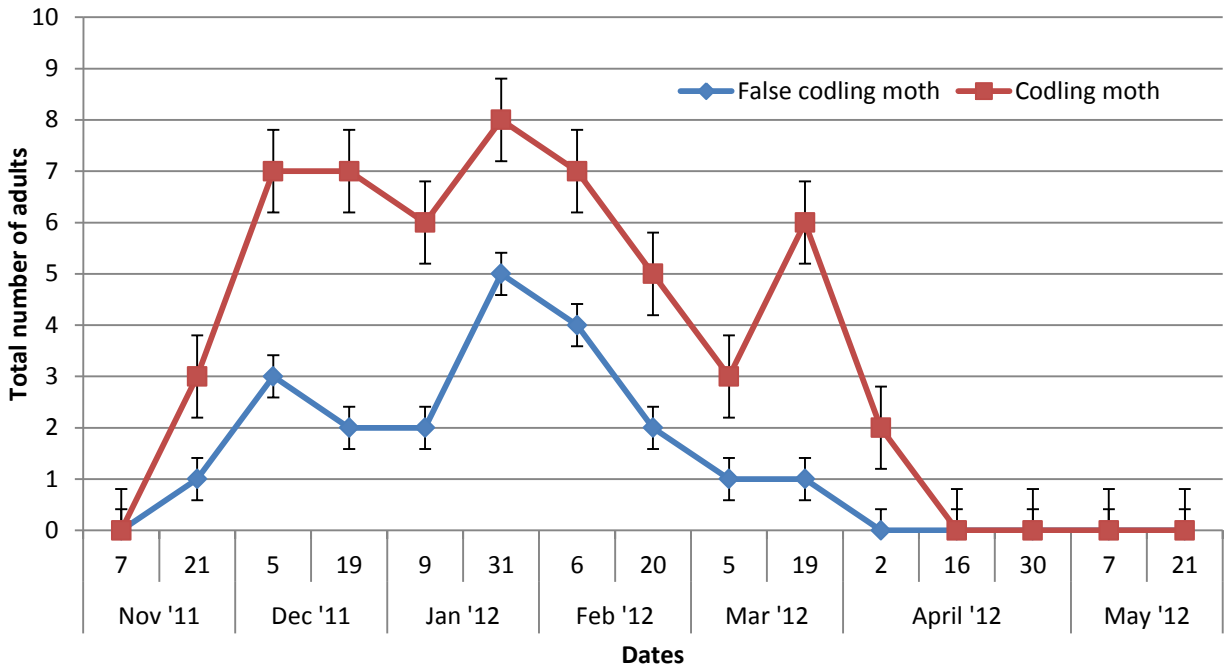

Fig. 3.9. Average (±SE) number of false codling moth and codling moth adults reared from damaged apples in one orchard block at Welgevallen experimental farm, Stellenbosch, over one growing season.

Fig. 3.10. False codling moth, *Thaumatotibia leucotreta*, (on the left) and codling moth, *Cydia pomonella*, (on the right) adult wing differences. Codling moth with clear dark band on hindwing (Timm *et al.* 2008).

The average number of FCM emergence per fruit kind was calculated, to compensate for varying numbers of orchard blocks within the different kinds of fruit (Fig. 3.11). The averages acted as the representation of the cumulative catches for one orchard block per fruit kind. Plums, for instance, comprised orchard block 1 (total = 24) and 6 (total = 16), which were then averaged. The number of FCM reared from apples, plums and pears, was similar, being 20 (±1.995), 21 (±2.103) and 22.33 (±2.05), respectively. Citrus had a higher damage total, adding up to 30 (±2.501). The only significant difference between the two orchard blocks was that orchard block 1 was adjacent to the citrus block, providing the fruit with a constant supply of FCM. Block 6 was also connected to the citrus block, but a windbreak could also act as a migration barrier between the orchard blocks.

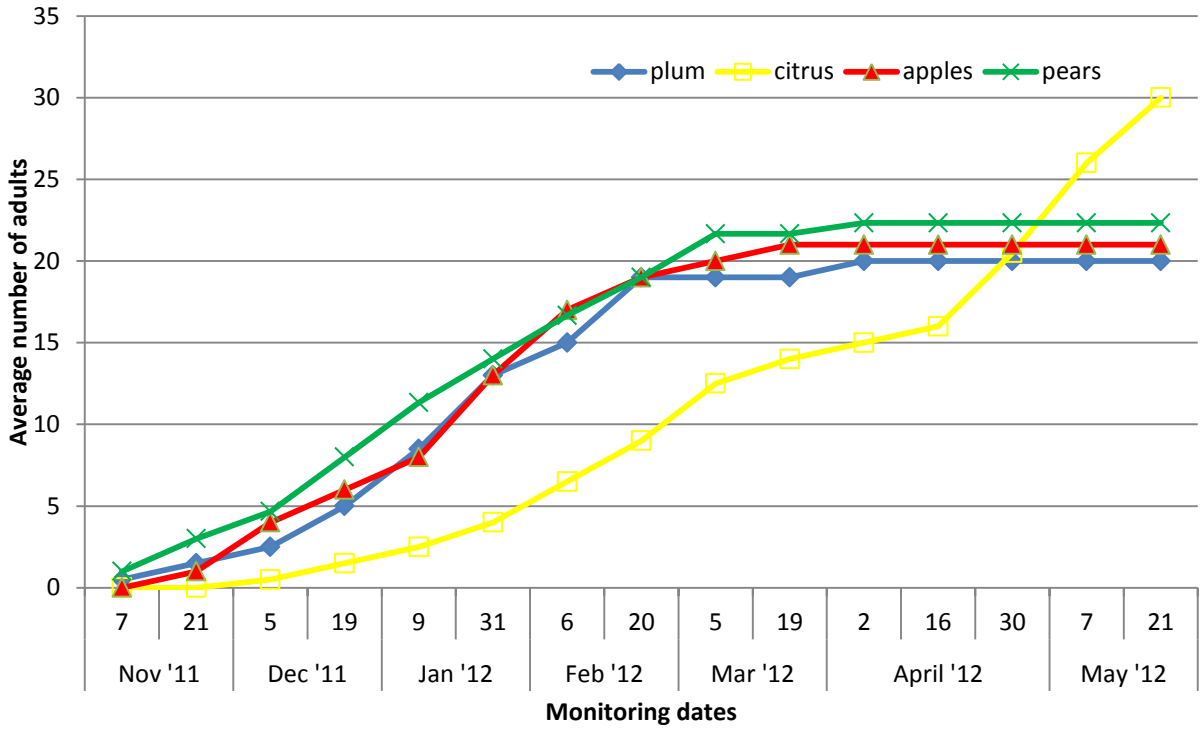

Fig. 3.11. The average cummulative number of FCM adults reared from four kinds of fruit (plums = blue; citrus = yellow; apples = red; pears = green) in eight orchard blocks on Welgevallen experimental farm, Stellenbosch from November 2011 to March 2012.

The spray programme is described in Appendix 2 (to this chapter) and lists the insecticides that were used during the 2011/2012 season. According to the spray programme, the only treatment that was specifically applied against FCM was Cryptogran+. This was insufficient to suppress the FCM levels, being totally inadequate for controlling the pest levels. However, Dursban and Azinphos could well have influenced FCM numbers. Azinphos (organophosphate) has a registration for FCM on peaches, while Dursban does not have a FCM registration but is also an organophosphate. (www.croplife.co.za). The orchards were also surrounded by naturally occurring, untreated vegetation, such as nearby oak trees, which might have acted as alternate hosts (Stotter 2009).

Conclusion

This study consisted of a flight study and of fruit damage assessments. The flight study is valuable, due to its contribution to a better understanding of how facility-reared sterile FCM could react when used in deciduous fruit orchards. The study illustrated that some movement did occur between orchard blocks, and that this movement appeared to be correlated with ripening fruits.. The study supports Stotter's (2009) findings that FCM are able to move between orchard blocks, and to seek a preferred and available host type. Integrated pest management systems should also focus on orchards and natural vegetation around the monitored orchard. Alternate hosts surrounding the orchard could either be removed, or treated. The flight ability of FCM was very sensitive to a change in temperature, again showing the importance of the correct SIT application timing to allow for distribution, once released. In a study by Daiber (1978) on male flight in fruit orchards, this author suggested that low temperatures and absence of hosts were more influential in determining activity patterns than high temperatures, although he did not record temperatures close to 40ºC in his studies. No studies have yet been conducted to determine upper lethal temperatures for false codling moth.

The poor recapture rates could be ascribed to poor moth quality as a result of the rearing process, in addition to stressed moths during the 2.5 hour transportation from Citrusdal to Stellenbosch shortly before release. Virus infections are relatively common in insect rearing facilities and have been documented before for false codling moth (Ludewig 2003).

Unfortunately these are logistic issues that could not be improved upon. Further research should investigate ways of improving moth quality for field release as these are critical issues for the success of an SIT programme (Simmons et al. 2009).

The damage assessments indicated the extent of FCM damage on hosts other than citrus, when the orchard blocks were in close proximity to one another. FCM is often misidentified, leading to its inadequate control. Isomers of α-faresene have been identified in Codling moth females as the compound which induces the individuals to oviposit on or near fruit. It is also the compound which helps the individual to find the fruit (Wearing & Hutchins 1973). Studies have however proven that no single factor can determine host specificity (Berenbaum 1983). We also see that there are three levels of resolutions when Lepidoptera search for a host. The first is the choice of habitat, the second is the choice of a specific plant and the third is the decision to oviposit after landing on the plant (Chew & Robbins, 1984;Douwes 1968)

It should be highlighted that FCM was reared from all four kinds of fruit, with the adults being reared from apples that were believed to have been affected by secondary damage. The fruits were most attractive to FCM when they were ripe, as also found by Daiber (1978). This period is crucial for the control and the monitoring of FCM, although the population levels should be suppressed throughout the growing season. Future research should focus on the ability of FCM to be reared from apples, specifically following on the presence of codling moth damage, as this might have major implications.

Additional stress is placed on orchard sanitation by the ability of FCM to move between orchards, as well as to infect, and to breed on, different hosts. More specifically, the fallen and infected fruit should not only be removed, but also destroyed.

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Map of the Welgevallen experimental farm, Stellenbosch, South Africa, retrieved on Nov 3, 2012 from website www.maps.google.co.za - Accessed 01/06/2012

www-naweb.iaea.org/nafa/ipc/public/ipc-codling-moth-sterile-insect-technique-2010 – Accessed 3/6/1012

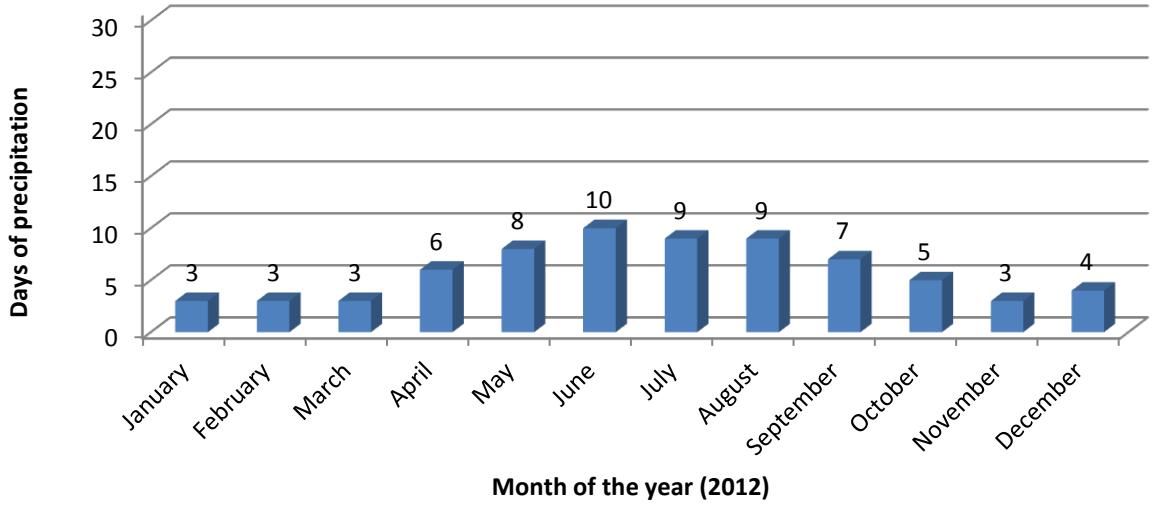
www.weather.sun.ac.za - Accessed 3/6/1012

www.maps.google.co.za - Accessed 20/08/2013

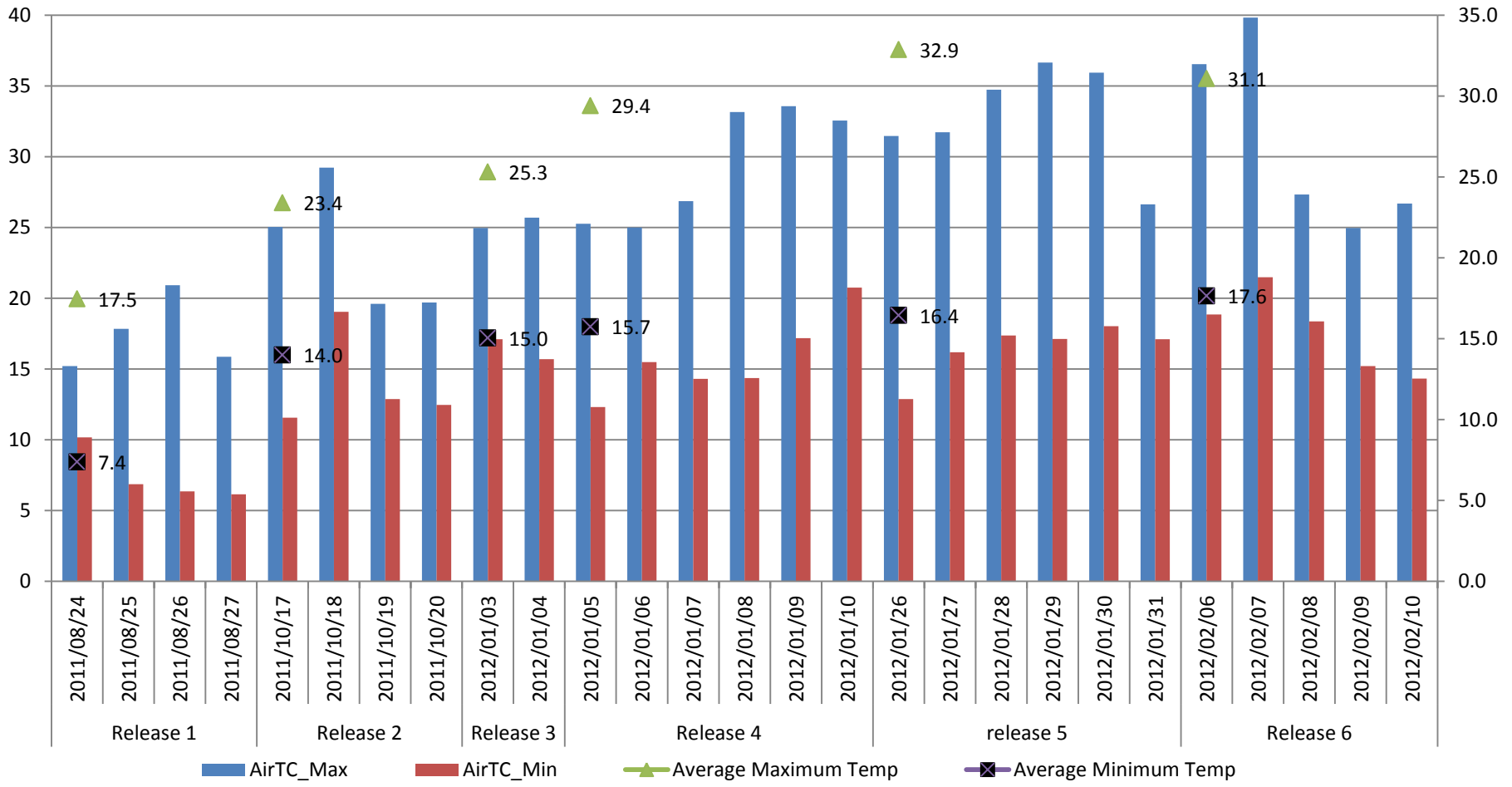
www.xsit.co.za - Accessed 20/08/2013

www.croplife.co.za – Accessed 06/09/2013

Appendix 1



Average days of precipitation (mm) for each month (2012) in Stellenbosch (weather.sun.ac.za).



Minimum and maximum daily and average temperatures for 6 release dates (2011/2012) on Welgevallen experimental farm, Stellenbosch (weather.sun.ac.za)

Appendix 2

Table 3.2. Spray programme for the 2011/2012 season on Welgevallen experimental farm, Stellenbosch, South Africa. Tabular data compiled from data provided by W Kerwel.

September 2011					
Date		**Cultivar (fruit type)**	**Product**	**Dosage**	**Target**
28/9/2011	1	Rosemary (pears)	Dithane WP (Mancozeb)	150 g / 100 L	Fusi
	2	Rosemary (pears)	Thioflo SC (Endosulfan)	100 ml / 100 L	Boll-worm
	3	Forelle (pears)			
October 2011					
5/10/2011	2	Rosemary (pears)	Rimon EC (Novaluron)	35 ml / 100 L	Codling moth
	3	Forelle (pears)			
	8	Packham's Triumph (pears)			
13/10/2011	1	Rosemary (pears)	Carpovirusine (Codling moth granulosis virus)	35 ml / 100 L	Codling moth
	2	Rosemary (pears)			
	3	Forelle (pears)			
	8	Packham's Triumph (pears)			
17/10/2011	4	Pink Lady® (apples)	Spraybor (Boron)	120 g / 100 L	Trace element
		Royal Gala (apples)	Carpovirusine (Codling moth granulosis virus)	35 ml /100 L	Codling moth
17/10/2011	6	Laetitia (plum)	Hunter SC (Chlorphenapyr)	35 ml / 100 L	Thrips
	7	Songold (plum)	Akito EC (Beta-cypermethrin)	12.5 ml / 100 L	Oriental fruit moth
		Laetitia (plum)			
	9	Larry Anne (pears)			
		Songold (plum)			
27/10/2011	1	Rosemary (pears)	Checkmateflo ((E,E)-8,10-Dodecadien-1-ol)	100 ml / 100 L	Mating disruption –

					Codling moth
	2	Rosemary (pears)			
	3	Forelle (pears)			
	8	Packham's Triumph (pears)			
27/10/2011	4	Pink Lady® (apples)	Dithane WP (Mancozeb)	150 g / 100 L	Fusi
		Royal Gala (apples)	Checkmateflo ((E,E)-8,10-Dodecadien-1-ol)	100 ml / 100 L	Mating disruption – codling moth
28/10/2011	6	Laetitia (plum)	Akito EC (Beta-cypermethrin)	12.5 ml / 100 L	Oriental fruit moth
November 2011					
1/11/2011	6	Laetitia (plum)	Azinphos+	70 ml / 100 L	Oriental fruit moth
	7	Songold (plum)	Dithane WP (Mancozeb)	150 g / 100 L	
		Laetitia (plum)			
	9	Larry Anne (pears)			
		Songold (plum)			
7/11/2011	10.1	Satsuma (citrus)	Nemesis EC (Pyriproxyfen)	30 ml / 100 L	Red scale
			Pyriproxyfen	100 g /L	Red scale
29/11/2011	10.1	All fruit	Cryptogran+	10 ml / 100 L	False codling moth
	10.2	All Citrus	Voermolas (Crude protein, calcium, phosphorus)	250 ml / 100 L	Microorganism growth
	11.1		Cryptogran+	10 ml / 100 L	False codling moth
			Voermolas (Crude protein, calcium, phosphorus)	250 ml / 100 L	Microorganism growth
December 2011					
6/12/2011	1	Rosemary (pears)	Calypso SC (Thiacloprid)	15 ml / 100 L	Codling moth

	2	Rosemary (pears)			
	3	Forelle (pears)			
	8	Packham's Triumph (pears)			
6/12/2011	4	Pink Lady® (apples)	Calypso SC (Thiacloprid)	15 ml / 100 L	Codling moth
		Royal Gala (apples)			
July 2012					
17/7/2012	1	Rosemary (pears)	Dursban EC (Chlorpyrifos) +Wetcit (Borax + Orange oil)	75 ml / 100 L+	Mealybug
	2	Rosemary (pears)		100 ml / 100 L	Pre-season
	3	Forelle (pears)			
	3	Forelle (pears)			
17/7/2012	6	Laetitia (plum)	Dursban EC (Chlorpyrifos)	75 ml / 100 L	Mealybug
	7	Songold (plum)	Wetcit (Borax + Orange oil)	100 ml / 100 L	Pre-season
		Laetitia (plum)			
	9	Larry Anne (pears)			
		Songold (plum)			
17/7/2012	4	Royal Gala (apples)	Dursban EC (Chlorpyrifos)	100 ml / 100 L	Mealybug/Scale
		Pink Lady® (apples)	Wetcit (Borax + Orange oil)	500 ml /100 L	

Chapter 4: Developmental parameters of false codling moth, *Thaumatotibia leucotreta*, (Meyrick) on four commercial hosts

Introduction

False codling moth (FCM), *Thaumatotibia leucotreta*, is indigenous to Southern Africa, as well as to many islands lying off the African continent (Stofberg 1954). FCM has adapted from its wide indigenous host plant range to cultivated crops. It prefers citrus as a cultivated crop, but also attacks a range of deciduous, subtropical, and tropical plants (Economides 1979).

To study population dynamics, it is important to have precise knowledge of the relationships between temperature, the rate of development, and reproduction (Golizadeh *et al*. 2009). The population dynamics of an insect population are influenced by the critical climatic factor, temperature. Temperature plays a major role, as it is able to set the limit of biological activities in arthropods (Roy *et al.* 2002). The effect of temperature can be attributed to the set rate functions of temperature for survival, reproduction, development, and population growth (Roy *et al.* 2003).

Temperature-dependent models can be developed to describe the relationship between temperature and development (Briere *et al*. 1999). Knowledge of a change in population dynamics due to temperature is important in pest management, due to the prediction that it allows in the timing of development and reproductive potential (Roy *et al*. 2002). The effect of temperature on life table parameters may vary, depending on such other ecological factors, such as host plants (Gilbert & Raworth 1996). The effect of the host plant on the life table parameters is dependent on the quality of certain components, such as carbon, nitrogen, and defensive metabolites (Gould *et al.* 2005).

The importance of a demographic study was stated by Vargas *et al.* (1997), as illustrated through several applications, such as the analysis of population stability and structure; the

estimation of extinction probabilities; the predicting of a pest outbreak of a particular species; and the analysis of the dynamics of colonising or invading species (Golizadeh *et al*. 2009).

Life table parameters allow for the measurement of population growth capacity under specific conditions. A key life table parameter is the intrinsic rate of natural increase (r_m), which is defined by Birch (1948) as being "the rate of increase per head under specified physical conditions, in an unlimited environment where the effects of increasing density do not need to be considered". The r_m, therefore, allows for the prediction of population growth potential under given environmental conditions (Southwood & Henderson 2000).

For the development of an effective pest management system, it is important to have a thorough understanding of the biology of FCM on all deciduous fruit kinds, including studies of the natural mortality of all developmental stages on different hosts, to establish which kinds of fruit to target for control. The Western Cape can be seen as a centre for fruit production in South Africa, with the stone fruit production areas in the country being illustrated in Fig 4.1 below. Due to the high agricultural potential of the area, few farmers have only one kind of fruit on their farm, and there tends to be more than one kind of fruit grown in an area. The variety of fruit grown tends to lead to increased pressure on the effectivity of any integrated pest management programme used, specifically for polyphagous pests such as FCM. It would be expected that the different kinds of fruit would exhibit clear differences in their ability and their potential to facilitate the complete FCM life cycle due to differences in FCM affinity for different citrus cultivars, with navel varieties being attacked more severely (De Villiers & Grové 2006) compared to Valencia oranges (Hofmeyr 1998).

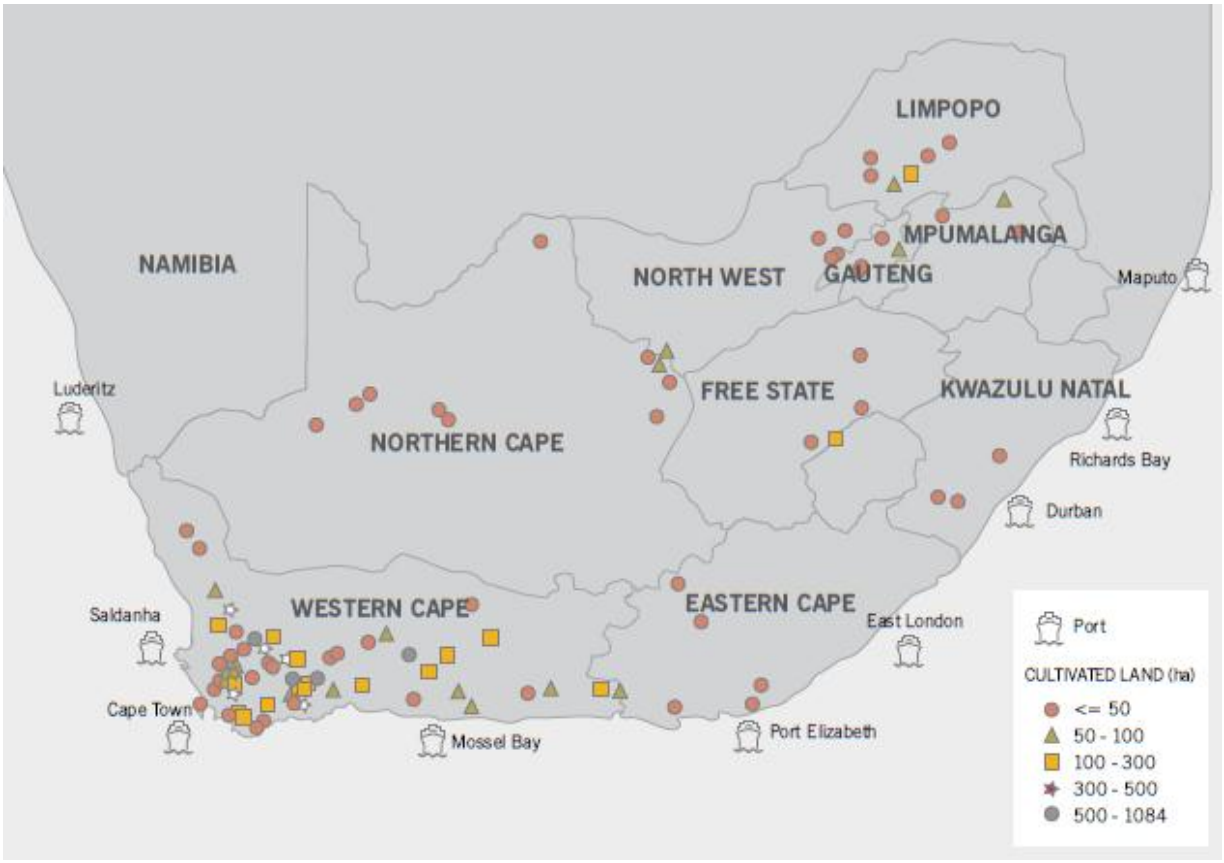


Fig. 4.1. Profile of stone fruit in the South African fruit industry (www.hortgro.co.za).

The aim of the experiment was to define the population parameters of various kinds of fruit in the laboratory. Egg fecundity and mortality, and larval and pupal mortality for FCM were investigated to determine which crops are more susceptible to FCM and therefore where management strategies should be focused.

Material and Methods

Population dynamics testing egg-laying ability and adult longevity at constant temperature.

The trial was conducted using a constant diet medium, supplied by Entomon Pty (Ltd), Stellenbosch, Western Cape, South Africa. The adults were taken directly after emergence from the pupal state, as supplied by the XSIT rearing facility in Citrusdal, Western Cape, South Africa. A 350 ml clear plastic honey jar (Plastics for Africa, South Africa) was used to house a newly emerged adult mating pair. The pair was then allowed to lay eggs until both the male and female inside the container had died. The containers were placed in an incubator (MRC, BOD-150, Israel) at 25°C, measured with Hydrochron DS1923-F5

temperature/humidity iButtons, with an accuracy of ±0.6% (Maxim/Dallas Semiconductor, Sunnyvale, CA, USA) The daylength was regulated at a constant 16:8 [L:D]. This trial was replicated 25 times.

Measurements

The following was monitored daily:

- the number of eggs that were laid
- the survival of males and females, measured in the number of days survived.

Population dynamics using different host treatments at constant temperature.

Experimental layout

The experimental layout consisted of five treatments, with twenty repetitions for each treatment. The five treatments used were apples, pears, grapes, oranges, and an artificial diet (Guennelon *et al*. 1981), as described in the experiment above. These were used as different developmental mediums. The diet medium was used as a baseline comparison for FCM development. Each treatment, except for those of the apples and the artificial diet, consisted of an individual fruit. In pre-runs, it was found that the apples were the only fruit that had not been penetrated by FCM. As apples were also the hardest fruit tested, an attempt was made to mimic field conditions, by placing the fruit in tandem. Doing so allowed for possible burrowing between the fruit where contact was made (Bentley & Viveros 1992) which is where codling moth, *Cydia pomonella* (Lepidopetra: Tortricidae), damage was found to be present. The individual fruit were placed in a 1-L plastic ice cream container (Plastics for Africa, South Africa), while the apples, in tandem, were placed in 5-L containers (Plastics for Africa, South Africa). The lid of the container was perforated, allowing for adequate air movement, and it was covered by a mesh with a diameter of 2 mm. A wax paper sheet with 2 FCM eggs was placed on the fruit surface inside the allocated container. Prior to inserting the wax paper into the container it was sterilised using Sporekill® in a 5% solution.

Measurements

Eggs and larvae

The containers were monitored daily for the following:

- the developmental period until hatching occurred;
- the first instar mortality, as larvae that failed to penetrate fruit;
- the larval mortality from egg hatch to fifth instar emergence;
- the period up to larval emergence from the fruit (i.e. the amount of time that was spent inside the fruit);
- the amount of time from larval emergence until cocoon formation (i.e. the amount of time that was spent both inside and outside the fruit);
- the number of larvae that emerged, and the number of larvae that entered the fruit; and
- the location of the point of entry of the larvae into the fruit.

The cocoon

The base of each container was lined with two materials. The first was a layer of cat litter crystals (Marltons, South Africa), to assist with the absorption of fluid expelled by the deteriorating fruit. The second was a layer of sawdust, to assist with pupation.

The following was monitored:

- the time from cocoon formation until the cocoons emerged;
- the number of cocoons formed after the larvae emerged; and
- the number of cocoons that emerged into adults, as well as the time until emergence.

Statistical analysis

The data were analysed using Statistica version 10.0 (Statsoft, Oklahoma, USA). A one-way ANOVA with post hoc comparisons of means, using Bonferroni's method, was used whenever the residuals were found not to be normally distributed (Efron & Tibshirani 1993). The non-parametric Kruskal–Wallis ANOVA test was performed to test for differences in the average FCM developmental parameters for three kinds of fruit. Each parameter tested was measured as part of the total developmental period. Levene's test for the homogeneity of

variances was performed for each of the FCM developmental parameters for the three kinds of fruit. A t-test assuming equal variances was performed to analyse the gender ratio. A chi-square 3×3 analysis was performed on the different kinds of fruit for the developmental periods considered.

Results and Discussion

Adult longevity and fecundity

The temperature was maintained at an average temperature and relative humidity of 25.7^{0}C and 49%, respectively. The adult female longevity was slightly longer than that of the male on diet (Table 4.1). An average of 172 eggs were laid, which coincided with results described by Stofberg (1939) and Stofberg (1954). This was far less than the 460 eggs per female that were laid at constant temperature of 25°C as described by Daiber (1980). Egg laying peaked after three days, which was also stated by Catling & Aschenborn (1974) and Newton (1998), due to the fact that the females tend to mate shortly after emergence (Stofberg 1954). Parameters such as R_0, r_m and T can be used to predict population dynamics. Studying the rate of increase per female under the above mentioned conditions allows us to determine the intrinsic rate of natural increase (r_m) (Birch 1948, Carey 1993). The calculated value provides information on biotic potential and survival of the species. The value is indicative of the species longevity, developmental speed and fecundity (Carey 1993). Calculating R_0 gives us the value for net reproductivity, while T is the relevant generation time (Vranken & Heip 1983). The intrinsic rate of natural increase for FCM (r_m= 0.1247) can be seen in Table 4.1. The r_m value was much lower than that of the Diamond back moth (*Plutella xylostella* (Lepidoptera: Plutellidae) where it ranged between 0.241 and 0.304, during life table studies conducted on Canola at similar temperatures (Soufbaf *et al.* 2010). The value is however between that of the diamond back moth on cabbage at temperatures of 20°C (r_m= 0.177±0.002) and 25°C (r_m= 0.285±0.006) respectively (Golizadeh 2009). We know FCM can survive a broad temperature range (Boardman *et al*. 2011), if the r_m values for these temperature ranges are constructed it can be used as the fundamental basis to the development of population dynamics models.

Table 4.1. Life table parameters for false codling moth on artificial diet.

Parameter	Diet medium	
	Male	Female
Adult longevity (days)	15.24	16.4
Number of eggs per female	171.68	
R_0	397.459	
r_m	0.125	
T	48.37	
Proportion of females: males	2.33:1	

Population dynamics, using different host treatments at constant temperature.

Developmental parameters for different host treatments

The developmental parameters for FCM on four host fruits at 25°C are given in Table 4.2 below.

Apples proved to be an unviable host for FCM, with no fruit being penetrated, leading to no offspring. Kruskal-Wallis multiple comparison test confirmed the developmental period for eggs on grapes differed significantly from that on pears (p= 0.0309) and oranges (p= 0.000001), with pears also differing significantly from oranges (p=0.0438). The developmental time that the larvae spent inside the fruits did not differ significantly between grapes and pears, although both differed from oranges (Fig. 4.2). The total larval developmental times (spent both inside and outside the fruit) on grapes differed significantly from those on oranges (p= 0.00006) (Fig. 4.3).

It could be deduced that the longer developmental time, and, thus a higher feeding potential as a result of better food quality during larval development inside the fruit, could lead to a higher survival potential (in numbers) for the prepupae forming a cocoon (Daiber 1979a).

The sex ratios were found to be more than 1:1 for citrus, pears and grapes. When comparing the sex ratio across the three kinds of fruit using a t-test, it was found that the means did not differ ($\rho>0.3$) between males and females. Females could easily be distinguished from males by the densely packed elongated scales on the hind tibia of the males (Newton 1998). Adult longevity was, on average, longer than was described in Hepburn & Bishop (1954), with the average life span on citrus being shortest for the females (8.5), and longest on the pear for the females (17.5).

The developmental periods of cocoons were substantially longer for pears, compared to the grapes. The total developmental times up to adult emergence were also calculated (Table 4.2). Oranges had, on average, the shortest cocoon duration before adults emerged. It is also clear that the cocoon stage is a less sensitive stage, as all the cocoons formed led to adult emergence. The amount of time involved was much shorter in the current study than was previously described in Daiber (1979a), ie. 21 to 80 days in the field, probably due to more fluctuating temperatures occurring in the field. Further, the only treatment with multiple entry points was the oranges, with only one case, although only one larva exited the orange. Newton (1998) states that larvae may be cannibalistic toward eggs and other larvae. It is very rare that more than one larva completes its development in a single fruit (Catling & Aschenborn 1974).

The egg-laying potential of the adults emerging from the fruit could not be determined, as was done for the adults on the diet medium. The adults emerged at very different times, with there being days where only males emerged and vice versa. The delayed mating period would, therefore, influence the mating ability. It was also not possible to measure mating ability for the adults in the pear treatment, as only females emerged.

Table 4.2. Developmental parameters (in days ±SE) of false codling moth on oranges, grapes, pears and apples at a constant temperature of 25^{0}C and a 12:12 (L:D) photoperiod.

Developmental stage	Treatments *							
	Oranges		Grapes		Pears		Apples	
Eggs	12,42 b (±0.53)		6,47 a (±0.48)		9,46 c (±0.55)		-	
Larval development inside fruit	12 b (±0.87)		11 a (±0.81)		10 a (±1.05)		-	
Larval development outside fruit	19 ab (±1.16)		18 a (±1.12)		23 b (±2.97)		-	
Last prepupae observed (days)	34		29		34		-	
Period in which cocoons were observed (days)	11		12		5		-	
Average longevity of cocoons	♀	♂	♀	♂	♀	♂	♀	♂
	8.5 (±1.16)	9.2 (±1.16)	13 (±1.12)	12.5 (±1.12)	17.5 (±2.97)	0	-	-
Total developmental time	40.92 c (±1.09)		37 a (±1.05)		50 b (±2.78)		-	
Gender ratio (male:female)	5:8		2:5		0:2			

*Numbers followed by the same letter in a row do not differ significantly ($\rho \leq 0.05$).

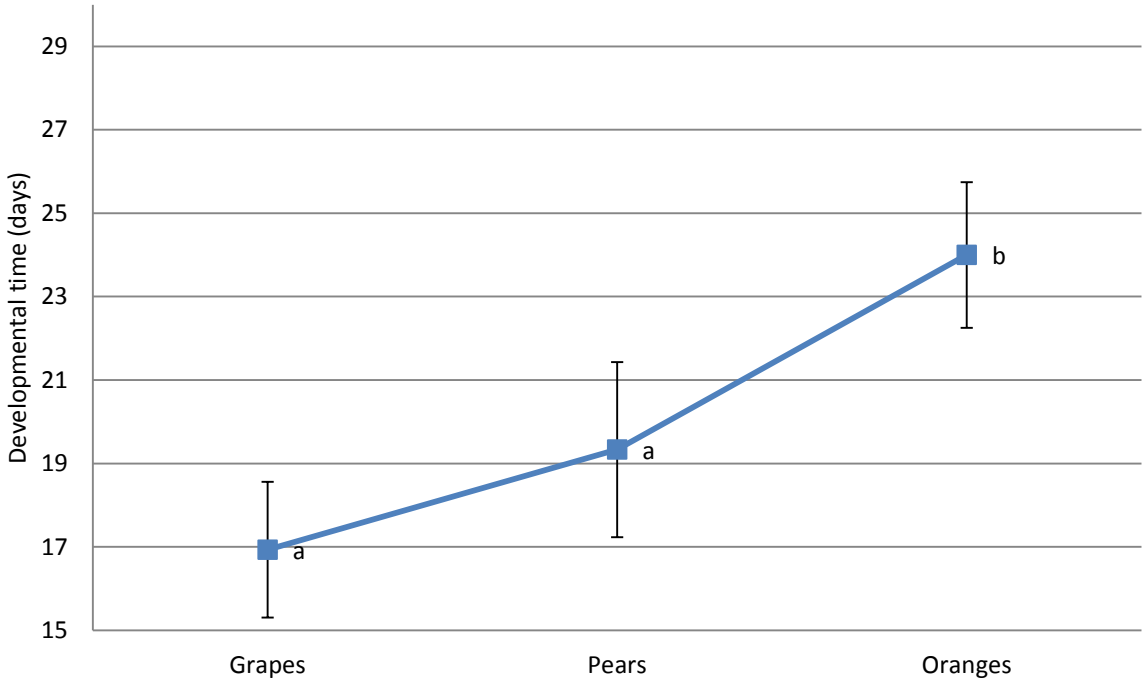


Fig. 4.2. Mean period for false codling moth larvae development (days) inside fruit, after egg development on three kinds of fruit at 25^{0}C, with 95% confidence intervals. Bars with the same letter show that the differences were not significant ($\rho \leq 0.05$).

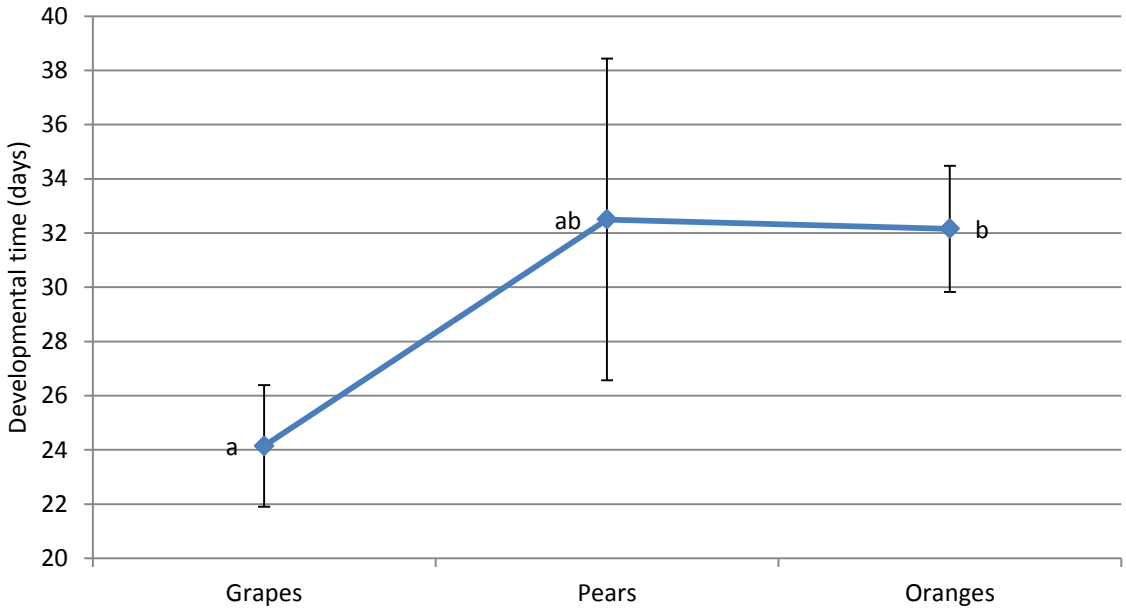


Fig. 4.3. Mean period for false codling moth larvae development (days) on three kinds of fruit at 25^{0}C, with 95% confidence intervals. Bars with the same letter show that the differences were not significant ($\rho \leq 0.05$).

FCM survival for the different kinds of fruit at the respective life stages is shown in Table 4.3. The low survivorship on pears could be due to the high moisture content and membrane deterioration of the over-ripe pears possibly being detrimental to FCM larval development inside the fruit, such as the sensitivity to wet soils (>20% moisture) for non-diapausing pink bollworm larvae (Clayton and Henneberry 1982) and ≥60% moisture for diapausing pink bollworm larvae (Venette *et al*. 2000). Nine larvae emerged from the deteriorating pear samples, but only two cocoons were formed. The chi-square analysis proved that the most deviation from the theoretical frequencies occurred in the case of the pears, from the pupa stage. The theoretical frequency (6.23) being much larger than the observed frequency (2). The fruit was found to have a significant influence (Pearson's value = 5.516964, p>0.2) on the different developmental periods, as the two categorical variables (fruit kind and developmental period) were related, The successful development of FCM in oranges, grapes and pears could facilitate the pest being active throughout the year, with a continual supply of fruit (Newton 1998).

Table 4.3. False codling moth survival (total n = 20) at different life stages on four kinds of fruit.

Variable	Grapes	Pears	Oranges	Total
Number of eggs completing development	17	13	14	44
Number of larvae	15	9	13	37
Number of cocoons formed	14	2	13	29
Number of adults emerging from cocoons	14	2	13	29

According to Hofmeyr (pers. comm.) the first instar larvae generally enter the navel oranges through the navel end of the fruit. The larvae are also opportunistic, entering through wounds and cracks on the fruit. As the fruit ripen, the larvae may enter through the cheeks of the fruit (Stotter 2009). Table 4.4 below indicates the exact entry point of the larvae into the specific fruit kind. This is visible from the frass that is left on the fruit surface, and from the clear entry hole. In the oranges, most entries occurred at the navel end, whereas, in grapes, most entries occurred at the top (stalk) end / in the middle section. Entry points for pears were more evenly distributed between the bottom and the bottom/middle sections.

Table 4.4. Locations of false codling moth entry points on oranges, grapes, pears and apples, at a constant temperature of 25^{0}C, and at a 12:12 (L:D) photoperiod.

	Treatments			
Entry point	Oranges	Grapes	Pears	Apples
Top	8	7	0	-
Top/Middle	4	8	1	-
Middle	2	1	2	-
Bottom/Middle	0	1	5	-
Bottom	0	0	5	-
Total	14	17	13	

Conclusion

The developmental parameters illustrated the ability of FCM to complete its life cycle from egg to adult stage on grapes, oranges, and pears, but not apples. This is of importance for post-harvest treatments and for management recommendations. Although the fruit differed greatly in terms of potential to host FCM, they were all viable hosts although FCM larvae were not able to penetrate apples, without prior damage having been done to the fruit. The larvae did manage to reproduce at a faster rate on the grapes compared to their reproduction rate on citrus, which should be taken into account when damage is noted. However, FCM is often not correctly identified in the field.

Future research should focus on the egg-laying ability of the adults reared from the individual fruit, which would thus determine the population growth potential. Such potential is relevant to orchard sanitation.

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Chapter 5: Conclusions and suggestions for future research

False codling moth (FCM), *Thaumatotibia leucotreta*, has been researched covering a wide variety of topics, mainly because of its relevance to the export market as a phytosanitary pest (Bloem *et al.* 2003). Adequate control of this elusive pest remains a concern. Integrated pest management (IPM) strategies, including chemical control, sterile insect technique and cultural control methods are often implemented, but the study is aimed at better understanding the pest biology and survival on alternate hosts to incorporate into the control package.

The objective of the thesis was to 1) evaluate diapause, if present, in FCM: 2) to determine population dynamics on different fruit kinds in the lab; and 3) to further examine FCM mobility and host preference between orchard blocks in the field. These are key issues which can improve the efficacy of an IPM program, which may be complicated by the polyphagous nature of this pest. A better understanding of FCM pest biology should fine-tune the target time for control actions and determine the best spatial strategy to use for an area-wide management programme. The results are discussed below as separate chapters:

An experimental test of diapause induction in false codling moth.

Larvae were exposed to a temperature treatment simulating the start of winter, as an attempt to induce diapause. The physiological assays conducted were not indicative of diapause. However this should be interpreted with some caution as it is possible FCM may nevertheless enter diapause in the wild, but our experimental methodology did not reproduce the precise set of abiotic conditions which may possibly trigger diapause under natural conditions (Tauber & Tauber 1976; Hunter & McNeil 1997). No studies have previously attempted to induce diapause in this species using a controlled change in photoperiod and temperature. To this end, the present work is both novel and important, particularly for pest management of the species in the region. For example, uncertainty around factors that should be incorporated into predictive modelling (e.g. day-degrees) can be reduced to improve forecasting efforts (Tobin *et al.* 2003). Another potential methodological limitation is that in some Tortricidae species the parental generation must experience a specific set of altered seasonal conditions for the F1 generation to be able to enter diapause (Hunter & McNeil 2000; Mousseau &

Dingle 1991; Dixon 1971). Thus, this work could be further improved by expanding the range of conditions and generations assayed for their potential ability to induce diapause in FCM. If FCM do indeed not undergo diapause, they should be susceptible to pesticides throughout the year (Denlinger 2008). This will make a good understanding of distribution between fruit kinds during the season even more important.

Distribution of the false codling moth in stone fruit, pome fruit and citrus orchards.

The orchard blocks in the trials had a very low recapture rate. This could be due to low temperatures and poor moth quality. The windbreaks could have contributed to impaired FCM distribution (Pasek 1988). The fruit kinds in the trial area also varied in their ability to host FCM as seen by damage assessments, with damage increasing as the fruit kinds ripen. The damage assessments were fairly similar in the prevalence of FCM between the fruit kinds that were present at the given assessment dates. FCM was reared from the damage assessments in apples. This is important because FCM has not yet been documented to occur on apples (USDA 1984, in USDA 2007). However, in addition to the apples the artificial diet was provided to optimize the development of larvae into adults for easier identification. FCM emergence could be attributed to FCM eggs possibly being on the fruit or present as secondary pests on the apples. This can be determined if the damage assessments are repeated and the larvae that are removed from the infested fruit are identified.

Future research should be focused on the mating potential of FCM emerging from alternate hosts as this could give a clear indication of the potential FCM population size. Cultivated crops could play a significant role in supporting FCM populations (Stotter 2009). It should also be examined whether the FCM life cycle can be completed in an in-field study, where it is exposed to all natural conditions which might not be optimal for its development. Movement between different cultivated crops could act as a bridge between successive seasons (Stotter 2009). Understanding the seasonal movements will contribute to improved control methods by interrupting the population dynamics at their most vulnerable stages.

Developmental parameters of the false codling moth on four commercial hosts.

The first part of the chapter is focused on adult longevity and fecundity. Egg laying peaked after three days, which was also stated by Catling & Aschenborn (1974) and Newton (1998). This is due to the fact that the females tend to mate shortly after emergence (Stofberg 1954). The calculated r_m value gives us insight into the species longevity, developmental speed and

fecundity (Carey 1993). The generation time under ideal environmental conditions was calculated to be 48.37 days. These results could be incorporated into predictive modelling to improve forecasting efforts (Tobin *et al.* 2003).

We then examined population dynamics, using different host treatments at constant temperature**.** Oranges, grapes and pears were viable hosts for FCM. Future research should look at life table studies on the different fruit kinds. The parameters would differ from these found on the artificial diet. This will be a valuable tool in predicting population dynamics. Cultivar preferences within the fruit kinds examined should be further examined. This could be done through choice, no-choice methods (Greenberg *et al*. 2002).

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