

**THE PEST STATUS AND INTEGRATED
MANAGEMENT PROGRAMME OF CAROB MOTH,
Ectomyelois ceratoniae ZELLER, ATTACKING
CITRUS IN SOUTH AFRICA**

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ABSTRACT

The carob moth, *Ectomyelois ceratoniae* Zeller, is a pest of agricultural commodities and stored products around the world. Carob moth is known to infest citrus in the Mediterranean region and in southern Africa. In grapefruit cultivars, carob moth infestations are associated with high levels of mealybug. However, although this relationship has been observed in other citrus types such as Navel oranges, this has never been quantified. A recent survey of infested fruit from various production areas in South Africa indicated that the pest status of carob moth on Navel oranges may have been underestimated. As a result of the incidental pest status of carob moth on citrus in South Africa in the past, a species specific integrated pest management (IPM) programme does not exist. Therefore, the overriding aim of this theses was to evaluate the pest status of carob moth in citrus and establish a species specific IPM programme by determine the autecology of carob moth in citrus.

Reliable methods for monitoring carob moth in citrus orchards both for producers and for research purposes were developed. A user-friendly monitoring method for determining weekly carob moth infestation through dropped fruit was suitable for producers. A timed scouting method was also developed; although the accuracy of this method varied with the experience of the scout. The pest status of carob moth was highest in the Loskop Valley, Nelspruit and the Vaalharts production areas and economic injury to growers ranged from R512.35 to R3 719.80 per hectare as a direct result of infestation. No infestation was recorded in the Sundays River Valley and Citrusdal production areas over both the 2014-15 and 2015-16 growing seasons.

A laboratory study showed the survival of carob moth larvae infesting citrus is less than 10% in the absence of mealybug. However, this increases to almost 40% in the presence of mealybug residues and sooty mould. There was a significant relationship between carob infestation at harvest and mealybug infestation in the middle months of the growing season. The relationship between carob moth and mealybug indicates that current production guidelines for the management of mealybug in citrus may need to be amended. Consequently, it is proposed that an orchard with a history of carob moth infestation and a high mealybug infestation in the previous season should be subjected to an early season preventative application of a registered control product. Also, if mealybug infestation in December is higher than a 5% of fruit per tree, then a corrective application of a registered product is recommended. The application of 2,4-D at petal drop reduced the size of the navel-end opening, decreasing

the proportion of mealybug found in the navel-end, subsequently reducing carob moth infestation, resulting in a direct benefit for producers.

Products registered for the control of false codling moth (FCM), *Thaumatotibia leucotreta* Meyrick, were effective in reducing carob moth infestation. In a spray trial conducted over two seasons, Delegate® and Runner® reduced infestation significantly in the 2014-15 season (over 80%), while only Delegate® was effective in the 2015-16 season (over 80%). If a late season corrective chemical application is targeted at both FCM and carob moth, this application should take place between 6-7 weeks prior to harvest. The mating disruption product, SPLAT® EC, reduced carob moth infestation by 70% compared to the untreated control.

A laboratory culture was established and head-capsule size categories were determined for all five carob moth instars. A parasitoid survey indicated that parasitism of carob moth larvae is generally less than 5% in citrus orchards and a new species of Braconidae was described as *Phanerotoma carobivora* van Achterberg and Thackeray. Carob moth fifth instar were found to be the most cold-tolerant larval stage, and were shown to be more cold susceptible than the most cold-tolerant FCM instars at -0.55°C for eighteen days. This cold treatment resulted in a mortality of 94.6% fifth instar carob moth compared to a combined fourth and fifth instar mortality of 87.8% for FCM after eighteen days. These results indicate that post-harvest cold treatments targeting FCM will be as, if not more, effective against carob moth, suggesting that current phytosanitary legislation for carob moth should be amended to incorporate this study's findings.

DECLARATION

The following thesis has not been submitted to any university other than Rhodes University, Grahamstown, South Africa. The work presented in this thesis is the original work of the author, and in the case of Chapter 6 contributions were made by co-authors of the published article.

Sean Robin Thackeray

RESEARCH OUTPUTS

The research conducted in this thesis has resulted in the following scientific outputs at the time of submission:

PEER REVIEWED PUBLICATIONS

Van Achterberg, C., Thackeray, S. R., Moore, S. D. and Hill, M. P. 2016. A new species of *Phanerotoma* Wesmal (Hymenoptera: Braconidae: Cheloninae) reared from carob moth in South Africa. *Zootaxa*, In press.

ARTICLES IN POPULAR PRESS

Thackeray, S. R. 2015. Carob moth on citrus: where do we stand? *Rostrum*, December issue.

SCIENTIFIC CONFERENCE OUTPUTS

Thackeray, S. R., Kirkman, W., Moore, S. D. and Hill, M. P. The development of an IPM programme for carob moth on citrus in southern Africa.

- Presented at the 9th Citrus Research Symposium 2016 (Drakensburg, South Africa) and the International Congress of Entomology 2016 (Orlando, USA).

GROWER WORKSHOPS

Thackeray, S., Kirkman, W. and Moore, S. IPM of carob moth on citrus.

- Presented at the Citrus Research International Spring Pest Complex Growers Workshops (2015) in Hoedspruit, Groblersdal, Nelspruit, Jefferies Bay and Citrusdal.

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CHAPTER 1: LITERATURE REVIEW AND STUDY AIMS

1.1 Introduction and motivation for this study

South Africa has over 100 citrus pests that are of economic importance and these are often split into major and minor pest complexes due to the varying climate in which citrus is produced (Bedford 1998). These pests include a variety of mealybugs, scale insects, leafhoppers, beetles, thrips, fruit flies and various moth and butterfly species. The major pest of citrus in southern Africa is the false codling moth (FCM), *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae), which is endemic to sub-Saharan Africa. Control of FCM in southern Africa takes the form of a multi-faceted integrated system, which includes orchard sanitation, chemical applications, sterile insect technique (SIT), biological control through the augmentation of parasitoids and application of microbial products (Moore and Hattingh 2012). Citrus fruit infested with FCM larvae undergo a physiological response which results in the early ripening and subsequent abscission of the fruit from the tree (Newton 1998). Although this has caused major monetary loss for growers in the past, in recent years this has become almost negligible due to improved control measures.

The South African citrus industry is the second largest global exporter of citrus, worth an estimated R11.5 billion annually (CGA 2016). Certain pests of citrus in South Africa are of phytosanitary concern to potential target markets of exported citrus. Due to FCM's endemism to the region, there are phytosanitary restrictions associated with this pest. An interception at the receiving port can result in the particular consignment being rejected and repeated interceptions could result in the closure of that particular export market. It is common for target markets of South African citrus to require cold sterilisation of fruit at a set temperature over a certain period of time to ensure that no pests within the shipment are left alive, but this comes at huge cost and significantly reduces profit margins.

Navel oranges are a preferred citrus host of FCM and are highly susceptible (Love *et al.* 2014). A recent study, by Citrus Research International, was undertaken to evaluate current cold sterilisation techniques against FCM in Navel oranges (Moore *et al.* 2014b). Large samples of infested Navel oranges, collected from a number of different orchards throughout South Africa were subjected to cold treatment trials and the opportunity was taken to identify each larva from fruit dissected throughout the study. Carob moth (*Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae)) larvae and FCM larvae are very similar in appearance; both

share a preference for certain cultivated hosts, and may be confused by producers (Honiball and Catling 1998). They are both pink in colour and of similar size, but have distinct morphological differences that can be used to distinguish between them (Honiball and Catling 1998). Using the diagnostic tool developed by Rental (2012), it was determined that up to 60% of fruit infestation in the Loskop Valley production area (Limpopo Province, South Africa) was by carob moth, and not FCM as originally thought. With up to 40% in Nelspruit (Mpumalanga Province, South Africa) and over 10% in the Eastern Cape Province (Moore *et al.* 2014a). This has brought the current pest status of carob moth on Navel oranges into question.

In southern Africa the carob moth is currently considered a minor pest of citrus, with outbreaks generally associated with mealybug on grapefruit varieties (Honiball and Catling 1998). This has resulted in little research conducted on the ecology and pest status of carob moth on other citrus types. Morland (2015) has been the most comprehensive study to date, however, this study was restricted to the Western Cape Province and focused mainly on describing diagnostic features of different life stages. With no treatment thresholds or registered methods for control (Grout and Moore 2015), there is a need to resolve the pest status of the carob moth on citrus throughout South Africa's major growing regions, and to establish an effective management programme for the control of this species in citrus orchards. The text that follows will provide a review of available literature on carob moth, focusing on its pest status, distribution, life history, phytosanitary status and control methods.

1.2 Nomenclature

Ectomyelois ceratoniae is a phytophagous pyralid belonging to the subfamily Phycitinae. Phycitine moths are usually small to medium sized and have a wide range of colours, sometimes being mimetic (Munroe and Solis 1998). Phycitine prefer warmer areas and are restricted to the tropics and subtropics. This subfamily consists of many economically important pests of stored products (Aitken 1963). Larvae belonging to this subfamily are usually concealed feeders, often making detection difficult (Munroe and Solis 1998).

The species *ceratoniae* has been placed in four genera throughout its taxonomic history; *Ectomyelois*, *Myelois*, *Spectrobates* and *Apomyelois* and 15 synonyms are listed for the species name. Morland (2015) writes in-depth on the prolific generic history of the carob moth, concluding that “misconceptions made by all authors were because descriptions were based on wing colouration, which is a poor character trait”. The carob moth (Gothilf 1968) is also known

as the locust bean moth (Goater 1986), the carob bean moth (Gonzalez and Cepeda 1999), the pomegranate fruit moth (Krasil'nikova 1964, Moawad 1979), and the pomegranate fruit worm (Al-Jamali 2006).

1.3 Distribution and pest status

The carob moth originates from the Mediterranean and is widely distributed in Europe, Africa, Arabia and Australasia where it is a pest of economic concern. However, the pest status of this moth differs between regions and commercial hosts (Table 1.1), mainly due to local conditions which influence a range of population demographics (Mehrnejad 2001). Carob moth is a major pest in date gardens in the Coahella Valley in California, United States of America, accounting for up to 40% of crop loss and costing stake holders millions of dollars in damage every season (Warner 1988, Nay and Perring 2006, 2008). In Australia it is a major pest of almonds (Madge *et al.* 2013) and in South America it is a minor pest of walnuts (Lange 2011). In the Near and Middle East it is a major pest of pomegranates, dates and the carob tree, while being a minor to major pest of citrus in the Mediterranean (Gothilf 1969, 1975, Orphanides *et al.* 1996). In South Africa carob moth is regarded as a minor or sporadic pest, and is only of slight economic concern in pecans (Moore *et al.* 2014a, Morland 2015), citrus (Catling 1970, Moore and Grout 2015), pomegranate (Giliomee and Barnes 2015), and is present in macadamia orchards (De Villiers 2001, Schoeman and De Villiers 2015).

Table 1.1 The global pest status of the carob moth on various agricultural commodities

Host	Country	Stored	Field	Pest status	Source
Citrus	Israel		x	**	Gothilf 1975, 1969
Citrus sp.	Turkey		x	**	Ozturk <i>et al.</i> 2011
	Cyprus		x	***	Orphanides <i>et al.</i> 1996
	Swaziland		x	*	Catling 1970
	South Africa		x	*	Catling 1970, Honibal and Catling 1998, Grout and Moore 2015
	Egypt			**	Hashem and El - Halawany 1996
Carob tree <i>Ceratonia siliqua</i>	Israel			****	Gothilf 1969
Almonds	Israel	x		***	Calderon <i>et al.</i> 1969, Navarro <i>et al.</i> 1986
<i>Prunus dulcis</i>	Australia		x	****	
Pistachio	Rafsanjan	x	x	***	Mehrnejad 1993
<i>Pistachio vera</i>	Iran		x	*	Mozaffarian <i>et al.</i> 2006
Dates	USA		x	****	Warner 1988, Nay and Perring 2006
<i>Phoenix dactylifera</i>	Tunisia	x	x	***	Mediouni and Dhouibi 2007

Pomegranate	Iraq	x	***	Al - Izz 1987
<i>Punica malus</i>	Iran	x	***	Fesharaki <i>et al.</i> 2011
	Tunisia	x	****	Mediouni and Dhouibi 2007
	South Africa	x	*	Giliomee and Barnes 2015
Walnuts	Argentina	x	*	Lange 1991
<i>Juglans sp.</i>				
Pecans	South Africa	x	*	Morland 2015, Moore <i>et al.</i> 2014a
<i>Carya illinoensis</i>				
Macadamia	South Africa	x	*	De Villiers 2001, Schoeman and De Villiers 2015
<i>Macadamia integrifolia</i>				
* = Minor , **** = Major				

1.4 Carob moth attacking citrus

In South Africa, carob moth was first reported on citrus in the Clanwilliam district of the Western Cape Province, where larvae were found in splits of the rind of ripening Navel oranges (Catling 1970). In 1969 it was observed in the Lowveld of Swaziland and in 1970 this species had been recorded in Navel oranges and Grapefruit from Citrusdal, Nelspruit and Pretoria (Catling 1970). Today carob moth is regarded as a minor pest of citrus, favouring grapefruit varieties (Bedford 1998, Grout and Moore 2015). However, very little is known about the life cycle and behaviour of the carob moth in citrus orchards in South Africa (Morland 2015).

In the eastern Mediterranean region, the ecology of carob moth on citrus is better understood. Israel did not consider the carob moth a pest of citrus until the late 1950s when it was found that infestation in grapefruit caused high levels of economic loss though fruit drop (Avidov and Gothilf 1960). In Cyprus and Turkey there is an understanding of the ecology of carob moth on grapefruit varieties where many studies have been undertaken to investigate the seasonal occurrence, economic impact and suitable conditions for outbreaks of this species (Avidov and Harpaz 1969, Gothilf 1964, 1969, Sergiouh 1983, Orphanides *et al.* 1996). However, in the western Mediterranean, where other citrus varieties are attacked, little is known about the moth's autecology on citrus (Gothilf 1964). Carob moth has been reported as a minor pest of Washington Navel oranges in Egypt (Hashem and El - Halawany 1996) and a pest of Navel oranges in Spain (Ebling 1959).

Gothilf (1969) described infestation of grapefruit by carob moth in Israel. The carob moth overwinters in natural hosts such as the carob tree and acacia pods and egg laying in citrus groves begins in mid-summer and lasts as long as temperatures permit adult activity. In susceptible orchards roughly 15% of fruit were infested with usually one larva per fruit. Females will oviposit one or two eggs beneath the calyx and neonate larvae will enter the fruit at this point, a physiological response by the fruit causes gum to be exuded and this usually results in the death of the larva. However, later on in the season when gum exudation is less intense, larvae are able to survive. Larvae will feed on the albedo and the rind but do not enter far into the flesh, and this feeding damage causes fruit to abscise from the tree, resulting in economic losses for the grower. Larvae will then pupate on the fruit epidermis and pupae were never recorded within citrus fruit (Gothilf 1969). The life cycle of carob moth infesting Navel orange cultivars is not as well documented, but the literature is in agreement that larvae will feed on the rind but seldom penetrate far into the flesh of the fruit (Catling 1970, Honiball and Catling 1978, Grout and Moore 2015). However, oviposition and pupation sites are unknown.

The increase in incidences of carob moth being found in southern African citrus orchards in the 1970s is thought to be attributed to an increase in the use of pesticides which are detrimental to beneficial insect communities, and resulted in outbreaks of secondary infestations including most honeydew producing insects (Honiball and Catling 1978). In Cyprus, carob moth is known as a pest of citrus in the form of a mealybug - carob moth complex. Serghiou (1983) conducted numerous chemical control trials over several years in Cyprus, and found that if mealybug was under good control, fruit drop as a result of carob moth infestation would be negligible. Over and above these field trials, an un-replicated laboratory trial involving the placement of neonate larvae onto grapefruit with and without mealybug, revealed that carob moth larvae could not survive on grapefruit in the absence of mealybug (Serghiou 1983). Gothilf (1964) noted that carob moth females will oviposit 1-2% of eggs on mealybug free fruit, however, on grapefruit infested with mealybug, up to 14% of fruit were infested by carob moth larvae.

Citrus is the only known host that gravid carob moth females oviposit their eggs on the surface of the host. Usually fruit or pods which have been damaged or have a favourable opening for oviposition, such as pomegranates, are selected. This leaves the reasoning behind carob moth's selection of citrus as a host unresolved i.e. Are they attracted to citrus itself? The mealybug, the honeydew they produce or the sooty mould which grows on the honeydew?

1.5 Ecology and life history

The global pest status and economic importance of the carob moth has resulted in a considerable amount of research being conducted on various aspects of this moths' biology. This research has mainly focused on basic aspects of fitness on its various hosts and factors that are reported to effect the developmental biology (Table 1.2). However, despite over 40 years of research there is still no sound consensus as to which are the main factors responsible for the extreme variation seen, especially under field conditions (Nay and Perring 2006). This variation of findings can have serious implications on decision making when attempting to control carob moth using an Integrated Pest Management (IPM) approach.

Table 1.2 Studies that have dealt with life history parameters of carob moth and factors that influence these parameters

Life History Parameter	Factors influencing life history parameters
<u>Number of Generations per year</u>	<u>Host nutritional quality</u>
Gothilf 1970, 1974	Alrubeai 1987
Al-Izzi <i>et al.</i> 1985, 1987	Gothilf 1968, 1969, 1984
Warner 1988	Navarro <i>et al.</i> 1986
	Al-Izzi <i>et al.</i> 1988
<u>Developmental rates of egg, larval and pupal stages</u>	<u>Amino acids</u>
Gothilf 1968, 1969, 1970, 1984	Al - Izzi <i>et al.</i> 1988
Cox 1976, 1979	
Moawad 1979	<u>Fungi and other micro-organisms</u>
Navarro <i>et al.</i> 1986	Gothilf 1969, 1970
Alrubeai 1987	Cox 1979
Al - Izzi <i>et al.</i> 1988	Warner 1988
Warner 1988	
Al - Izzi and Al - Maliky 1996	<u>Relative humidity</u>
Nay and Perring 2006	Gothilf 1969
Nay and Perring 2008	Cox 1976
	Moawood 1976
<u>Adult longevity</u>	<u>Host moisture content</u>
Gothilf 1969, 1984	Gothilf 1968, 1969
Moawod 1979	Cox 1976
Navarro <i>et al.</i> 1986	Navarro <i>et al.</i> 1986
Al - Izzi <i>et al.</i> 1987	Warner 1988
Alrubeai 1987	Nay and Perring 2006

Larval Mortality

Gothilf 1968, 1969, 1970, 1984

Vavarro *et al.* 1986Al - Izzi *et al.* 1987, 1988

Alrubeai 1987

Female Fecundity

Gothilf 1968, 1969, 1970, 1984

Navarro *et al.* 1986Al - Izzi *et al.* 1987, 1988

Alrubeai 1987

Light regime

Cox 1979, Warner 1988

Adult weight on eclosion

Gothilf 1968

Cox 1979

Alrubeai 1987

1.5.1 Adult

The carob moth adult is a small, inconspicuous greyish moth with variable wing markings, body size and genital structures (Catling 1970). The wingspan is approximately 19-26 mm with the forewing being grey in colour with two faint and variable oblique stripes. The rear wing is light grey to white and fringed with long hairs. Wing shape of adults varies with nutritional availability in host plants and sexual dimorphism in wing size exists with females having larger wings than males (Mozaffarian *et al.* 2006, 2007). A lack of genetic basis for this variation in phenology was confirmed using AFLP primer combinations which showed high levels of variation within populations of carob moth in Iran, but non-significant genetic distances among sympatric host associated populations (Zare *et al.* 2012).

Adult longevity is also variable and can range from 5-15 days (Gothilf 1969), with female longevity (8.8 days) being slightly longer than males (5.6 days) (Mediouni and Dhouibi 2007). Adults emerge in the early hours of the scotophase with male moths eclosing on average two days before females (Cox 1976). Adult moths show little activity during daylight hours and will start to vibrate wings and antennae as light intensity diminishes (Cox 1976). Females exhibit a broad late scotophase peak in calling activity and as age increases, calling is initiated earlier each night and the time spent calling increases on successive days (Soofbaf *et al.* 2007). The majority of mating occurs between the fifth and sixth hour of the scotophase, which corresponds with the calling behaviour, and coupling lasts two and a half hours (Vetter *et al.* 1997).

Female carob moths oviposit approximately 200 eggs and this is variable according to larval diet, temperature and host moisture content (Gothilf 1969, Nay and Perring 2006). Eggs are oviposited during the twilight and dark periods (Al-Izzi 1987, Cox 1976), favouring the

first hour of the scotophase (Vetter *et al.* 1997), and females oviposit the greatest number of eggs during the third, fourth and fifth days of the adult life stage (Al- Izzi 1987; Vetter *et al.* 1997), of which roughly 80% of fertilised eggs will hatch (Alrubeai 1987). Fecundity can be as high as 90% and this is correlated to female weight which can differ significantly with larval diets and environmental conditions (Gothilf 1969; Nay and Perring 2008). Olfactory stimuli may play an important role in mediating oviposition. Female moths show an ovipositional preference to fruit infested with fungus (*Phomopsis* sp.) (Gothilf *et al.* 1975). Gothilf *et al.* 1975 observed that an extract of the steam distillate of carob pods infected with the fungus *Phomopsis* sp was more effective in stimulating female moths to oviposit than an extract of uninfected carob pods. The extract from fungus infested pods was composed mainly of simple alcohols: ethanol 60%, 1-propanol (15%), 2-propanol (2.5%), 2-methyl-1-propanol (15%), 1-butanol (2.5%) and 3-methyl-1-butanol (5%). With the exception of isopentanol, all the alcohols stimulated oviposition. However, when tested separately, the response elicited by the female carob moths was reduced (Gothilf *et al.* 1975). Ethyl hexanoate, a volatile compound extracted from fungus infected date fruit, stimulates upwind flight of female moths (Cosse *et al.* 1994). Carob moth females select pods that are damaged through cracking during the ripening season (Gothilf 1969, Mehrnejad 1993), or insect inflicted damage, such as the emergence holes created by the carob midge *Eumarchalia genadii* Marchal, which spends its larval stage within carob pods and then chews an emergence hole before adults leave the carob pods. This is also the case for *Acacia farnesiana*, where *Virachola livia* (Lepidoptera) and *Pseudopachymerus lallemandi* (Coleoptera) create suitable oviposition sites on the pods for carob moth females via their respective emergence holes (Gothilf 1969).

1.5.2 Eggs

Eggs of the carob moth are approximately 0.7 mm in length and 0.05 mm wide, ovoid in shape and generally oviposited individually or in clusters of two to three eggs (Morland 2015). Once oviposited, eggs appear white/yellow in colour and if fertilised will turn pink 12-24 hours after oviposition under a constant temperature of 25°C (Gothilf 1969). Unfertilised eggs tend to be fully rounded in the shape of an ellipse while unfertilised eggs appear as an empty sac. Development of eggs ranges from 1 to 3 days at 30°C and as temperature decreases developmental time may extend to 8 days (Gothilf 1969; Mediouni and Dhouibi 2007). Eggs held at temperatures below 20°C fail to hatch (Gothilf 1969; Cox 1976).

1.5.3 Larvae

Larvae are slender, elongate, cream white to light pink in colour with rugose integument and a head-capsule that is yellow-red-brown in colour. Morland (2015) discussed the morphology of the larvae and made comparisons to that of FCM, which carob moth larvae may be confused with in the field. There are usually five instars (Mehrnejad 1993) and in some cases a sixth instar may be reached during diapause (Gothilf 1969). To the author's knowledge a head-capsule size range to distinguish instars is not available in published literature. However, Gothilf (1969) lists larval instar sizes in larval length. These cannot be relied on, as four of the six instar sizes listed overlap in size ranges. Diapause, or overwintering, occurs in the larval form when day length is shorter than thirteen hours and average temperature below 20°C (Cox 1979). Carob moth is a chill intolerant insect, since overwintering larvae perish above their super cooling point (SCP), and the larval SCP varies from diapausing (-17.3°C) to non-diapausing (-12.0°C) (Heydari and Izadi 2014).

Developmental rates of the carob moth larval stage can differ due to variation in light regime, temperature and diet/host plant properties such as nutritional quality, temperature, relative humidity and moisture content. On unripe acacia pods, larval and pupal development took roughly 32 days at 25°C, while on ripe dry pods development could take close to three months (Gothilf 1969). If water was added to these dry pods, the growth of micro-organisms was enhanced, which in turn increased the speed of larval development, especially when naturally occurring *Phomopsis* sp. fungus was present (Gothilf 1969). Under mass-rearing conditions at the sterile insect technique (SIT) rearing facility in Tunisia, the mean duration of larval development was 23.7 days at 30°C, resulting in six generations per year (Mediouni and Dhouibi 2007).

The upper developmental threshold is 38°C (Ahmad and Ali 2005) and the lower developmental threshold is 12.5°C (Warner 1988). These thresholds have assisted in attempting to compile a degree-day model to assist in timing of chemical applications in date gardens (Nay and Perring 2008). However, there is a distinct sexual differentiation in developmental rates that is influenced by the host stage and moisture content, which is directly related to agricultural practices and the immediate environment (Nay and Perring 2006). Host moisture content has been shown to be the overriding factor in terms of population doubling time, suggesting that development is not solely temperature dependant and illustrates a possible confounding factor in previous research (Nay and Perring 2006, 2008).

1.5.4 Pupae

Pupae are approximately 11 mm in length, yellow to red-brown in colour with darker abdominal marking ventrally. Male pupae are identified by abdominal segments 8-10 being fused with two bullae which are present on segment 8, while female pupae are identified by abdominal segments 7-10 being fused and on the 9th segment a slit-like opening is distinguishable (Underwood 1994). Pupal development is seven days at 30°C with male pupae weighing 35-37g and females weighing 41-43g (Mediouni and Dhouibi 2007). Unlike the larval stage, pupal development is not influenced by host characteristics (Nay and Perring 2006).

1.6 Rearing carob moth in a laboratory environment

Having a laboratory culture of an insect pest is invaluable for experimentation that cannot easily be done in the field. The first attempt at rearing the carob moth in the form of a mass culture was by Gothilf (1969), who noted that if one was to be successful in establishing a laboratory culture of this species, a continuous supply of larvae from the field was a prerequisite for success. It takes six generations until a carob moth laboratory culture is regarded as stable (Al-Izzi 1987). As generations pass, the culture will become suited to a laboratory environment. Al-Izzi (1987) showed that only 22% of mated females oviposited fertilised eggs, by the sixth generation this had increased to 42%. This was also experienced by Cox (1979) who noted that the major difficulty in establishing a laboratory culture of carob moth was ensuring that the adults mate, allowing females to oviposit fertile eggs. Two factors have been regarded as important in allowing for successful mating when attempting to establish a carob moth laboratory culture. Firstly, it is necessary to provide a large space for newly eclosed moths to mate in (Gothilf 1969, Cox 1976). Secondly, a higher percentage of successful mating occurs in a more competitive environment, with multiple pairs within a mating chamber opposed to only one or two mating pairs (Al-Izzi 1987). Observations by Cox (1976) also suggest that simulations of dawn and dusk in the laboratory through artificial lighting can contribute to a successful mating environment. Mediouni and Dhouibi (2007) found no difference in developmental parameters between mass reared and single reared carob moth larvae and contrary to the experience with adults, found that individual rearing was preferable.

Environmental conditions used when sustaining a laboratory culture are similar to that of most sub-tropical pest species: suitable temperatures used ranged from 25-30°C, 65-85% relative humidity (RH) and a 16:8 h (light: dark) cycle (Gothilf 1969, Cox 1976, Al-Izzi 1982).

The least complicated diet for the laboratory rearing of carob moth consists of soy bean flour (43.5%), sugar (43.5%) and distilled water (13%) (Cox 1979, 1979, Gothilf 1969). There was no apparent difference in larval development and adult fecundity when comparing different types of commercially available soy bean flour: regular (high fat), extracted (low fat or low fat and heated) (Gothilf 1969). To date the carob moth SIT programme in Tunisia has been responsible for the only large scale mass rearing facility for carob moth (Mediouni and Dhouibi 2007). The larval diet used at this facility is complex compared to those previously mentioned. Diet composition per 1000g is as follows: wheat bran (600g), sucrose (120g), yeast (23g), salt mixture (20g), vitamin C (6.7g), aureomycin (6.7g), methyl paraben (1.3g), lysine (3g), glycerine (150ml), distilled water (240ml) and calco red dye 41ml). Methyl paraben and aureomycin are used to suppress bacterial and fungal contamination (Mediouni and Dhouibi 2007).

1.7 Alternative hosts of carob moth

Carob moth is a phytophagous insect which is known to feed on over thirteen different plant families, attacking a variety of both cultivated and wild hosts, a favourite being leguminous plants in the family Fabaceae (Nay and Perring 2008). Natural or alternate hosts are considered to be of great importance in providing overwintering sites and refuges that are not subjected to control methods for arthropod pests. Often the cultivated host is not a suitable host for infestation until a certain stage of the growing season, and the pest will undergo multiple generations on natural hosts before moving into orchards. As is the case in Israel, where carob moth completes generations on acacia and carob trees before entering citrus orchards (Gothilf 1964). Therefore, it is advantageous to growers to be aware of potential or existing alternate hosts in close proximity to orchards.

Morland (2015) lists all recorded hosts of carob moth which consists of over 50 species. However, not all of these are of concern to this study, as most of them do not occur in South Africa. Known hosts in South Africa are, *Ceratonia siliqua*, *Ximenia caffra*, *Quercus* sp. (Catling 1970), *Acacia karoo* and *Englerophytum magalismontanum* (Grout and Moore 2015). Table 3 shows both confirmed and potential hosts of carob moth that occur in South Africa, most of which are non-native to the region, such as *Acacia farnesiana* (Gothilf 1969) and *Tamarindus indica* (Neunzig 1979), although no information is available as to whether these are utilised by carob moth in South Africa.

It is important to note than when listing a host of a particular insect that is mentioned in previous research, one must take care to ensure that the information is valid and has scientific justification, and not assumed due to anecdotal evidence (Moore *et al.* 2015).

Table 1.3 Existing and potential hosts of carob moth in southern Africa.

Species	Common name	Native	Non Native	Source
<i>Acacia farnesiana</i>			x	Gothilf 1969
<i>Acacia karroo</i>	Sweet thorn	x		Grout and Moore 2015
<i>Annona</i> sp.			x	Solis 1986
<i>Caesalpinia sappan</i>			x	Zimmerman 1956
<i>Ceratonia siliqua</i>	Carob tree		x	Catling 1970
<i>Cereus</i> sp.			x	Solis 1986
<i>Dioscorea</i>			x	Solis 1986
<i>Englerophytum magalismontanum</i> *	Transvaal Milkplum	x	x	Grout and Moore 2015
<i>Ficus</i> sp.		x	x	Heindrich 1956, Gothilf 1984
<i>Prunus</i> sp.			x	Mehmejad 1995
<i>Robinia</i> sp.			x	Heinrich 1956
<i>Sesbania</i> sp.			x	Solis 1986
<i>Tamarindus indica</i>	Tamarind		x	Neunzig 1979
<i>Ximenia caffra</i>	Sourplum	x		Catling 1970
<i>Quercus</i> sp.	Oak/acorn		x	Catling 1979

1.8 Natural enemies of carob moth

There are a number of natural enemies of *E. ceratoniae* that have been reported including, egg parasitoids, larval parasitoids, pupal parasitoids and other predacious species (Gothilf 1969, Davarci 1996, Nay and Perring 2005, Ksentini *et al.* 2010, Kishani-Farahani *et al.* 2012, Ksentini *et al.* 2013, Nobakht *et al.* 2015). In South Africa only one species of parasitoid has been recorded: *Phanerotoma ornatulopsis* De Seager (Braconidae), which was reared from infested acorns in the Citrusdal area of the Western Cape (Honiball and Catling 1998). In Israel, Gothilf (1969) conducted an extensive survey of carob moth natural enemies in carob plantations, acacia hedges and grapefruit orchards. In carob pods parasitism rates reached 56% towards the end of the season, although in citrus orchards and acacia hedges parasitism was present at low levels, concluding that the percentage of parasitized insects

seems to be influenced by the host plant and the positioning of the individual plantation (Gothilf 1969).

1.9 Population monitoring and control methods

1.9.1 The use of semiochemicals

Semiochemicals are signalling chemicals used to carry information between living organisms and these generally cause changes in their behaviour (Nordlund 1981). These chemicals are emitted by one individual and generate a response in another. Various terminology has been attached to certain types of chemicals to enable a clear understanding of the types of interactions one is referring to when this terminology is used. Pheromones act within the same species and consist of sex, alarm, aggregation or territory marking signals and have evolved for communication purposes (Smart *et al.* 2013). Allelochemicals act between species and can be divided into three different groups: kairomones which benefit the receiver of the signal, allomones which benefit the emitter, and synomones which benefit both the emitter and the receiver (Nordlund 1981).

1.9.2 Monitoring populations

Sex pheromones are used as monitoring tools worldwide to evaluate population size and to ensure economic thresholds are recognised and adhered to (Rodriguez and Stelinski 2009). This monitoring technique has proven to be very effective in British Columbia on apples; where application of pesticides targeting codling moth (*Cydia pomonella*) were reduced by up to 50% by allowing growers to make sound decisions on whether application of insecticide is necessary (Madsen 1981). All over the world the use of sex pheromones for monitoring has become an integral part of IPM strategies (Baker 2008) and chemical compositions of thousands of species are readily available on websites such as The Pherobase (www.pherobase.com).

The sex pheromone for carob moth was first isolated from the female sex gland by Baker *et al.* (1991), using a variety of techniques which included coupled gas chromatographic electroantennographic recordings, micro-ozonolysis, electroantennographic assays of monosaturated standards and evaluated using wind tunnel bioassays and field trials. The pheromone was determined to have three components, consisting of an 8: 1: 1 ratio, with the major component being identified as (Z, E)-9, 11, 13-tetradecatrienal and the two minor components (Z, E)-9, 11-tetradecadienal and (Z)-9-tetradecanal. When either one of the two minor components was added to the major component, the upwind flight response of males

was improved compared to the major component alone (Baker *et al.* 1991). These authors went on to synthesise these compounds, creating a parapheromone. However, this was inferior relative to the gland extracts eliciting male responses, especially in the field trials. It was thought that the trienal, a major component of the pheromone, lost its integrity rapidly and in order to prepare reliable field lures for monitoring, the problem of the decomposition of the trienal needed to be overcome. Todd *et al.* (1992) synthesised (Z, E)-7, 9, 11-dodecatrienyl formate, a more stable analogue of (Z, E)-9, 11, 13 tetradecatrienal, and demonstrated that it effectively mimicked the major component of the carob moth pheromone at both the cellular and behavioural levels, and that it was equally or more effective than the synthetic blend of the natural pheromone components in field trapping studies.

In South Africa there are two available male carob moth pheromone lures: Insect Science Carob lure (Tzaneen, South Africa) and Chempac Carob moth (Paarl, South Africa) lure (Active ingredient in both products: 7, 9, 11-dodecatrien-ol, formate, (7Z, 9E)), designed for monitoring male flight activity in orchards. However, no action thresholds have been determined for carob moth on citrus or any other crop in South Africa.

It has been shown that a species pheromone communication system can vary between populations in different geographical regions (Noldus and Potting 1990). Morland (2015) showed that the efficacy of the Insect Science and Chempac pheromone lures for carob moth were varied in different citrus orchards in the Western Cape of South Africa, suggesting that these lures may perform differently in separate geographic areas. Apart from chemical variation, adaptations to different environmental conditions and host plants might also lead to behavioural variation between populations in different areas (Noldus and Potting 1990). Although the sex pheromone of carob moth has been identified (Baker *et al.*, 1991; Todd *et al.* 1992), and commercial products containing the parapheromone are available, it is necessary to determine whether these products are suitable for use in South Africa.

1.9.3 Mating disruption

Mating disruption involves dispensing relatively large amounts of sex pheromone over a large area and suppressing the male's ability to locate females for mating (Sanders 1997; Wenninger and Averill 2006; Wang *et al.* 2011). Since the introduction of the first commercial pheromone mating disruptant in 1979 against the pink bollworm on cotton, use of the mating disruption technique has grown slowly but steadily (Minks 1997). Worldwide over 400 000 hectares of various agricultural crops and forests have been under commercial mating

disruption, targeting a wide variety of insect pests (Witzgal *et al.* 2010). For mating disruption to be effective, the mating behaviour of the insect must be understood, including the chemicals involved and how the synthetically produced chemicals behave within an airspace; and the population dynamics of the target pest must also be understood (Sanders 1997).

Vetter *et al.* (2006) reported mating disruption field trials using (Z, E) 7, 9, 11-odecatrienyl formate deployed in hollow fibres. It was found to be effective in causing trap shutdown and in most cases reduced damage in date gardens, gardens is the accepted term when referring to date plantations. However, the disruptant did not have a long efficacy in field conditions. A more recent mating disruption product, SPLAT® EC (ISCA Technologies, Riverside, CA, U.S.A), is available for use against carob moth and has been shown to be effective in date gardens in California. However, the product did not provide superior control to chemical treatments (Mafra-Neto *et al.* 2013).

1.9.4 Mass trapping and attract and kill

Another use for sex pheromones is in the technique of mass trapping, which is an extension of the use of species specific monitoring traps. This aims to reduce or eradicate populations of target pests by capturing as many individuals as possible. Mamay and Dag (2016) reduced carob moth infestations by 60% in pomegranate orchards in Turkey through placing traps baited with carob moth lures at a rate of twenty traps per hectare. LAST CALL® CAROB (Insect Science) is an unregistered product which contains an attractant and insecticide, attracting male carob moths which come into contact with the droplet containing a lethal dose of permethrin.

1.9.5 Chemical control

Carob moth is a cryptic pest, spending almost its entire larval stage within its host with no wandering of late instar larvae before pupation. This has resulted in varying success in obtaining effective control using pesticide applications (Catling 1978, Soofbaf *et al.* 2007). Currently there are no chemicals registered for use against carob moth in South Africa and it is assumed that in citrus orchards, chemical control methods that are implemented for other pests such as FCM contribute to the control of carob moth (Honiball and Catling 1998). Some of the products currently available for use against lepidopterans in citrus orchards in South Africa include Delegate® (active ingredient: spinetoram) (Dow AgroSciences), Coragen® (active ingredient: rynaxapyr) (DuPont), Runner® (active ingredient: methoxyfenozide) (Dow AgroSciences), Cypermethrin® (active ingredient: cypermethrin) (Arysta Lifescience), and

Broadband® (active ingredient: *Beauveria bassiana*) (BASF Crop Protection) (Agri-intel 2016, Moore and Hattingh 2012a). The efficacy of such products against carob moth attacking citrus have not been evaluated and due to the mode of action (i.e. contact), the timing of application is difficult with no proven method of monitoring.

Harpaz and Wysoki (1984) found that a 1% concentration of *Bacillus thuringiensis* (Bt) wettable powder (16 000 iu/mg), applied at a rate of 48 000 iu/cm³ killed 94% of fourth instar larvae after 66 hours and 100% after 88 hours. In Turkey, carob moth infestation was reduced by up to 95% when Bt was applied at the start of the second half of the growing season and reapplied at twenty day intervals (Davarci 1996, Ozkan *et al.* 2001). Bt has also been used effectively to control carob moth in pomegranate orchards and date gardens in Tunisia (Mediouni and Dhouibi 2007). Boukedi *et al.* (2015) found that carob moth larvae that ingested Bt showed vacuolisation of the cytoplasm, brush border membrane destruction and cellular disintegration in the midgut. Similar histopathological effects were described by Mnif *et al.* (2013) when carob moth larvae were treated with *Bacillus subtilis* biosurfactant, demonstrating that the mid gut tissue is a primary site of action of these biological toxins (Boukedi *et al.* 2015).

Cypermethrin has been used effectively against carob moth on citrus in Egypt (Hashem and Halawany 1996) and on dates in Israel (Blumberg 2008). Carbaryl, Naled and Malathion applied as dust applications were also effective in controlling carob moth in date gardens in California (Warner *et al.* 1990, Nay and Perring 2008). Although not exclusively to control carob moth, Serghiou (1983) conducted pesticide application trials against the carob moth-mealybug pest complex in Cyprus and found that Chlorpyrifos, Pirimiphos-methyl, Mecarbam, Methomyl, Methidathion, Oxamyl, Omethoate, Permethrin and Carbofuran were most effective. In Australia, Methoxyfenozide has been registered for use against carob moth on Almonds. However, to the best of the author's knowledge, there is no published literature that evaluates its efficacy against this pest. Kaolin clay applied to pomegranate orchards has also been effective in controlling carob moth (Zohdi 2012).

In laboratory trials, Barkhordar (2006) showed that *Ferula assafoetida* essential oil successfully altered carob moth adult behaviour, acting as a repellent. When evaluated under field conditions within pomegranate orchards, infestation was slightly reduced in treated orchards but not significantly (Peyrovi *et al.* 2010).

1.9.6 Cultural control

On citrus in South Africa, cultural control via collecting dropped fruit and picking fruit exhibiting early ripening off the tree (orchard sanitation) is the only recommended method of control against carob moth infestations (Grout and Moore 2015). In citrus orchards in Turkey, orchard sanitation resulted in a reduction of carob moth infestation by over 80% (Davarci 1996). Orchard sanitation is also recommended in pomegranate orchards and date gardens (Dhouibi 1982).

Other cultural control methods used to combat carob moth infestation include the bagging of fruit clusters (Nay and Perring, Dhouibi 1982), abscising rotten fruit within date bunches, which removes favourable egg laying sites in which larvae are offered optimal conditions to reach the adult life stage (Nay *et al.* 2006, Perring and Nay 2015). In date gardens and pomegranate orchards, cultural control practices to negate carob moth damage and infestation include orchard sanitation and bagging of fruit clusters, which has been shown to reduce infestation by roughly 5% in Tunisia (Dhouibi 1982). Pomegranate necking reduces favourable egg laying sites and reduces the risk of larvae entering the fruit from the neck; although this method of cultural control is very labour intensive it has been shown to be effective (Shakeri 2004).

1.10 Phytosanitary status and post-harvest control

Carob moth is a phytosanitary organism when exporting to China, one of South Africa's most important citrus export markets. The protocol for phytosanitary requirements for the export of citrus from South Africa to China, signed between the governments of the two countries (SA-DAFF 2016), states that the mere presence of carob moth in an orchard, packhouse or during phytosanitary inspections, will lead to the expulsion of the relevant orchard from the China export programme for the duration of the season. The protocol states that if any carob moth infestation of fruit is recorded on inspection in China, then the consignment will be returned or destroyed and the relevant orchard and packhouse suspended.

In South Africa, protocols for the post-harvest control of carob moth in citrus do not exist. However, carob moth is a major pest of crops other than citrus, especially stored products, and there is a considerable amount of literature on its post-harvest control. In dates, methyl bromide remains the primary fumigant used to kill carob moth in date fruit (Dhouibi *et al.* 2001). However, methyl bromide has been associated with depletion of the earth's ozone layer and is under restriction through the Montreal Protocol (United Nations Environment Program

(UNEP)) (Ozone Secretariat 2016). In an attempt to find an alternative to methyl bromide, Bessi *et al.* (2015) tested the efficacy of ethyl formate as a fumigant against carob moth in dates and found it to be very effective. Fumigation with essential oils is a very common method of post-harvest control of carob moth. Essential oils derived from *Eucalyptus* species are the most commonly used against carob moth. Examples are *E. camadulensis* (adults, final instar) (Mediouni *et al.* 2013, Ben Jemea *et al.* 2012), *E. leucoxydon* (adults, final instar) (Mediouni *et al.* 2013, Ben Jemea *et al.* 2012), *E. radis* (adults) (Haoel *et al.* 2010), *E. dumosa* (adults, larvae, eggs) (Khemira 2012), *E. transcontinentalis* (adults, larvae, eggs) (Khemira 2012), *E. astringens* (adults) and *E. lehmanii* (adults) (Ben Jemea *et al.* 2012). Bachroach *et al.* (2010) showed that *Pistacia lentiscus* was also effective against adults, larvae and eggs.

Other methods of post-harvest control of carob moth in dates include irradiation (Abo-El-Saad *et al.* 2006), microwave (Zouba *et al.* 2010), ozone (Abo-El-Saad *et al.* 2011, Jemni *et al.* 2014), vacuum-modified atmosphere packaging (Achour *et al.* 2003) and heat treatments (Ben-halli *et al.* 2013, Ben-Amor *et al.* 2016).

1.11 Problems faced by the southern African citrus industry

The southern African citrus industry has a large number of potential pests that warrant consideration with the main pest being FCM. However, it is thought that FCM may not always be the main culprit and carob moth larvae may sometimes be misidentified as FCM. This may be leading to an overestimate of FCM infestation levels or an underestimation of carob moth infestation levels. This is of concern to the industry, firstly due to the phytosanitary status of carob moth when exporting to China, and secondly, although there is a wealth of information available on carob moth in other parts of the world, clarity is required on the pest status of carob moth citrus in South Africa. Additionally, there are no registered products available for population monitoring or control and a method for effectively monitoring infestation in orchards is yet to be established. There is a need to address these issues in a manner that produces reliable, scientifically sound information which can be translated into an effective management plan.

1.12 Aims for this research

A laboratory culture enables researchers to conduct controlled experiments in the laboratory that may not necessarily be able to take place in field conditions. The aims, addressed in Chapter two, were therefore to develop methods for establishment of a laboratory

culture, to determine basic rates of development of larval instars and lastly to establish a reliable method of determining larval instars through establishing the head-capsule size categories for each larval instar.

Carob moth is regarded as a sporadic or secondary pest of citrus, consequently a reliable method to monitor this pest in citrus has not been developed. However, the pest status of carob moth has been brought into question. Therefore, the aim addressed in Chapter three, was to establish a reliable method of monitoring carob moth in citrus and subsequently to determine the pest status of carob moth in citrus in various production areas.

Although there is literature describing a qualitative relationship between carob moth and mealybug in grapefruit cultivars in the Mediterranean region, it has not been determined whether the presence of mealybug plays a significant role in carob moth infestation of other citrus types. The aim in Chapter four was thus to generate an understanding of the relationship between carob moth and mealybug in citrus orchards, by determining whether carob moth was able to develop on citrus in the absence of mealybug and whether the position of mealybug on the fruit influenced the level of carob moth infestation.

There are no registered chemical or semiochemical control methods for carob moth attacking citrus in South Africa. The aim in Chapter five was therefore to firstly determine whether chemical products registered for other lepidopteran pests have any efficacy against carob moth and secondly, to evaluate the efficacy of a mating disruption product against carob moth attacking citrus.

Biological control is the cornerstone of IPM. Therefore, an understanding of the biological control agents of a pest in an agricultural landscape is important. Thus the aim in Chapter six was to establish the levels of carob moth larval parasitism in citrus orchards and neighbouring infested crops.

Current phytosanitary legislation for carob moth when exporting citrus to China states that if any carob moth infestation of fruit is recorded on inspection in China, then the consignment will be returned or destroyed and the relevant orchard and packhouse suspended. All citrus exported to China undergoes compulsory cold treatment disinfestation aimed at other quarantine pests. In order for these cold treatments to be accepted for the disinfestation of carob moth, its cold susceptibility must be determined. Preliminary studies suggest that carob moth is less cold-tolerant than FCM. The aim in Chapter seven was firstly to determine the most

cold-tolerant carob moth instar, and secondly to demonstrate equivalence by comparing the cold susceptibility of the most cold-tolerant carob moth and FCM instars.

Results are discussed within each chapter. However, Chapter eight aims to provide a synthesis of all results, provide recommendations for production practices and future research on carob moth and other parameters which may influence carob moth infestation in citrus orchards.

CHAPTER 2. THE ESTABLISHMENT OF A CAROB MOTH LABORATORY CULTURE

2.1 Introduction

In order to conduct basic biological studies of an insect pest, a laboratory culture is often required. Carob moth laboratory cultures have been established in many regions for different types of research. These include understanding the influence of hosts on developmental rates and fecundity (Gothilf 1969, Naverro *et al.* 1986, Nay and Perring 2008, Zare *et al.* 2012, Mortazavi *et al.* 2015), establishing the biology of mating behaviour (Cox 1976, Vetter *et al.* 1987, Soofbaf *et al.* 2007), conducting bioassays for the efficacy of plant protection products (Al-izzi and Al-maliky 1996, Harpaz and Wysoki 1984, Medouini *et al.* 2013, Mnif *et al.* 2013), understanding their chemical ecology (sex pheromones (Baker *et al.* 1991, Todd *et al.* 1992) and ovipositional stimulants (Gothilf *et al.* 1975, Cosse *et al.* 1994) and mass rearing for the sterile insect technique studies (Dhouibi and Abderahmane 2002, Mediouni and Dhouibi 2007).

To conduct basic research on carob moth in South Africa it was deemed necessary to establish a laboratory culture. Attempts have been made in the past by Morland (2015) and by some commercial companies in order to bioprospect for microbial outbreaks which could be harnessed as biopesticides (John Opoku-Debrah, River Bioscience, pers. comm.). However, these attempts at establishing a culture have been unsuccessful. The purpose of this chapter is to provide an outline in the methods used to establish a carob moth laboratory culture, determine basic developmental parameters, assign head-capsule size categories for larval instars and to assess the rearing conditions in which microbial outbreaks, leading to larval mortality, are most likely to occur.

2.2 Materials and Methods

2.2.1 The establishment of a laboratory culture

This section aims to provide a description of the methods that were used for the duration of this study and outline those that were most effective.

Source of insects

Pecans infested with carob moth larvae (Fig. 2.1) were sourced from the Vaalharts region in the Northern Cape Province in South Africa. In order to maximise the emergence of eclosed adults from these pecans, the nuts were cracked with a metal nut cracker. When cracking these nuts, care was taken to only apply enough force to split the shell and not shatter it, as this may inflict damage to the larvae inside. Often one could tell if a nut was infested when using these nut crackers as when the shell was split it would make a hollow popping sound, while un-infested nuts would sound distinctly denser. If a good site for the collection of infested nuts was found, it was possible to recover over 400 individuals in 8kg of nuts, with many nuts often having over 10 larvae per nut.

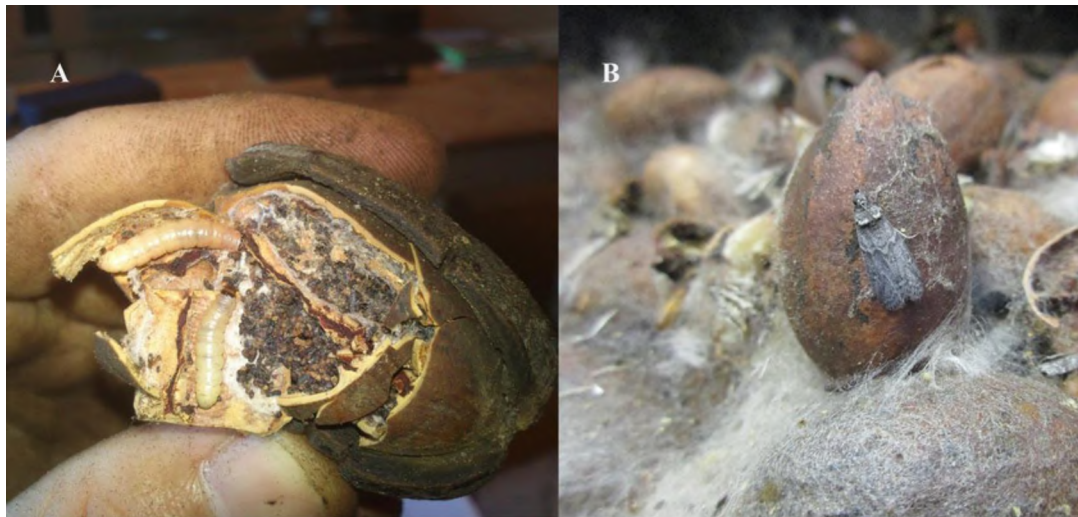


Fig. 2.1 Carob moth infesting pecans. **A:** larvae feeding **B:** Adult ovipositing on split in pecan shell.

Once nuts were split, these were placed in emergence boxes of various sizes (Fig. 2.2) and adults were collected on a daily basis. One problem with the emergence boxes was that in cases of high infestation, larvae would spin mats of silk webbing across the top layer of nuts (Fig. 2.3), often trapping eclosed moths. In some cases, this webbing would block funnels leading to emergence jars. This meant that emergence boxes needed to be monitored frequently and any blockages due to webbing were cleared and this was done by carefully removing webbing with forceps.



Fig. 2.2 Emergence boxes used in establishing a carob moth laboratory culture.



Fig. 2.3 Webbing from carob moth larvae covering pecans in emergence boxes.

Mating chambers

Establishing a method for successful mating was done through trial and error. Al-izzi *et al.* (1987) showed that with each passing generation, successful mating and the number of fertile eggs per female increases. The ratio of males to females has been shown to play a role, and as competition increases with an increase in males, fertilisation of eggs increased (Al-izzi *et al.* 1987, Alrubeai 1987). However, efforts to pair individual females with five males in one litre mating chambers was not successful. Cox (1976) showed that successful mating was more likely when one increased the mating chamber size. Lighting plays a significant role in inducing female calling behaviour, as they respond to the stimulus of the setting sun and often need sites in which to call from within the mating chamber (Cox 1976).

Taking this into consideration, the most successful method of producing fertile eggs was using the following method: for mating chambers, 25 L capacity clear plastic buckets were used with five to seven strands of kitchen paper towelling stuck to the lid of the bucket to

provide calling sites for females (Fig. 2.4). These strands of paper towel were lightly sprayed with a 5% sucrose solution to provide moths with a source of nutrition (Alrubeai 1987). Paper towel lined the bottom of the bucket for egg laying. This was used due to the thigmotactic behaviour observed in the oviposition of females as eggs are preferably oviposited in clusters in crevices (Fig 2.5). The paper towel used has a dimpled texture which provided a suitable contact stimulus.

The room was set at $25 \pm 2^{\circ}\text{C}$, 30% relative humidity (RH) and a 16:8 light: dark cycle (LD). Lighting in the room was set on a step-up step-down system in order to stimulate dawn and dusk (Cox 1976). Three light sources of equal strength were used and light intensity would increase or decrease incrementally by lights turning on or off individually at twenty minute intervals for the first and last hour of the 16-hour light period.



Fig. 2.4 Mating bucket with paper towel providing calling sites for female carob moth.

Newly eclosed adults were placed into a mating bucket, with numbers ranging from 15 to 50 individuals, were left to mate and oviposit and were checked daily. When fertile eggs were first observed through seeing a colour change from white to pink (Fig. 2.5), moths were left to oviposit for a further two days in order to maximise the number of fertile eggs present on the egg sheet. The egg sheet was then collected and placed into a 500ml clear sealable plastic container and monitored until the first neonate larvae emerged. Any surviving moths were

collected and placed into a fresh mating bucket with any newly eclosed adults. Due to the low numbers of moths available, no set protocol was in place for optimising egg laying through moth density or accounting for general moth condition, however, these aspects were always considered throughout the rearing process.

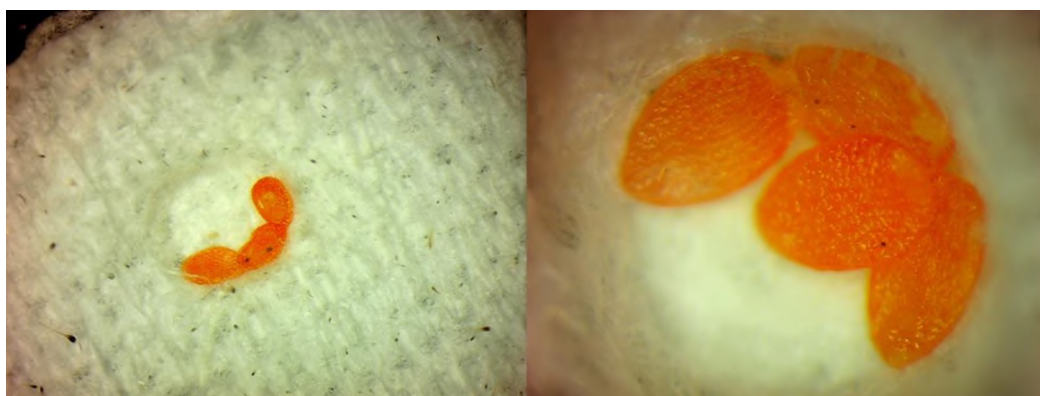


Fig. 2.5 Fertilised carob moth eggs oviposited in a thigmotactic manner by gravid females.

Larvae and artificial diet

Neonate larvae were collected from egg sheets three times a day and placed directly onto the surface of artificial diet with a camel hair paintbrush. Larvae were reared individually in 30ml Polytop vials (Bonpak, South Africa) with 5g of diet sealed with a sterilised cotton wool plug at $25 \pm 2^{\circ}\text{C}$, 30% RH and a 16:8 L: D. The artificial diet consisted of 25% sucrose, 25% soy flour and 50% distilled water, which had been autoclaved at 203 kPa for fifteen minutes before adding the distilled water. Although larvae took to feeding on this diet, there was often a high level of fungal contamination on the surface of the diet. To reduce the occurrence of microbial contamination 0.1% of the total diet weight of Nipagin and sorbic acid (Ibhayi Laboratory Suppliers, Port Elizabeth, South Africa), were added to the dry diet before autoclaving. These products are common anti-microbial agents in artificial diets for insect rearing (Dhouibi and Abderahmane 2002, Moore *et al.* 2014b). Once the pupal casing had formed, pupae were extracted from vials and placed into clean vials to ensure satisfactory adult emergence (Al-izzi 1987). Microbial contamination could have been reduced through sterilising eggs as these are generally contaminated by various microorganisms present in the rearing environment (Inglis and Sikorowski 2009). However, attempts at sterilization of egg sheets by rinsing in a 1: 10 solution of 3.5% sodium hypochlorite and distilled water resulted in zero egg hatch, which may have been due to the absorbent nature of the paper towel on which the eggs were oviposited.

2.2.2 Head-capsule sizes for larval instars

Carob moth larvae were collected at three day intervals from egg hatch to pupation from the laboratory culture ($25 \pm 2^{\circ}\text{C}$, 30% RH and a 16:8 L: D) and from field samples over a twelve month period. Head-capsule widths were measured using a Dewinter Caliper Pro 4.6 (Dewinter Optical Inc. New Delhi, India) and these measurements were plotted according to Dyar (1890).

2.2.3 Single versus multiple larvae per vial

After six generations the laboratory culture was deemed established (Al-izzi 1987) and the number of individuals began to increase. A pilot study was initiated to evaluate whether there would be any beneficial or detrimental outcome if larvae were reared collectively per vial compared to individually. Treatments consisted of single larvae and groups of three larvae per vial. Ten vials were used for each treatment and the experiment was replicated four times with different cohorts of the same generation.

The developmental time from neonate to pupal stage was monitored at $25 \pm 2^{\circ}\text{C}$, 30% RH and a 16:8 L: D, along with the pupal period for each sex. Vials were inspected daily and any pupae were removed and sexed according to Underwood (1994): male pupa's abdominal segments 8-10 are fused with two bullae, which are present on the 8th segment, while female pupae are identified by abdominal segments 7-10 being fused and on the 9th segment a slit-like opening is distinguishable. On the third day of pupal development, pupae were weighed using a PW-184 Adam® Analytical Balance Scale (Max 180g, d = 0.0001g). When a larva had died, it was noted whether there were signs of microbial infection such as discolouration and flaccidness or shrinking (Fig 2.6).

Parameters evaluated were compared between rearing treatments using a General Linear Model Analysis of Variance in Statistica (Statsoft 2016).



Fig. 2.6 Dead larvae which show typical symptoms of microbial infection.

2.3 Results and discussion

2.3.1 Head-capsule size categories for carob moth larval instars

Five distinct size classes were visible when plotting head-capsule measurements of carob moth larvae in 0.5mm categories. Head-capsule measurements for carob moth larval instars were determined to be as follows: first instar (0.0-0.34mm), second instar (0.35-0.64mm), third instar (0.65-0.94mm), fourth instar (0.95-1.14mm) and fifth instar (0.15mm and wider) (Fig. 2.7).

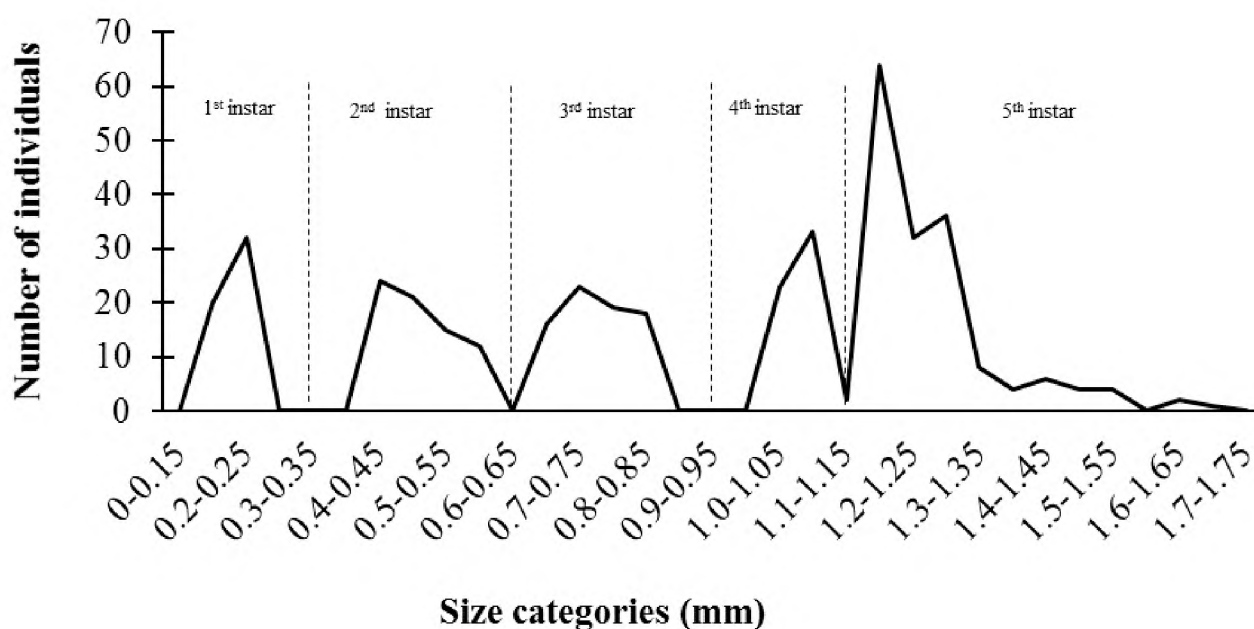


Fig. 2.7 Frequency of head-capsule sizes falling into categories of 0.5mm to determine the number of instars and size categories of instars for carob moth.

Head-capsule sizes indicated five larval instars and a prepupal period. This is congruent with Gothilf (1969) who observed five head-capsule moults, but went on to define instar size categories in larval length. Mediouni and Dhouibi (2007) conducted experiments to establish the development of carob moth larvae in a mass rearing facility for the sterile insect technique, however, they failed to mention how instars were separated. To the best of the author's knowledge, head-capsule size categories of the carob moth larval instars have not been previously established. In two instances, there was a gap between instars (first-second and third-fourth). In a similar study on the false codling moth, Daiber (1979) also recorded gaps, which resulted in confusion when head-capsule measurements did not fall within a specific instar category. Hofmeyr *et al.* (2016) amended these categories by removing gaps to avoid further confusion. Therefore, the same approach was taken in this study and gaps between instars were avoided by extending the relevant instar size categories to a midpoint between instars.

2.3.2 Single versus multiple larvae in rearing vials

Mediouni and Dhouibi (2007) compared larval and pupal rate of development between mass reared and single reared individuals and found that there was no significant difference between the rearing densities. However, in this study larval developmental time was significantly reduced when larvae were reared in groups as opposed to individually ($F_{1, 56} = 16.34$, $P = 0.000$) (Table 2.1). The male pupal period was slightly shorter than female pupal periods at both rearing treatments, however, there were no significant differences in rate of development between rearing conditions for males ($F_{1, 58} = 0.0001$, $P = 0.174$) or females ($F_{1, 47} = 0.028$, $P = 0.154$).

Female pupal weights were higher than males at both rearing densities, however, there were no significant differences in pupal weight between rearing conditions for males ($F_{1, 58} = 0.0027$, $P = 0.124$) or females ($F_{1, 47} = 0.03$, $P = 0.168$). Male carob moth pupae have been found to be lighter in weight than females in other studies (Navarro *et al.* 1986, Arubeai 1987, Mediouni and Dhouibi 2007). There is a strong relationship between pupal weight and adult fecundity in insects (Leather 1988). Therefore, the similarity in both male and female pupal weights for both rearing treatments suggests that rearing density does not alter fecundity in carob moth. However, other studies have shown that carob moth reared collectively were more

fecund and produced higher numbers of fertile eggs than larvae reared individually (Mediouni and Dhoubi 2007).

Mortality due to diseases was significantly higher in rearing vials with multiple larvae per vial compared to single rearing jars ($F_{1,6} = 14.99$, $P = 0.0082$). This mortality was mainly due to a microsporidian, most likely a *Nosema* species (Lloyd *et al.* unpublished). A *Nosema* microsporidian has previously been recorded infecting carob moth infesting walnuts in Argentina (Lange 1991). Microsporidian contamination in laboratory cultures is often a result of a pre-existing infection introduced into the culture from field collected individuals. Symptoms are expressed when individuals are stressed and transmission of infection apparently occurs vertically (van Frankenhuyzen and Liu 2007). In this study multiple larvae in 30ml vials were more susceptible to infection. This may have been a result of increased stress at high densities, increased likelihood of horizontal transmission of infection from larvae reared in close proximity, or a combination of these two possibilities. This microbial infection resulted in the culture collapsing and not being re-established.

Table 2.1 Mean (\pm SE) values of biological parameters for single and multiple carob moth larvae per rearing vial. Different letters in the same row indicate a significant difference ($P < 0.05$).

Biological parameter	Rearing condition	
	Single	Multiple
Development time from neonate to pupa (days)	38.18a \pm 1.2	24.6b \pm 0.65
Male pupal development time (days)	6.82a \pm 0.58	6.9a \pm 0.42
Female pupal development time (days)	7.15a \pm 0.43	7.22a \pm 0.6
Male pupal weight (mg)	2.22a \pm 0.13	2.02a \pm 0.4
Female pupal weight (mg)	3.58a \pm 0.15	3.39a \pm 0.6
Mortality due to disease (%)	24.9a \pm 9.6	76.25b \pm 11.87

2.4 Conclusion

Carob moth head-capsule sizes were established for five larval instars and a prepupal stage was evident. A suitable rearing method was developed, which enabled the establishment of a laboratory culture. Larvae reared in single vials developed significantly slower than multiple larvae per vial. However, the reduced fatalities due to microbial infection indicated that single rearing may be more effective in establishing a laboratory culture, unless a method can be developed to adequately or suppress or control a microsporidian infection.

CHAPTER 3: DEVELOPING A MONITORING METHOD AND ESTABLISHING THE PEST STATUS FOR CAROB MOTH IN CITRUS ORCHARDS

3.1 Introduction

Monitoring (scouting and trapping) is the cornerstone of integrated pest management (IPM) because IPM requires information concerning the pest status of the insect or disease in question to make timely decisions about management activities (Gonzalez 1971). Sampling information is traditionally used in IPM to make decisions regarding the preventative or corrective control measures required to reduce the risk of pests inflicting damage which could result in monetary loss. Therefore, reliable and practical scouting and trapping systems are essential for effective implementation of monitoring programmes within an IPM based production system (Buntin 1994).

When attempting to develop a usable and effective sampling plan, it is important to understand and define the key objectives as these will largely determine the methods incorporated into the sampling plan (Southwood 1978). Sampling programmes can be classified as having three general objectives: (1) detecting the presence of a target species, (2) providing information on the pest status of the target species and (3) providing accurate density estimates with a high level of precision (Buntin 1994).

Within citrus production in South Africa, the majority of produce is destined for export as fresh unprocessed product (CGA 2016). Citrus pests, which can hinder the exportability of the fruit, can be crudely separated into the following categories: phytosanitary pests, production pests (i.e. reduce yield), cosmetic pests (reduce exportability) and vectors (i.e. transmit diseases). A single pest can fall into multiple categories, however, the phytosanitary status of certain pests varies with the target market to which the fruit is exported. Therefore, the objective of a species specific monitoring programme may be determined by the categories that each pest falls into.

The carob moth, *Ectomyelois ceratoniae* Zeller, is a phytosanitary pest when exporting citrus to China (SA-DAFF 2016), and the repercussions of an interception at the destination port can result in large monetary losses for all stake holders involved (ie. growers, exporters and importers). Recently the pest status of carob moth on Navel oranges has been highlighted

in certain citrus producing regions (Moore *et al.* 2014) and in order to establish exactly what this pest status is, a reliable monitoring programme which includes sampling flight activity and infestation levels in orchards needs to be developed. Monitoring flight activity of male carob moths in agricultural landscapes has been undertaken through the use of trapping using virgin females (Serghiou 1983, Vetter *et al.* 1997, Al-Jamali 2006 Mortazavi *et al.* 2016) and sex pheromones deployed in various trap types (Mafra-Neto *et al.* 2013, Morland 2015, Mamay and Dag 2016). In South Africa, these sex pheromones are available for purchase, however, these are not registered and their efficacy for reliable use still needs to be determined.

Evaluating carob moth damage through larval feeding was largely limited to various hosts where it is a common pest of economic importance such as in nut crops (Calderon *et al.* 1969, Lange 1991, Mehrnejad *et al.* 2006), dates (Warner 1998, Nay and Perring 2006) and pomegranates (Memay and Dag 2016). In citrus, methods of evaluating damage in orchards have been restricted to grapefruit and consist of evaluating a number of fruit exhibiting gumming symptoms through random picking of a set number of fruit per tree, and later in the season recording the number of dropped fruit which showed signs of infestation (Serghiou 1983). However, observations of carob moth infestation in Navel oranges has revealed that it is different to that observed in grapefruit (Fig. 3.1). In Navels oranges, carob moth larvae of all instars are almost always found feeding within the fruit itself, entering through the navel-end or under the calyx and will then pupate within the fruit (Fig. 3.2). The adults will often eclose when the fruit is still on the tree.

In South African citrus orchards, false codling moth (FCM), *Thaumatotibia leucotreta* Meyrick, is a key phytosanitary pest. FCM larvae infest citrus fruit and are concealed feeders (Newton 1998). This destructive feeding behaviour results in a physiological response by infested fruit leading to premature ripening and fruit drop (Newton 1998), which allows infestation levels to be monitored through counting weekly dropped fruit infested with FCM larvae or simply the feeding damage, if the larva has already exited the fruit (Moore *et al.* 2015). This is the standard method in which citrus producers and research scientists accurately evaluate the level of FCM infestation and damage.

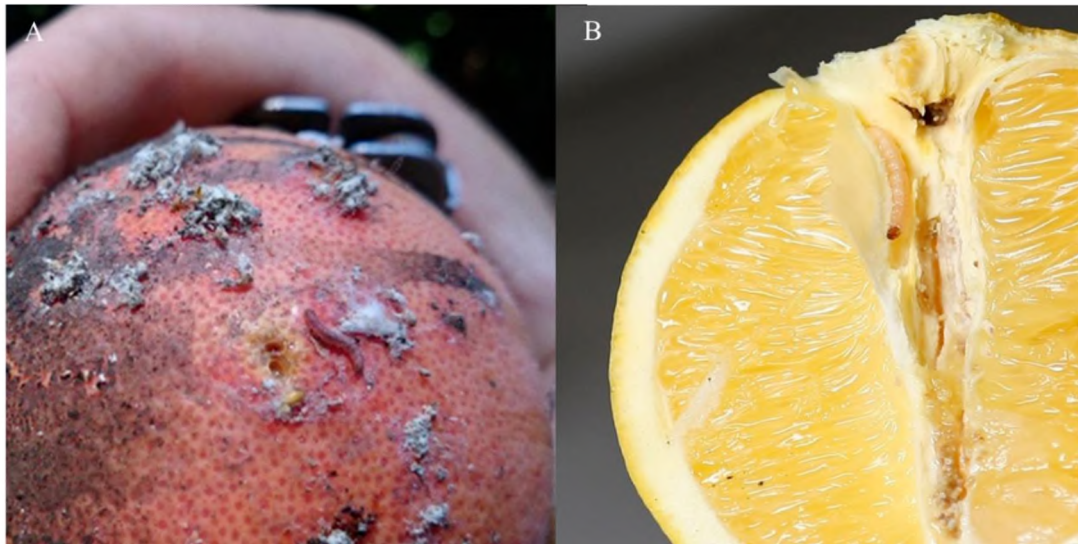


Fig. 3.1 Carob moth larvae infesting citrus fruit. A: carob moth larva foraging on the epidermis of a Star Ruby grapefruit, B: larva feeding within a Palmer Navel orange.

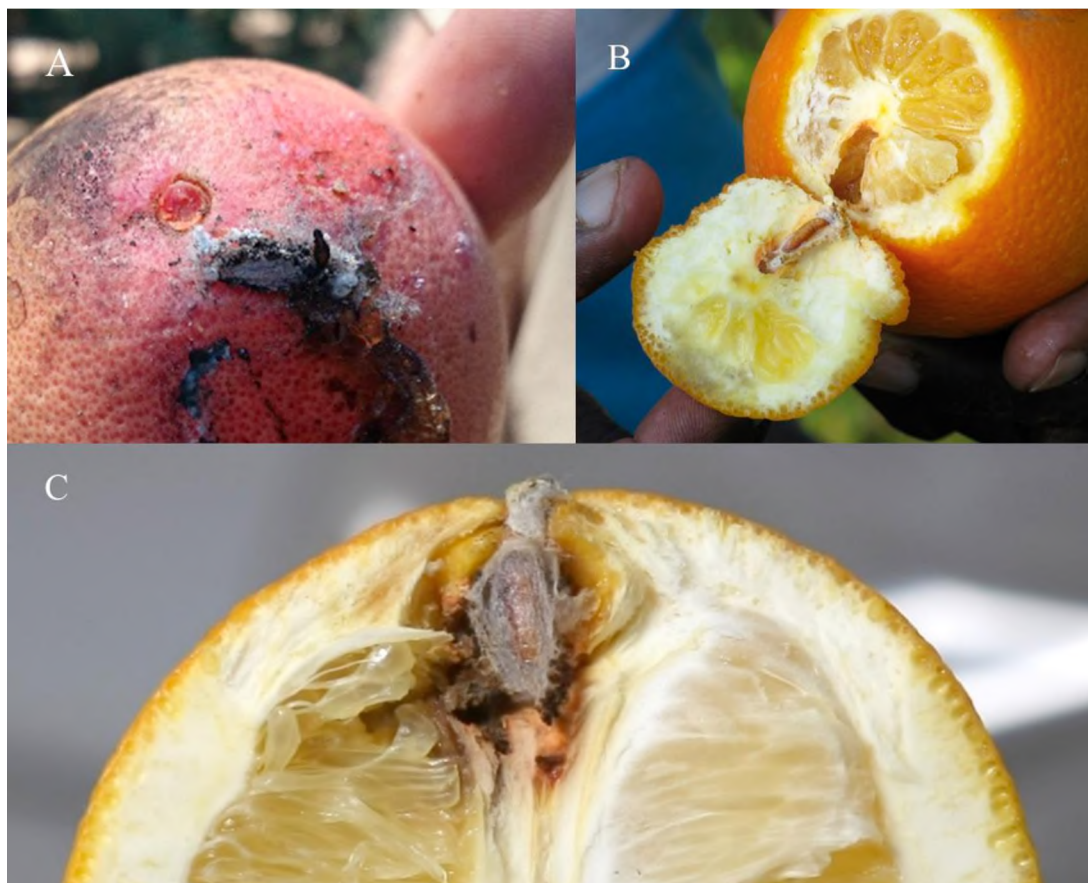


Fig. 3.2 Carob moth pupa on citrus. A: carob moth pupa on the epidermis of a Star Ruby grapefruit, B-C: carob moth pupae within the navel-end of Palmer Navel oranges.

It would be beneficial if carob moth infestations could also be detected and quantified with this method, allowing both FCM and carob moth infestation to be monitored simultaneously. However, carob moth infestation/feeding within Navel oranges is not as destructive as FCM, possibly prolonging the period between infestation and fruit abscission. Additionally, adults often eclose while fruit is still on the tree, potentially reducing the accuracy of this method for detection and monitoring of carob moth infestation. A more species specific method which takes these factors into consideration may be more appropriate.

The aims for this chapter were: (1) to determine whether there was a difference in preference of carob moth males to different sex pheromone lures and whether any of these lures are more suitable than others for population monitoring; (2) to establish whether carob moth infestation can be monitored through the current FCM monitoring method, or if a novel sampling method is more accurate in detecting and monitoring for infestation; (3) to monitor flight activity periods of male carob moth and larval infestation in Navel orange orchards in five citrus production areas within South Africa, and to describe the seasonal phenology, and inter-orchard variation within production areas, as well as comparing levels of infestation and flight activity between these production areas; (4) to determine whether a significant and reliable relationship exists between moth trap catches and larval infestation, in an attempt to establish a species specific predictive monitoring tool for the citrus industry; and (5) to evaluate the pest status of carob moth on Navel oranges in different production areas through comparing the levels of infestation of carob moth to FCM.

3.2 Materials and Methods

3.2.1 Evaluation of carob moth pheromone lures

Choice test

The attractiveness of three carob moth pheromone lures were compared. Two are commercially available in South Africa (Chempac, and Insect Science), while the third (ISCA) was imported as a test product by River Bioscience. All three lures possess the same active ingredient (7, 9, 11-dodecatrien-ol, formate, (7Z, 9E)). The attractiveness of these lures was compared in a choice test over the 2015-16 growing season in the Loskop Valley production area (Fig. 3.3) in a 10.5 hectares (ha) Palmer Navel (25° 13' 4.75"S 29° 25' 53.45"E) orchard with a history of carob moth infestation. Pheromone lures were placed in yellow delta traps (Insect Science, Tzaneen, South Africa) with sticky floors and five replicates existed for each lure type. Delta traps with lures were placed in the fifth tree of a row and in every second row

within an orchard. No two lures of the same type were positioned in successive rows. The lures were placed in traps in a random order which was generated from a random number table. Traps were inspected weekly from October 2015 to April 2016, with the number of carob moth caught per trap each week being recorded. Lures were replaced every six weeks as per product label guidelines.

No choice test

Insect Science and Chempac carob moth pheromone lures in yellow delta traps were placed on opposite ends of an orchard in the fifth row and the fifth tree from the edge. The trial took place in the Vaalharts production area (Fig. 3.3) in four Navel orange orchards (four replicates) ($28^{\circ}17'40.9''\text{S}$ $24^{\circ}35'13.4''\text{E}$) in the 2015-16 growing season. Lures were replaced every six weeks as per label guidelines and trap catches were recorded weekly from October 2015 to April 2016.

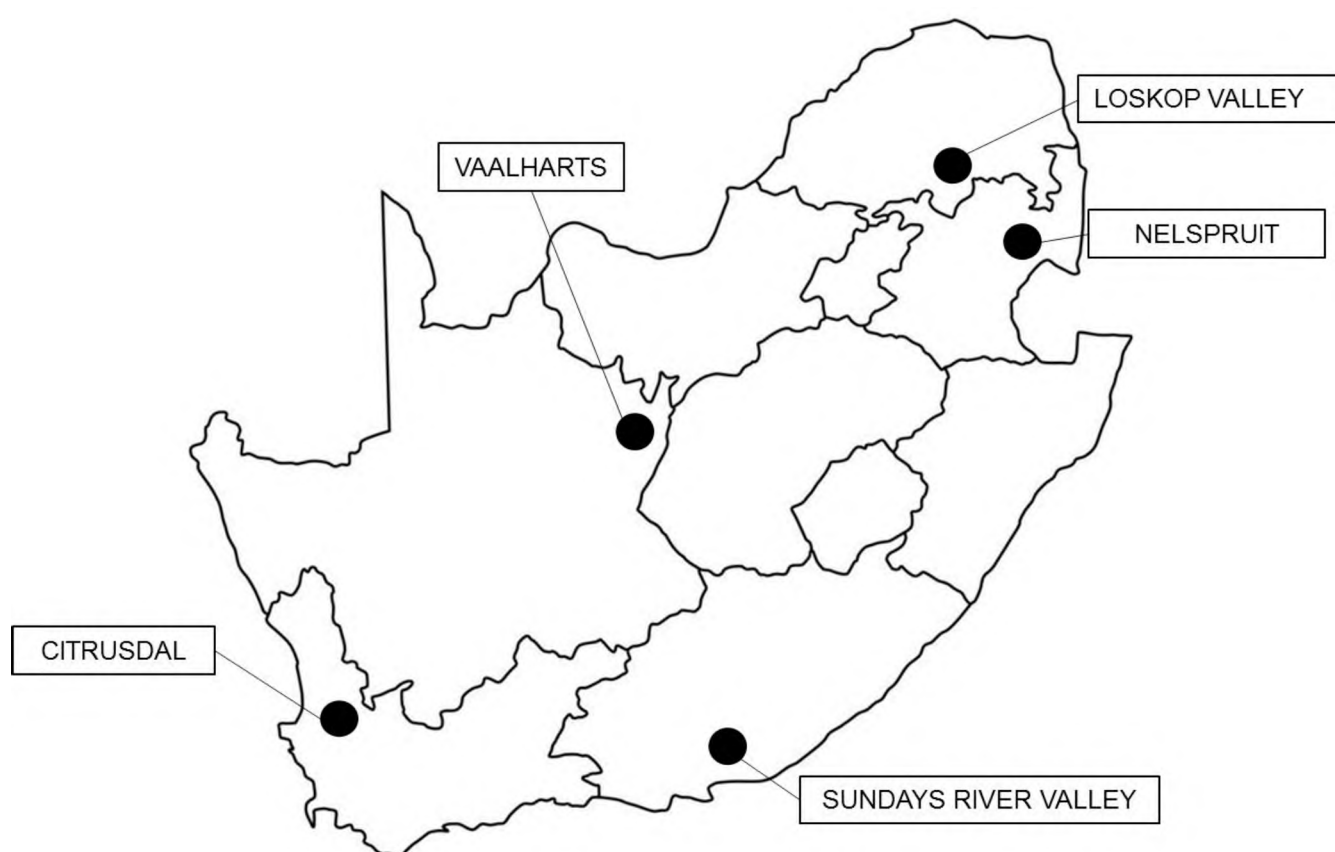


Fig. 3.3 Location of production areas where monitoring took place within South Africa

Statistical analyses

Due to the variance in the count data being about equal to the mean, trap catch data underwent square root transformation ($\sqrt{x + 0.5}$) and means were analysed using an analysis of variance (ANOVA) (Fadamiro 2004) in Statistica (Statsoft 2016).

3.2.2 Sampling methods for detecting infestation

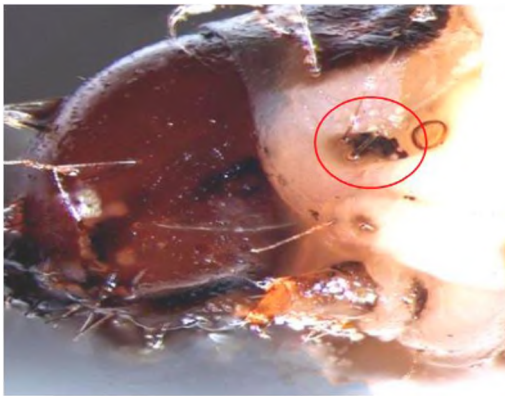
To determine whether the method used to evaluate FCM infestation through monitoring dropped fruit in citrus orchards is effective in monitoring or detecting carob moth infestation, this method was compared to a novel approach which accounts for key differences in lifecycle stages between carob moth and FCM, and key differences in the physiological response of fruit to infestation. Evaluation of these two methods took place for the duration of the 2015-16 growing season across three different production areas; Loskop Valley (four Palmer Navel orchards), Nelspruit (three Palmer Navel orchards) and the Vaalharts area (three Palmer Navel orchards) (Table 3.1, Fig. 3.3). Each orchard represents a replicate within the production area. Five consecutive data trees were allocated to each sampling method with the first tree for the dropped fruit method being the sixth tree in the fifth row and the picked fruit method starting in the eleventh tree in the same row (Fig. 3.5). Data trees were located in the fifth row from the corner closest to the direction of the prevailing wind direction.

Sampling methodology

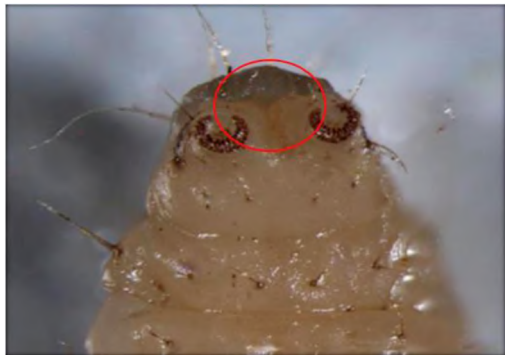
Dropped fruit method:

Five data trees were marked in each orchard in the first week of October 2015 and all dropped fruit under trees were cleared so that any fruit drop the following week took place in the previous seven days. On a set day of the week, all dropped fruit were collected and inspected for the presence of larval infestation. Any larvae that were recovered were placed into 70% ethanol and kept for identification. Larvae were distinguished between carob moth and FCM according to Rental (2012) and Morland (2015) (Fig. 3.4). The total number of larvae collected each week were then divided by the number of data trees, allowing infestation to be reported as number of infested fruit per tree per week (Moore *et al.* 2015).

Carob moth



Dark sclerotized block with 3 setae



Anal comb absent



Sclerotized ring around the SD1 seta on 8th abdominal segment

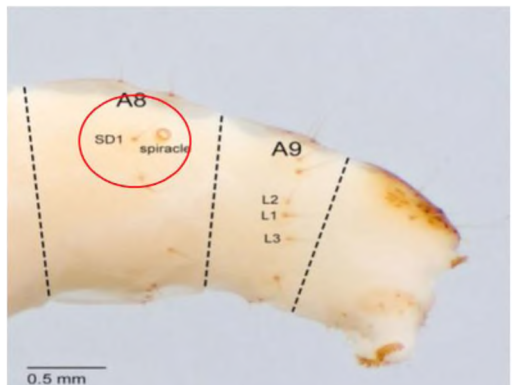
False codling moth



No dark sclerotized block with 2 setae



Anal comb present



No sclerotized ring around the SD1 seta on 8th abdominal segment

Fig. 3.4 Distinguishing features between carob moth and FCM larvae, adapted from Rental (2012) and Morland (2015).



Fig. 3.5 Schematic diagram of the position of picked and dropped fruit data trees and the position of the yellow delta trap with carob moth sex pheromone lure.



Fig. 3.6 Typical signs of carob moth infestation in Palmer Navel orange. A: infested fruit exhibiting the physiological response of premature ripening due to larval infestation. B: frass and webbing protruding from the navel-end of an orange. C: carob moth infestation.

Picked fruit method:

Five data trees were marked in each orchard in the first week of October 2015. Each data tree was scouted for sixty seconds, searching for fruit that showed typical signs of carob moth infestation, such as premature ripening along with frass and webbing protruding from the navel-end (Fig. 3.6), first circling the tree and then moving under the canopy. All fruit exhibiting these symptoms or which appeared suspicious were picked from the tree and then dissected. Due to the morphological similarity between carob moth and FCM larvae, all larvae or pupae were placed into 70% ethanol and identification was confirmed in the laboratory according to Rental *et al.* (2013) and Morland (2015) (Fig. 3.4). Pupae found in fruit were considered to be carob moth as FCM will pupate in the soil (Newton 1998). The mean number of fruit infested with carob moth per tree per week over a predetermined time period was determined.

Statistical analyses

Weekly carob moth infestation per tree were subjected to square root transformation ($\sqrt{x + 0.5}$). A main effects ANOVA was used to determine whether there was a significant difference in carob moth infested fruit recovered between the two sampling methods within each region and between orchards within each region.

3.2.3 Regional carob moth flight activity and infestation in citrus orchards

Flight activity of male carob moth adults and larval infestation of carob moth in Navel orange orchards in production areas throughout South Africa was conducted over the 2014-15 and 2015-16 growing seasons (Fig. 3.3) Three citrus producing regions in the northern part of the country were monitored and these included the Loskop Valley (Limpopo Province), Nelspruit (Mpumalanga Province) and Vaalharts (Northern Cape Province). In the southern parts of the country monitoring took place in Citrusdal (Western Cape Province) and the Sundays River Valley (SRV) (Eastern Cape Province). However, this only took place in the SRV for the 2014-15 season and due to low levels of trap catches and no infestation this did not take place in the 2015-16 season.

Orchards were selected in each production area due to having a history of conspicuous lepidopteran infestation in fruit. Flight activity of carob moth was monitored through the use of yellow delta traps placed in the fifth row in the fifth tree in the orchard corner closest to the prevailing wind (Fig. 3.4) and a single carob moth pheromone lure (Insect Science) was used to bait the delta trap. Lures were placed into traps in the first week of October in each season,

monitored weekly until the orchard was harvested and replaced every six weeks as the product label indicated. Infestation was monitored over this same period using the dropped fruit method outlined in section 3.3.2 above (Moore *et al.* 2015). Orchard details and the number of orchards monitored in each growing season for each production area can be seen in Table 3.1.

Table 3.1 The number of orchards where carob moth trap catches and infestation were monitored in each production area for the 2014-15 and 2015-16 growing seasons.

Production area	Orchard	Variety	Season monitored		Used in regression
			2014-15	2015-16	
<u>Loskop Valley</u>	L1	Palmer Navel	x	x	Yes
	L2	Palmer Navel	x	x	Yes
	L3	Palmer Navel	x	x	Yes
	L4	Lina Navel	x	x	Yes
	L5	Palmer Navel		x	Yes
	L6	Palmer Navel		x	Yes
		Bahianinha			
	L7	Navel		x	Yes
	S1	Palmer Navel	x		Yes
	S2	Palmer Navel	x		Yes
	S3	Palmer Navel	x		Yes
	S4	Palmer Navel	x		Yes
<u>Vaalharts</u>	MV1	Palmer Navel	x		Yes
	MV2	Palmer Navel	x		Yes
	MV3	Palmer Navel	x		Yes
	MV4	Palmer Navel	x		Yes
	JM1	Palmer Navel		x	Yes
	JM2	Palmer Navel		x	Yes
	JM3	Palmer Navel		x	Yes
	JM4	Palmer Navel		x	Yes
<u>Nelspruit</u>	PS1	Palmer Navel	x	x	No
	PS2	Palmer Navel	x	x	No
	PS3	Palmer Navel	x	x	No
<u>Citrusdal</u>	MG1	Palmer Navel	x	x	No

	MG2	Palmer Navel	x	x	No
<u>Sundays River valley</u>	ST1	Lane Late Navel	x		No
	ST2	Palmer Navel	x		No
	ST3	Palmer Navel	x		No
	ST4	Palmer Navel	x		No

Statistical analyses

Weekly trap catches and larval infestation data underwent square root transformation. An ANOVA was used to evaluate statistical differences in trap catches and larval infestation between seasons for each growing region as well as differences between orchards within each growing season for each region. Trap catches and infestation levels between regions were compared in an ANOVA using all data collected over the two growing seasons.

3.2.4 Establishing a relationship between trap catches and infestation

Navel orange orchards which had more than two separate trap catches and at least one week where carob moth infestation occurred were used to evaluate the relationship between trap catches and infestation (Table 2.1) over the 2014-15 and 2015-16 growing seasons. A simple regression analysis was used to compare trap catches (continuous predictor) and corresponding infestation for one to six weeks after each week's trap catch (dependant variable) (Fig. 3.6) in Statistica (Statsoft 2016). Country-wide data was combined and then each of the regions in which data were available for analysis (Loskop valley and Vaalharts) was evaluated separately. Each regression resulted in an R^2 value and a significant relationship was indicated by a P value of less than 0.05.

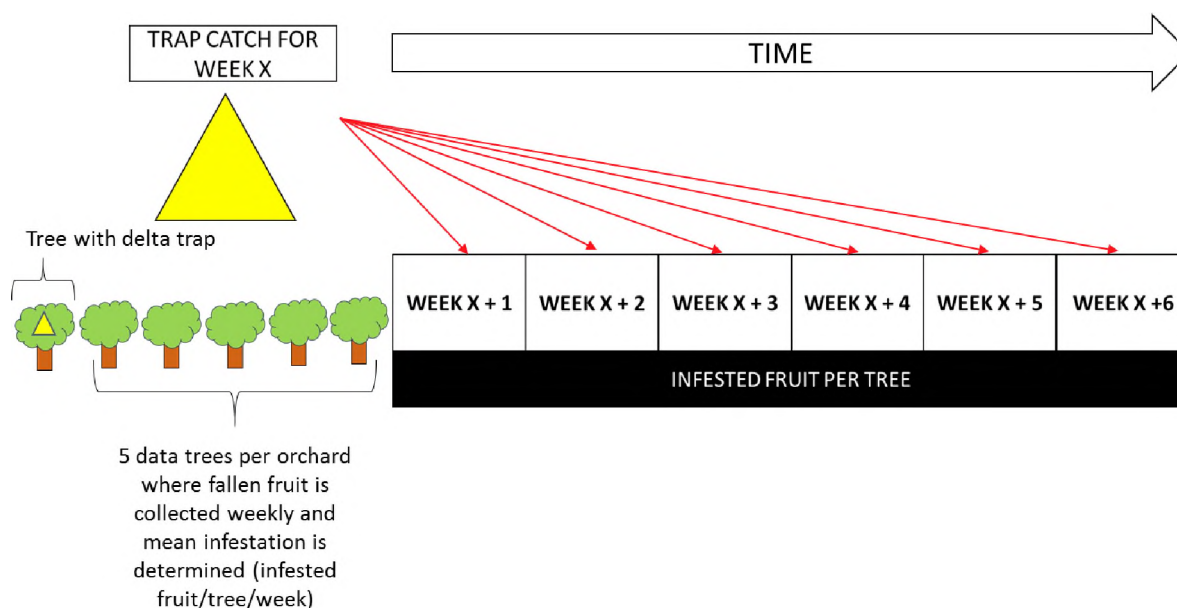


Fig. 3.7 The process involved in evaluating the relationship between carob moth trap catches and infestation in citrus orchards.

3.2.5 The pest status of carob moth on Navel oranges

The pest status of carob moth compared to FCM was determined through analysis of data collected in section 3.2.3. For this, only data collected in the Loskop Valley, Nelspruit and Vaalharts production areas were used, as only in these areas was infestation of both carob moth and FCM recorded in both the 2014-15 and 2015-16 seasons. All larvae collected over the sampling period were identified according to Rental (2012) and Morland (2015) (Fig. 3.4), allowing the number of fruit infested by either species (carob moth or FCM) per week per tree within each orchard to be quantified. The weekly cumulative mean levels of infestation were determined for each species in each orchard. Data were kept separate for production areas and a comparison of mean fruit infestation for each species between and within the 2014-15 and 2015-16 growing seasons was conducted using a factorial ANOVA, after data were transformed using a square root transformation.

To establish an estimate of the direct economic loss in Rands (R) incurred by producers over a growing season per hectare, through fruit drop as a result of carob moth and FCM infestation (Equation 1, below), was used. The definition of variables within said equation and the values incorporated along with sources and reasoning is presented in Table 2.2.

Equation 1 Formula used to establish an estimate of economic loss due to fruit infestation by carob moth and FCM in navel orange orchards.

$$\text{Economic loss} = \frac{\left(\frac{(MCI \times n. \text{ tree per ha})(\% \text{ packout})}{FPC} \right) \times CW}{1000 \text{ kg}} \times EGV \text{ per ton}$$

Table 3.2 The definitions for variables in equation one along with the values used to arrive at presented results and the source or reasoning associated with these values.

Variable in formula	Definition	Value used
<i>MCI</i>	Mean cumulative infestation per tree over a given period	
<i>n trees per ha</i>	Total number of trees per hectare	555 ¹
<i>% packout</i>	The expected volume of fruit harvested that will be of export quality	70% ²
<i>FPC</i>	Number of fruit per carton (determined by fruit and carton size)	48 ³
<i>CW</i>	carton weight (kg)	15
<i>EGV</i>	Economic gross value per ton	R5781 ⁴
Source of values		
¹ The most common tree spacing in production areas analysed is 6x3m which equals 555 trees per hectare		
^{2, 3} Pack out value was established as a mean across all orchards through communication with packhouse managers (Pers comm. Louis Nieman (Rosle Boerdery – Loskop Valley) and Johanna Matthewson (Saamfarm - Vaalharts))		
⁴ Mean gross value per ton returned for oranges exported in 2014 (CGA 2016)		

3.3 Results

3.3.1 Evaluation of carob moth pheromone lures

There was a significant difference between the mean number of carob moths caught with the different pheromone lures in the choice test ($F_{1,2} = 7.4$, $P = 0.00076$). Insect Science and ISCA had the lowest overall trap catches of 17 and 21 respectively, with Chempac having the largest trap catch (51) (Fig. 3.8). Post-hoc analyses showed that Chempac lures trapped significantly more moths than both Insect Science ($P = 0.001079$) and ISCA lures ($P = 0.006712$). However, in a no choice test, there was no statistical difference between mean weekly trap catches for Insect Science and Chempac pheromone lures (Fig. 3.9) ($F_{1,1} = 0.75$, $P = 0.387$).

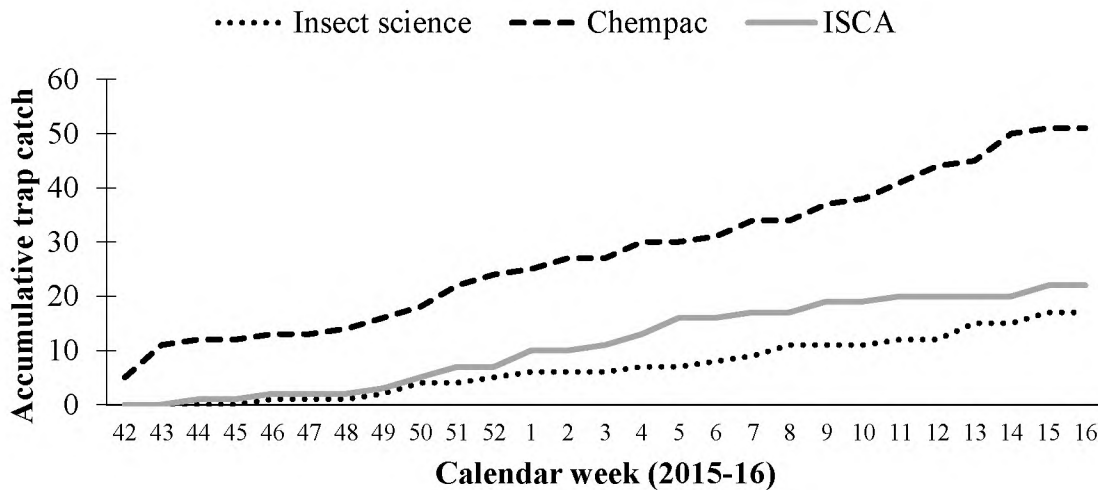


Fig. 3.8 Cumulative carob moth trap catches in a lure choice test conducted in the Loskop Valley production area over the 2015-16 growing season.

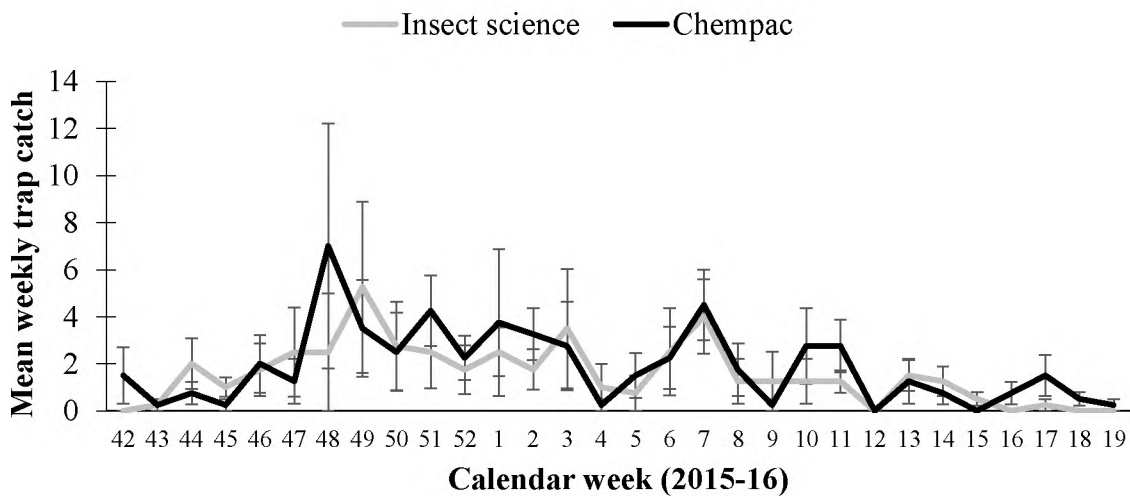


Fig. 3.9 Mean carob moth trap catches per week in a no choice lure test conducted in the Vaalharts production area over the 2015-16 growing season. Error bars represent standard error from the mean.

3.3.2 Sampling methods for detecting infestation

Detection of the first occurrence of a carob moth infested fruit was recorded in week two of 2016 for both sampling methods. There was a significant difference in mean weekly carob moth infested fruit per tree between sampling methods in Nelspruit production area ($F_{1,1} = 22.38$, $P = 0.000006$), with the picked fruit method producing the higher number of

infested fruit (Fig. 3.10). However, the trend in infestation was similar over the season for the two methods. There was no significant difference in the level of infestation between orchards within the Nelspruit production area for both sampling types ($F_{1,2} = 0.799$, $P = 0.452$).

In the Vaalharts production area the dropped fruit method produced significantly higher weekly numbers of carob moth infested fruit than the picked fruit method ($F_{1,1} = 12.9$, $P = 0.000429$). There was also a significant difference in infestation levels between orchards within the production area ($F_{1,3} = 8.00$, $P = 0.00051$). Carob moth infestation was observed in fallen fruit as early as week 49 while the first picked fruit with carob moth infestation only occurred in week 11 (Fig. 3.11).

In the Loskop Valley production area, the mean weekly level of carob moth infestation was significantly higher for the dropped fruit method than the picked fruit method ($F_{1,1} = 28.717$, $P = 0.0000001$). There was no significant difference between the orchards within this production area ($F_{1,3} = 2.56$, $P = 0.0558$). Carob moth infestation was first observed in week two in the dropped fruit and five weeks later (week seven) in the picked fruit sampling method. The overall trend in infestation observed over the season for the two methods was similar (Fig. 3.12).

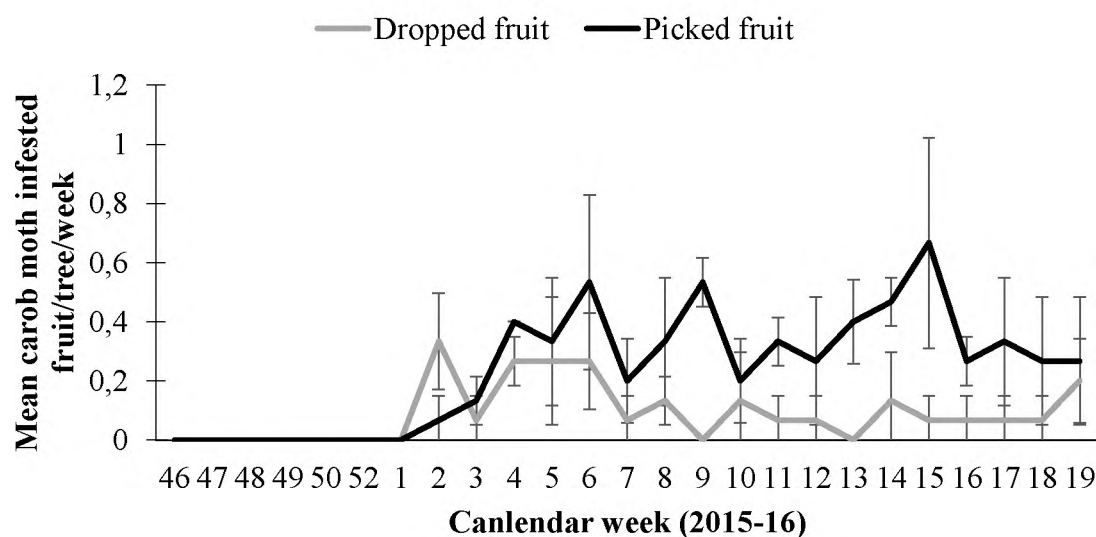


Fig. 3.10 Mean number of carob moth infested fruit per tree per week recovered from two different sampling methods over the 2015-16 growing season in the Nelspruit production area. Error bars show standard error from the mean.

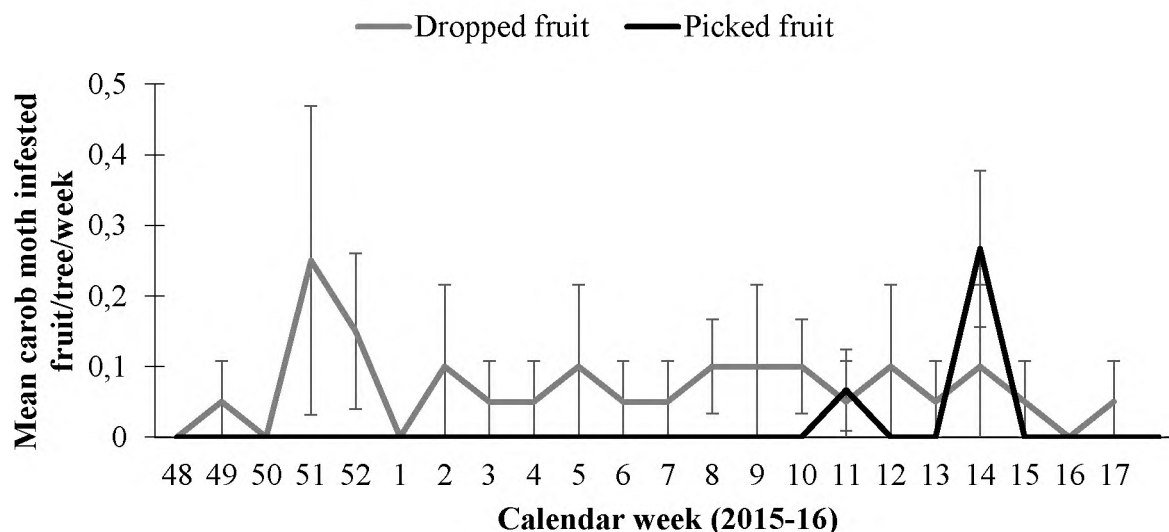


Fig. 3.11 Mean number of carob moth infested fruit per tree per week recovered from two different sampling methods over the 2015-16 growing season in the Vaalharts production area. Error bars show standard error from the mean.

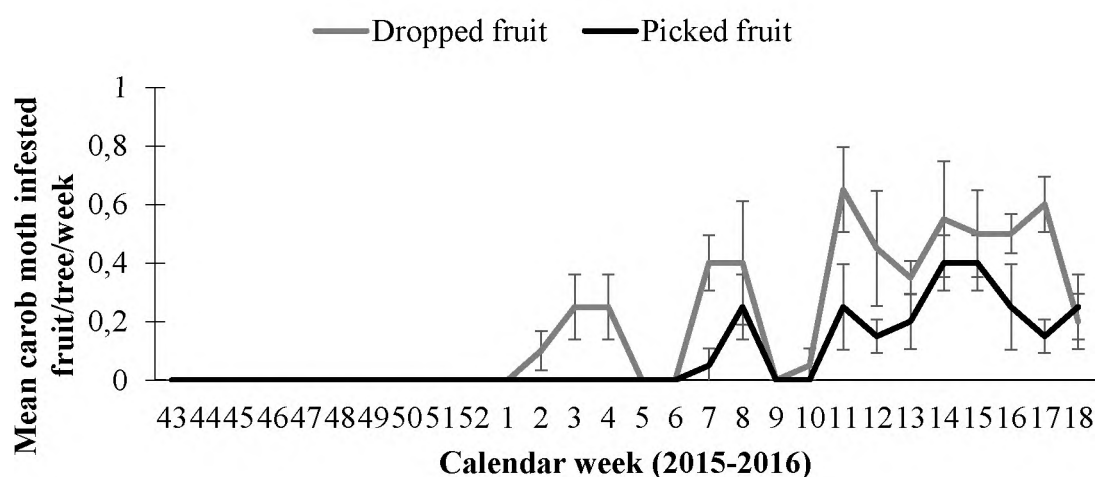


Fig. 3.12 Mean number of carob moth infested fruit per tree per week recovered from two different sampling methods over the 2015-16 growing season in the Loskop valley production area. Error bars show standard error from the mean.

3.3.3 Regional carob moth flight activity and infestation in citrus orchards

In the Loskop Valley production area male carob moths were caught throughout the both growing seasons (Fig. 3.13A). There was a significant difference in mean weekly trap catches between the two seasons with catches in 2015-16 season being significantly higher than the 2014-15 season ($F_{1,1} = 6.8$, $P = 0.00956$). When comparing variation in trap catches

between orchards within each season, there was no significant difference for both the 2014-15 ($F_{1,6} = 2.12$, $P = 0.057$) and 2015-16 season ($F_{1,3} = 1.57$, $P = 0.2$). In the 2014-15 season three distinct periods in activity were evident, recorded in weeks 43-47, 52-3 and 8-11. Lower numbers of moths were caught in all other weeks, barring week 50 (Fig. 3.13A). Mean peaks in activity in 2014-15 occurred in week 43 (1.57 ± 1.26), 45 (1.14 ± 0.83), 47 (1.14 ± 0.54), 2 (1.43 ± 0.62), 8 (1.71 ± 0.92), 10 (2 ± 0.86), 15 (0.85 ± 0.4) and 18 (1 ± 0.4). In the 2015-16 season, there were four time periods when moth flight activity was at its highest: weeks 45-47, 1-3, 1-7 and 9-16; while peaks in activity occurred in week 47 (1.5 ± 0.3), 2 (1.75 ± 0.73), 5 (2.25 ± 0.98), 9 (0.5 ± 2.74) and 15 (2.5 ± 0.74). Although moths were almost always present in orchards in both seasons, flight activity was at its peak in weeks 1-17.

Mean weekly fruit infestation followed a similar trend over the two seasons (Fig. 3.13B), however, there was a significant difference between the two seasons with 2015-16 having higher levels of mean weekly infestation levels than the 2014-15 growing season ($F_{1,1} = 9.12$, $P = 0.00273$). In the 2014-15 season there was a significant difference in infestation levels between orchards ($F_{1,6} = 4.74$, $P = 0.00029$), however, this was not the case in the 2015-16 season ($F_{1,3} = 0.69$, $P = 0.557$). The first fruit infestation was observed in week two (2015-16) and three (2014-15) and continued for three and two weeks respectively. Another peak in infestation occurred in week seven and eight and then every week from week 10-18. The highest mean weekly infestation occurred in week 11 for both seasons, $2.48 (\pm 1.02)$ (2014-15) and $2.74 (\pm 0.72)$ (2015-16) fruit per tree per week.

In the Nelspruit production area, there were very few male moths trapped over both seasons, with flight activity occurring in three distinct peaks in the 2014-15 season (week 43 (0.33 ± 0.41), 47 (0.33 ± 0.41) and 1 (0.33 ± 0.41)) and two distinct peaks in the 2015-16 season (week 47 (0.33 ± 0.41) and 16 (0.33 ± 0.41)) (Fig. 3.14A). There was no significant difference in trap catches between the two seasons ($F_{1,1} = 0.2$, $P = 0.65$), or between the orchards monitored over the 2014-15 ($F_{1,2} = 0.0$, $P = 1$) and the 2015-16 seasons ($F_{1,2} = 2.074$, $P = 0.132$).

Carob moth fruit infestation was first observed in week 2 (2015-16) and week 4 (2014-15) (Fig. 3.14B). In each season, after the first carob moth infestation was recorded, the presence of carob moth larvae in dropped fruit was almost continuous for the rest of the season with no extended period without infestation. There was no significant difference in infestation levels between the two seasons ($F_{1,1} = 0.0002$, $P = 0.96$) and this was also the case for levels of

infestation between orchards with both 2014-15 ($F_{1,2} = 1.04$, $P = 0.375$) and 2015-16 ($F_{1,1} = 0.74$, $P = 0.477$).

In the Vaalharts production area, carob moth flight activity differed significantly between the two seasons ($F_{1,1} = 14.775$, $P = 0.00001$), with constant moth activity in the orchards from week 43-16 in the 2015-16 season and week 50-10 in the 2015-16 season (Fig. 3.15A). Two peaks of moth activity occurred in 2014-15 (week 3 (0.75 ± 0.86) and 10 (1.25 ± 0.86)), while four were present in the 2015-16 season (week 44 (2 ± 1.24), 49 (5.52 ± 4.19), 4 (3.5 ± 2.92) and 8 (4 ± 1.83)). In both the 2014-15 and 2015-16 seasons trap catches were significantly different between the monitored orchards ($F_{1,3} = 3.35$, $P = 0.0214$ and $F_{1,3} = 25.9$, $P = 0.00001$ respectively).

Fruit infestation in the 2014-15 was significantly lower than the 2015-16 season ($F_{1,1} = 7.24$, $P = 0.00001$) (Fig. 3.15B). The first infested fruit was observed in week 4 of 2014-15 and a full seven weeks earlier in the 2015-16 season (week 48). Infestation in the 2014-15 was only recorded in five separate weeks with peaks occurring in week 4 (0.2 ± 0.1) and 9 (0.2 ± 0.1). Over the 2015-16 season, infestation was highest in week 50 (0.25 ± 0.21) and occurred weekly from week 2-15. There was no significant difference in infestation between orchards monitored in the 2014-15 season ($F_{1,3} = 1.42$, $P = 0.24$). However, a significant difference was observed between orchards monitored over the 2015-16 season ($F_{1,3} = 8.05$, $P = 0.00074$).

In the Citrusdal production area, there was no significant difference between the two seasons for mean trap catch ($F_{1,1} = 1.17$, $P = 0.28$). Moth flight activity in the 2014-15 season was recorded between weeks 48-10 and again towards the end of the season in week 16-17 (Fig. 3.16). Peaks in activity for the 2014-15 season occurred in week 49 (1.5 ± 0.71) and 8 (1.5 ± 0.71). In the 2015-16 season, moth flight activity was recorded slightly earlier than in the previous season (week 44 vs week 49) and the main period of activity was from week 52-9. Peaks in trap catches occurred in week 52 (2.5 ± 3.5) and 9 (2 ± 1.4). There was no significant difference in trap catches between the orchards monitored over both seasons ($F_{1,3} = 0.8$, $P = 0.45$).

Trap catches and infestation were monitored in the SRV production area only in the 2014-15 season. Moth activity consisted of five peaks in activity with no moths being trapped between these peaks (Fig. 3.17). The highest trap catches occurred in weeks 42, 43, 18 and 19. There were no significant differences in mean trap catch between orchards monitored ($F_{1,3} = 1.098$, $P = 0.352$). No fruit infestation by carob moth was recorded over the season.

When comparing mean carob moth trap catches for both seasons using combined data, there was a significant difference between production areas ($F_{4, 946} = 31.238$, $P = 0.0$) (Fig. 3.17). Post-hoc analyses revealed that there was no significant difference between the Loskop Valley and Vaalharts ($P = 0.79$), while both these regions were significantly different to Citrusdal (Loskop Valley $P = 0.000181$, Vaalharts $P = 0.0102$), Nelspruit (Loskop Valley $P = 0.00017$, Vaalharts $P = 0.00017$) and the SRV (Loskop Valley $P = 0.00017$, Vaalharts $P = 0.00017$). Citrusdal trap catches were significantly higher than Nelspruit ($P = 0.01303$), however, not significantly different to the SRV ($P = 0.977$).

There was a statistically significant difference in mean carob moth fruit infestation between production areas over both seasons ($F_{4, 946} = 28.530$, $P = 0.0$) (Fig. 3.18). Loskop Valley had the highest mean infestation and this was significantly different to Nelspruit ($P = 0.01662$), Citrusdal ($P = 0.00017$), Vaalharts ($P = 0.00017$) and SRV ($P = 0.00017$). Nelspruit had the second highest mean infestation and was significantly higher than Citrusdal ($P = 0.000025$), Vaalharts ($P = 0.0398$) and SRV ($P = 0.00022$), while infestation in Vaalharts was significantly higher than Citrusdal ($P = 0.05$) and SRV ($P = 0.05$).

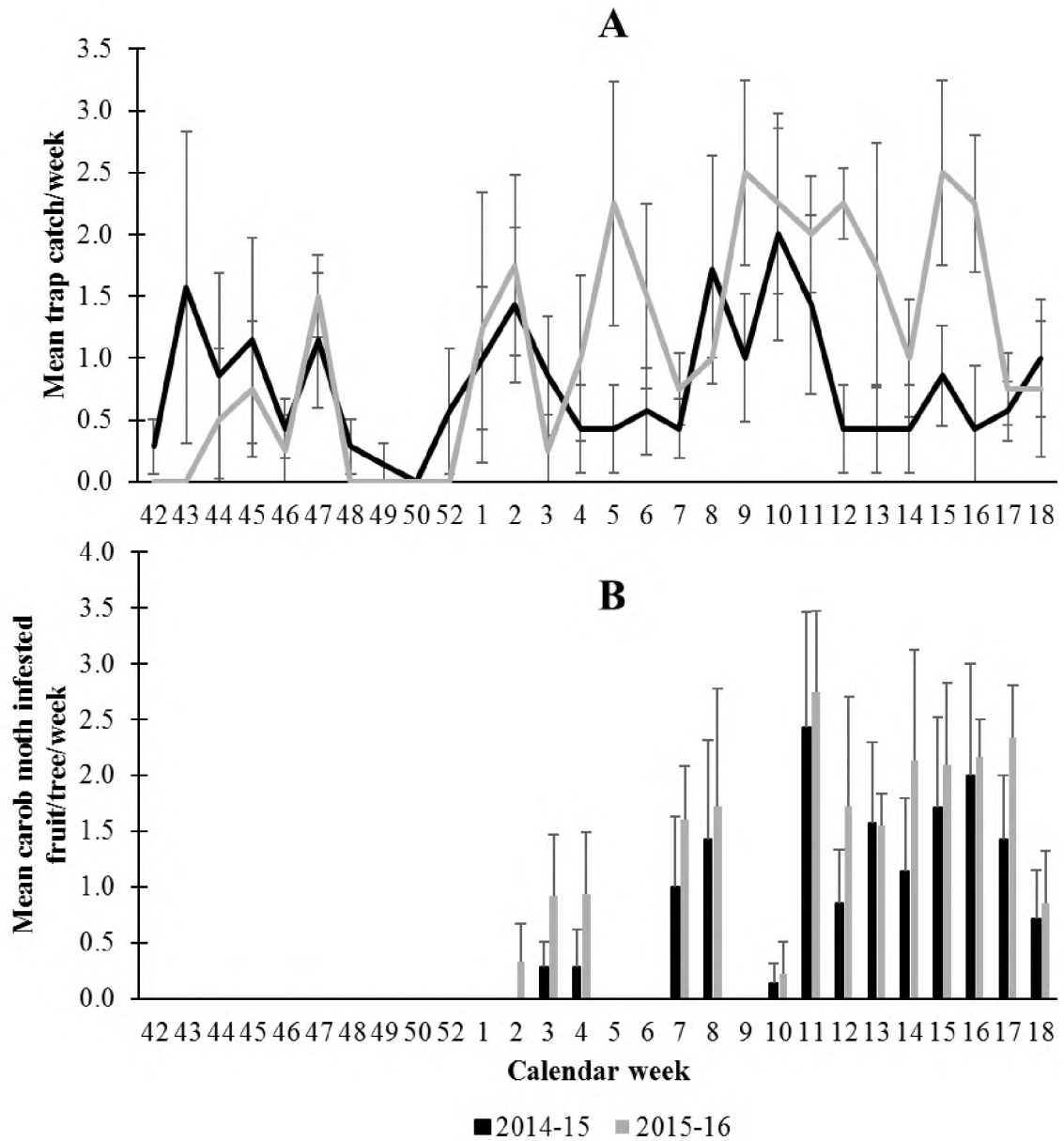


Fig. 3.13 Mean carob moth trap catches (A) and fruit infestation (B) using the dropped fruit method over the 2014-15 and 2015-16 seasons in the Loskop Valley production area. Error bars represent standard error from the mean.

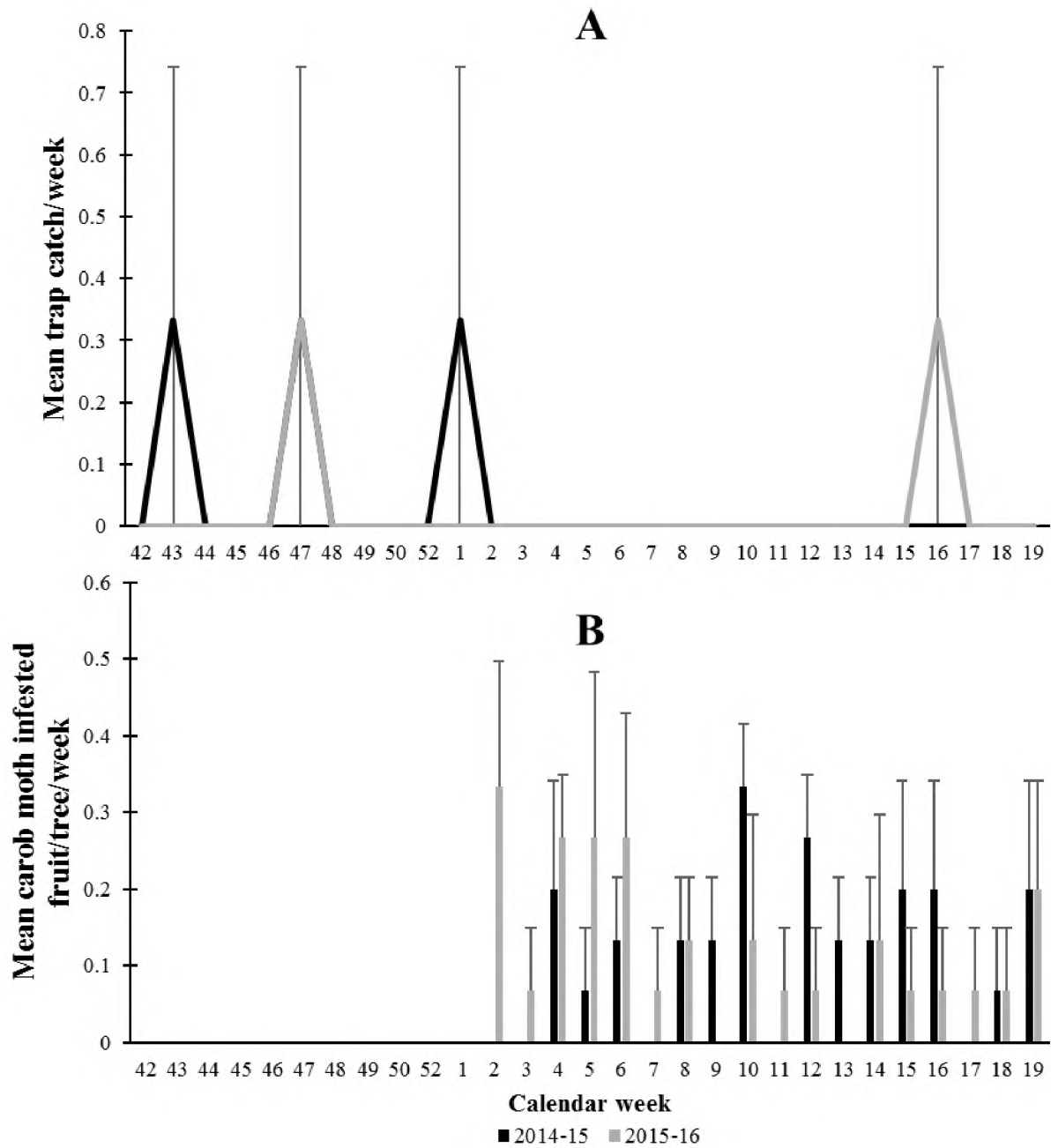


Fig. 3.14 Mean carob moth trap catches (A) and fruit infestation (B) using the dropped fruit method over the 2014-15 and 2015-16 seasons in the Nelspruit production area. Error bars represent standard error from the mean.

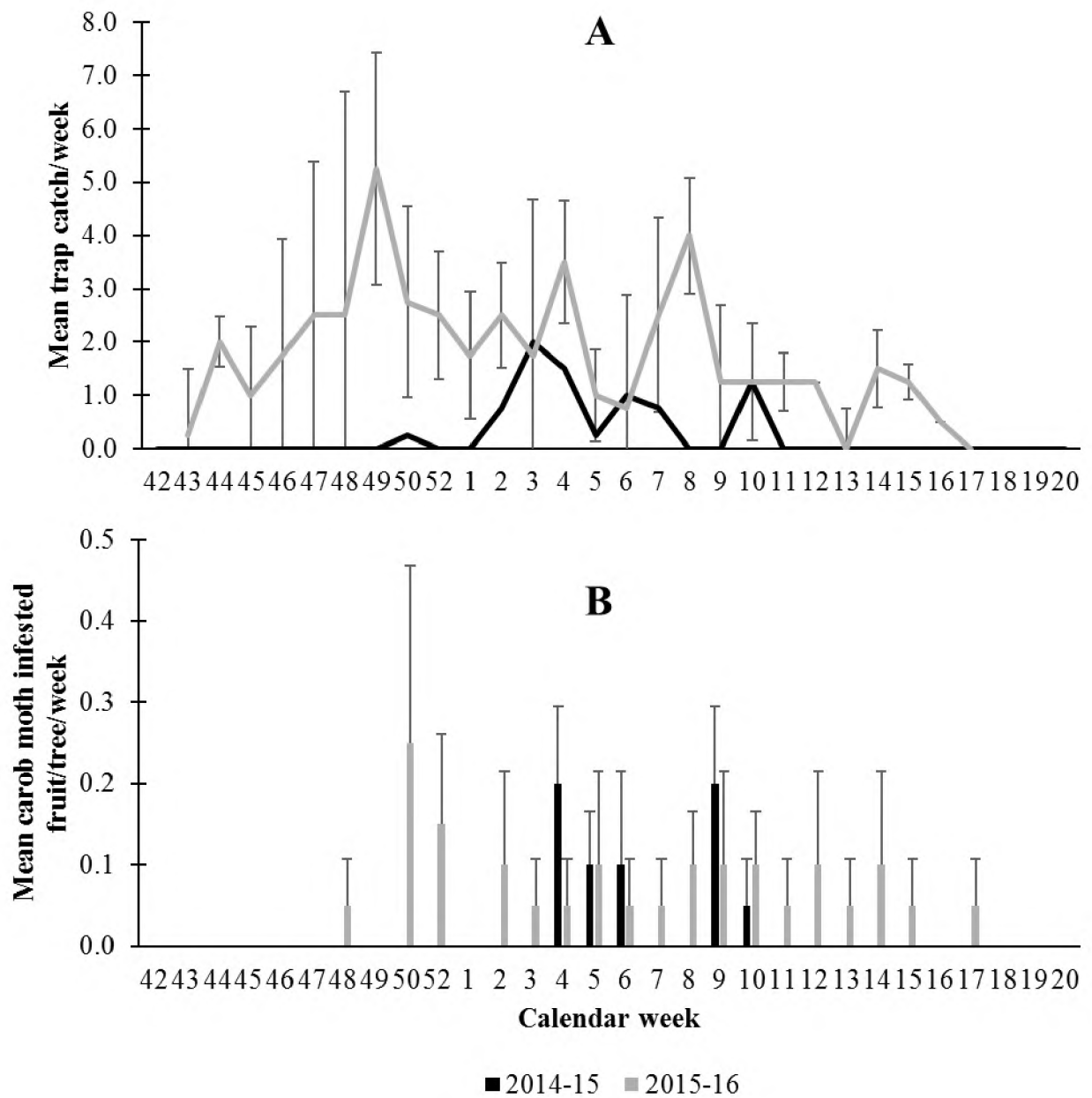


Fig. 3.15 Mean carob moth trap catches (A) and fruit infestation (B) using the dropped fruit method over the 2014-15 and 2015-16 seasons in the Vaalharts production area. Error bars represent standard error from the mean.

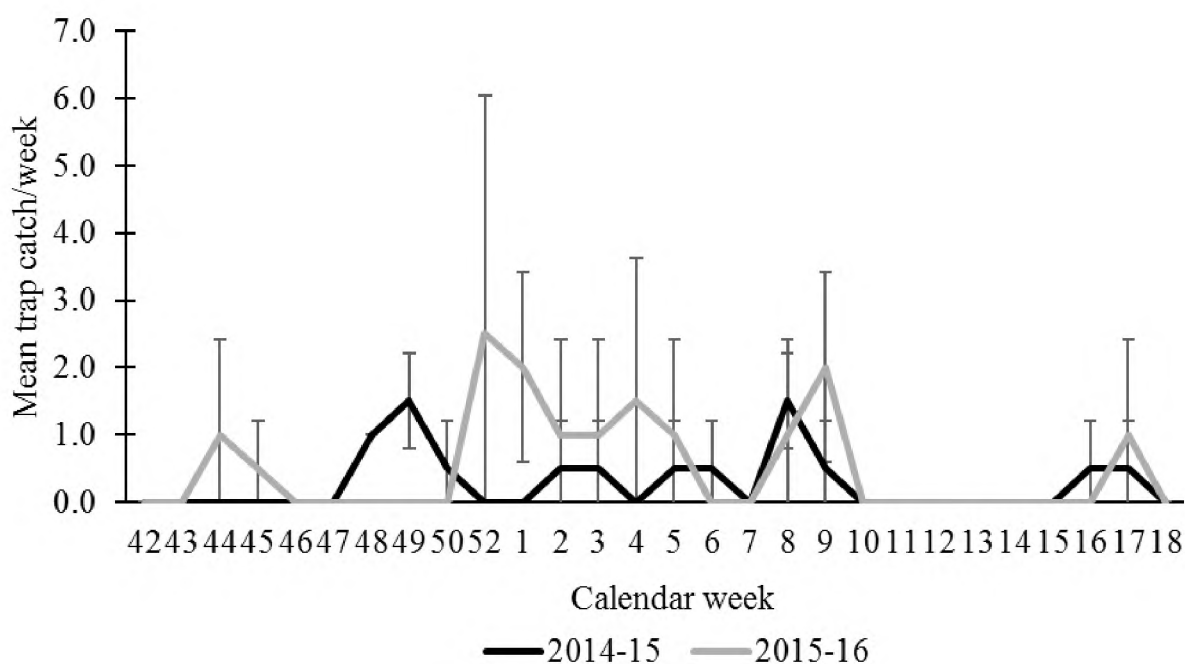


Fig. 3.16 Mean carob moth trap catches over the 2014-15 and 2015-16 seasons in the Citrusdal production area, infestation data are not shown due to zero infestation recorded over both seasons monitored. Error bars represent standard error from the mean.

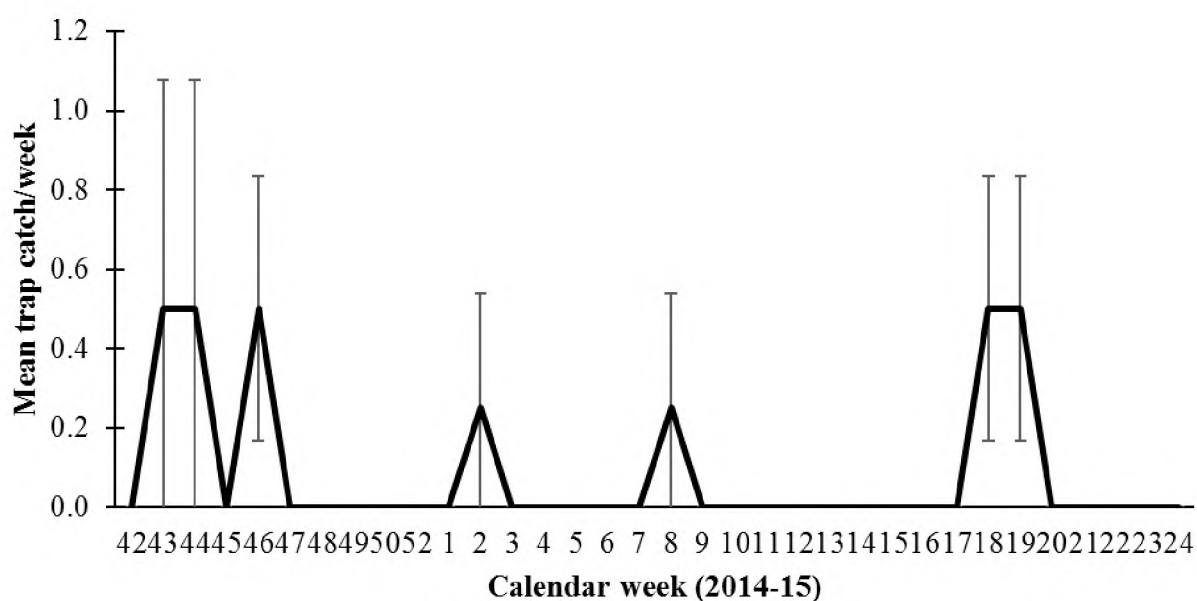


Fig. 3.17 Mean carob moth trap catches in the Sundays River Valley production area for the 2014-15 growing season. Infestation data are not displayed due to zero infestation recorded. Error bars represent standard error from the mean.

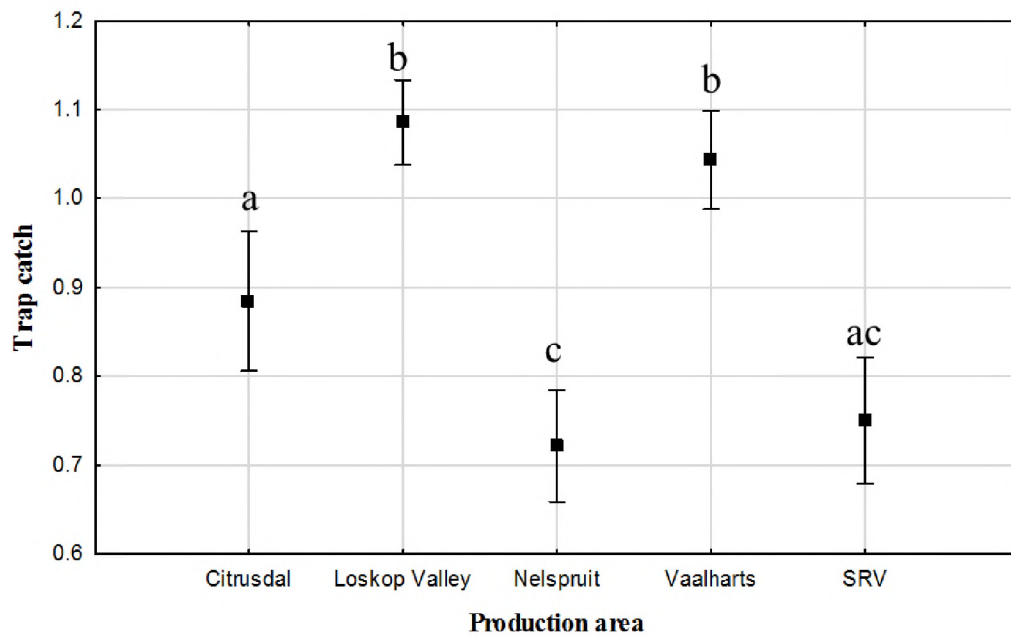


Fig. 3.18 Mean combined trap catch data for the 2014-15 and 2015-16 seasons with error bars representing 95% confidence intervals. Different letters denote statistically significant differences between means.

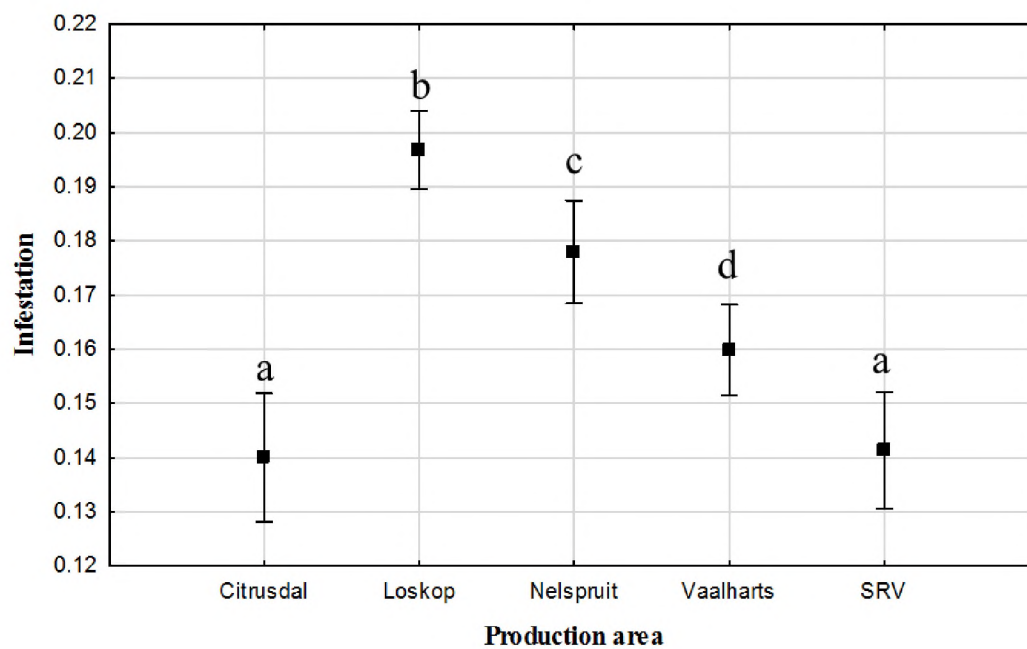


Fig. 3.19 Mean combined infestation data for the 2014-15 and 2015-16 seasons with error bars representing 95% confidence intervals. Different letters denote statistically significant differences between means.

3.3.4 Establishing a relationship between trap catches and infestation

Country-wide trap catches showed a significant relationship when regressed against infestation two ($P = 0.0001$), four ($P = 0.0001$), five ($P = 0.0001$) and six weeks ($P = 0.0001$) later (Table 3.3). However, although these relationships were significant, infestation two weeks after trap catch had the highest R^2 value (0.12) with weeks four, five and six having a lower R^2 value (0.04). When looking at the production areas individually, both the Loskop Valley and the Vaalharts showed a significant relationship between trap catches and infestation two weeks later ($P = 0.003$ and $P = 0.004$) with R^2 values of 0.14 and 0.25 respectively. Trap catches and infestation five weeks later showed a significant relationship in the Loskop Valley ($P = 0.002$, $R^2 = 0.11$), while in the Vaalharts infestation both four and six weeks after trap catch showed a significant relationship ($P = 0.0001$ ($R^2 = 0.17$) and $P = 0.0001$ ($R^2 = 0.13$), respectively).

Table 3.3 Shows R^2 values generated for the relationship between trap catches and infestation for country wide combined data and area specific data.

Production area	Weeks after trap catch					
	1	2	3	4	5	6
Country wide	0.07	0.12*	0.04	0.04*	0.04*	0.04*
Loskop valley	0.10	0.14*	0.09	0.03	0.11*	0.07
Vaalharts	0.22	0.25*	0.10	0.17*	0.05	0.13*
Nelspruit	-	-	-	-	-	-
Sundays River valley	-	-	-	-	-	-
Citrusdal	-	-	-	-	-	-

* indicates a significant relationship ($P < 0.05$)

3.3.5 The pest status of carob moth on Navel oranges

In the Loskop Valley production area, cumulative carob moth infestation was significantly higher than FCM in both the 2014-15 season (carob moth: 3.0, FCM: 0.8) ($F_{1,334} = 18.848$, $P = 0.00002$) and the 2015-16 season (carob moth: 5.3, FCM: 0.7) ($F_{1,222} = 41.036$, $P = 0.000001$) (Fig. 3.20). There was a significant difference in the level of infestation between seasons ($F_{1,556} = 3.15$, $P = 0.076$), with carob moth infestation increasing significantly in the 2015-16 compared to the 2014-15 season ($P = 0.028$). However, there was no significant difference recorded in levels of FCM infestation between seasons ($P = 0.99$). In the 2014-15 season the economic loss incurred by producers through fruit drop for FCM was R561.48/ha

and carob moth infestation resulted in almost four times this amount (R2 105.55/ha) (Table 3.4). Carob moth infestation was higher in the 2015-16 season, resulting in an economic loss of R3 719.80/ha while FCM decreased slightly (R491.29/ha).

For the 2014-15 season in Nelspruit, there was no significant difference in the level of infestation for carob moth and FCM ($F_{1, 112} = 0.023$, $P = 0.88$) (Fig. 2.20), with both resulting in similar economic losses for producers (R1 544.07/ha and R1 614.25/ha respectively). In the 2015-16 season FCM infestation was higher than carob moth infestation, but not significantly so ($F_{1, 112} = 1.74$, $P = 0.19$). Economic loss from FCM was R2 877.58/ha compared to R1 614.25/ha for carob moth. There was no significant difference in levels of infestation for the two species between the two growing seasons ($F_{1, 224} = 2.06$, $P = 0.43$).

In Vaalharts production area over the 2015-16 growing season, there was no significant difference between carob moth and FCM infestation ($F_{1, 198} = 0.249$, $P = 0.62$). Economic losses for both species were estimated at R512.35/ha. In the 2015-16 season carob moth infestation was significantly higher than FCM ($F_{1, 174} = 4.16$, $P = 0.0428$) and resulted in an average estimated economic loss of R2 105.55/ha compared to R789.58/ha for FCM. There was a significant difference between seasons ($F_{1, 372} = 7.39$, $P = 0.0069$) and post-hoc analyses showed that only carob moth infestation differed significantly between seasons ($P = 0.0253$).

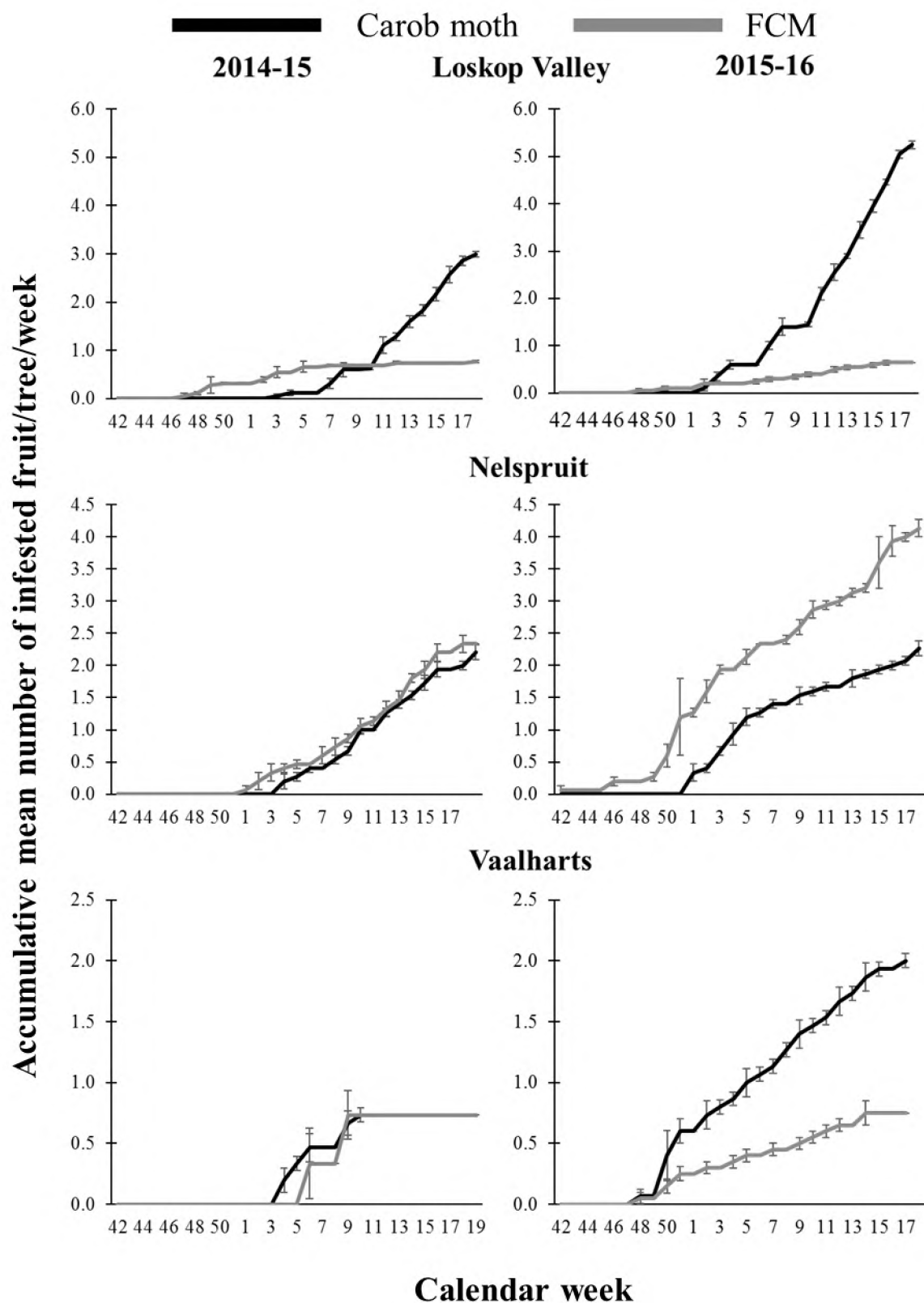


Fig. 3.20 Cumulative mean weekly infestation of carob moth and false codling moth in the Loskop Valley, Nelspruit and Vaalharts production areas over the 2014-15 and 2015-16 growing seasons. Error bars show standard error from the mean.

Table 3.4 Economic loss in Rands due to carob moth and FCM per hectare, estimated from infestation in weekly fruit drop of Navel oranges for three production areas.

Production area	Season	Species	Cumulative infestation/tree/season	Economic loss per hectare
Loskop Valley	2014-15	Carob moth	3.0	R 2 105.55
		FCM	0.8	R 561.48
	2015-16	Carob moth	5.3	R 3 719.80
		FCM	0.7	R 491.29
Nelspruit	2014-15	Carob moth	2.2	R 1 544.07
		FCM	2.3	R 1 614.25
	2015-16	Carob moth	2.3	R 1 614.25
		FCM	4.1	R 2 877.58
Vaalharts	2014-15	Carob moth	0.73	R 512.35
		FCM	0.73	R 512.35
	2015-16	Carob moth	2.0	R 1 403.70
		FCM	0.75	R 526.39

3.4 Discussion

The first step in establishing a reliable monitoring method for carob moth in citrus in South Africa was to evaluate the different commercially available pheromone lures for this species. This was important as the active ingredients within the lures were not identified from South African populations of carob moth and attraction of a species to sex pheromones isolated from geographically distinct populations can vary (Boo 1998, Haung *et al.* 1998). Morland (2015) found that in the Western Cape of South Africa, carob moth trap catches using Insect Science lures produced consistent results between two geographically distinct regions compared to the Chempac lure whose attractiveness differed between regions. Results from section 3.3.1 showed that in a competitive environment the Chempac lure was most attractive to male moths even though all products have the same active ingredient. However, results obtained in the no choice experiment showed that when not competing with each other, Chempac and Insect Science lures are comparable. This difference in trap catches in the choice test may have been a result of either the release rate of the active ingredient providing a higher

density of pheromone for moths to follow, or the stability of the active ingredient compounds (Witzgal *et al.* 2010). The carob moth sex pheromone is known to be highly volatile when applied in small amounts (a single pheromone lure), and the rate of degradation can be highly variable due to the instability of the chemistry which can influence results (Pers comm. William Uritia, ISCA technologies). This is so much so that almond growers in Australia are advised to vary the age of pheromone lures within monitoring programmes to reduce the risk of unreliable trap catch data (Madge *et al.* 2013). The ability of one product to outcompete others in a choice environment could be beneficial in certain environments. Mamay and Dag (2016) showed that using twenty sticky traps baited with carob moth pheromone lures per hectare of pomegranate orchards as a mass trapping technique (MTT), infestation was reduced by 50%. If one commercially available lure is more attractive, then it would most likely be more beneficial to use the Chempac lure if this MTT was to be tested in South Africa.

The picked fruit method recorded higher levels of carob moth infestation in the Nelspruit production area than the dropped fruit method, but the opposite was observed in the Loskop Valley and the Vaalharts. This can most likely be attributed to sampling error by the scouts. The scout in Nelspruit was a trained entomologist and experienced in identifying infested fruit on the tree and incorporated the use of picking tools to reach fruit on the tree that one could not reach without such tools. The scouts in the Vaalharts and the Loskop Valley were not as familiar with detecting infested fruit while still on the tree, resulting in the low level of detection. Although it was displayed that the picked fruit method can be more effective than the dropped fruit method, this experiment highlighted the limitations of the picked fruit method in the absence of a trained scout without the appropriate equipment, which is a reality on many farms in South Africa.

The initial detection of carob moth infestation did not differ between the two methods in Nelspruit, however, detection was recorded in dropped fruit significantly earlier than in picked fruit in other regions. When critiquing the two methods for practical use by industry stakeholders, this delay in detection using the picked fruit method would result in producers being unaware of the presence of carob moth infestation, and had producers been aware of this infestation, strategies to limit the risks associated with infestation could be implemented. These could include increased orchard sanitation, chemical applications or changing the export market. The dropped fruit method was able to detect carob moth infestation over the full growing season and the initial detection of infestation often being earlier than with the picked method. The dropped fruit method is already widely incorporated into commercial citrus

production in South Africa for FCM monitoring, therefore, this method is considered adequate for monitoring the presence and levels of carob moth infestation within orchards.

Seasonal monitoring of carob moth flight activity and infestation for each growing region has provided valuable insight into the ecology of carob moth in citrus in South Africa. The variation within and between seasons in each production area for trap catches and infestation can be attributed to changes in trial sites, as a concerted effort was made to conduct experiments where carob moth infestation was reported to be highly likely. When trial sites were not altered (Nelspruit and Citrusdal) or only a small portion were changed (Loskop Valley), there was no or minimal seasonal or inter-orchard variation. The highest levels of moth activity and fruit infestation were recorded in the northern production areas (Loskop Valley, Vaalharts and Nelspruit). This is consistent with early observations by Catling (1970) who noted that the occurrence of carob moth on citrus in the northern parts of South Africa was increasing. Interestingly, there was a distinct difference between regions when considering the levels of infestation and the apparent pest pressure through trap catches. It would be assumed that the areas with the highest trap catches would also have the highest infestation levels, however, this was not always the case. Trap catches were very low in Nelspruit, however, infestation in this region was the second highest. These low trap catches with high infestation levels could be a result of population reservoirs existing in natural vegetation or surrounding agricultural crops such as macadamias. Other possibilities to consider are differences in attractiveness or susceptibility of the fruit, which could be related to mealybug (or other sucking insect) infestation or any volatiles that we are unaware of. However, in the Vaalharts, pecans orchards surround the sites monitored and this contributed to high trap catches even though levels of infestation were low compared to Nelspruit. These conflicting results suggest that there is a lack of understanding in the role of natural and cultivated alternate hosts and the role these play in carob moth movement within an agricultural ecosystem. There is ongoing research being conducted on the migration of carob moth between pecans and citrus, however, results are still inconclusive (Moore *et al.* 2014a).

Periods of flight activity in all regions followed similar trends to FCM (Newton 1998), with the first peak in flight activity occurring relatively early in the growing season (week 42-15), when temperatures start to warm and the trees are in full blossom. Infestation only starts to occur later on in the season (week 50-2) and this continues until harvest. This suggests that Navel oranges are not suitable hosts until a certain point in the growing season. Another possible explanation for infestation occurring at this time is that weeks 50-4 (the middle of

summer) are when mealybug infestations are at their highest (Hattingh *et al.* 1998) and the honeydew and sooty mould associated with mealybug may provide a suitable ovipositional stimulus for gravid carob moth females, as *Phomopsis* sp. fungi do in carob pods (Gothilf 1975). Over and above the fungus' role as an oviposition stimulus, Gothilf (1970) showed that survival of carob moth on hosts or stages of hosts that were considered unsuitable for larval development, increased significantly in the presence of fungus. This sooty mould or honeydew may be a source of nutrition for carob moth larvae for a period, until either the citrus fruit are suitable for feeding, or until larvae have developed to an instar capable of surviving on a less favourable host as seen by Serghiou (1983), with neonate carob moth survival on grapefruit in the presence and absence of sooty mould.

Although there were significant relationships between trap catches and fruit infestation at various periods after the trap catch, these relationships were relatively weak, restricting their value as a predictive tool for determining levels of infestation through trap catches. These weak relationships can be attributed to the narrow range between weekly high and low points for both trap catches and infestation. Also, the assumption that catches of male moths will be indicative of infestation of larvae in the ensuing generation is a tenuous one from the outset, as there are numerous assumptions and unknowns that separate the two.

The comparison of carob moth and FCM infestation provides two valuable results. Firstly, the onset of fruit drop through infestation occurs at very similar stages in all cases, meaning that broad spectrum control applications may be timed more appropriately to effectively knock down both pest species simultaneously. Secondly, a better understanding of the true pest status of carob moth, measured by crop damage, is obtained. Economic losses due to carob moth are similar to that of FCM, with the exclusion of the experiences in the Loskop Valley. One must also consider that FCM is generally under stringent control through a multi-faceted management approach (chemical and biological (including microbial) treatments, orchard sanitation, mating disruption/attract and kill) which comes at great expense to growers, which is currently not the case with carob moth. However, even though it has been determined that carob moth is not a major pest, measured by fruit damage, it is a phytosanitary pest, at least for China, and if fruit is destined for such a carob moth sensitive market, the economic impact of a possible interception needs to be incorporated into the monetary values presented in this text. In this sense, effective control of the pest is immeasurably important and cannot be calculated based on direct economic losses, as rejections of entire consignments or even market closures could lead to multi-million rand losses.

3.5 Conclusion

Carob moth pheromone lures available for purchase are effective for monitoring male flight activity in orchards, and although differences exist between lures in some cases, for the purpose of monitoring, all lures are sufficiently comparable. Although the picked fruit sampling method is effective when the scout is trained and has the correct equipment, sampling error is too great for widespread implementation. However, with trained scouts this method is more accurate for research purposes, as a comparative rather than absolute measurement. The dropped fruit method reduced the possibility of sampling error and was capable of detecting and monitoring levels of infestation in orchards, making it more appropriate for routine monitoring. This along with its widespread implementation in citrus production in South Africa allows for easy adoption and minimal training for scouts, which would be limited training in differentiation between carob moth and FCM larvae. Traps caught carob moth for the majority of the season and infestation of Navel oranges was first recorded towards the middle of the season and continued until harvest. The pest status and economic loss to growers from carob moth is higher in the northern regions and not of concern in the south of the country. However, the phytosanitary status of a pest must be considered when estimating potential monetary losses.

CHAPTER 4: THE RELATIONSHIP BETWEEN CAROB MOTH AND MEALYBUG WITHIN CITRUS ORCHARDS

4.1 Introduction

There are seven species of mealybugs (Hemiptera: Pseudococcidae) that attack citrus in southern Africa, of which four are most prevalent: the long-tailed mealybug (*Pseudococcus longispinus* Targioni Tozzetti), citrus mealybug (*Planococcus citri* Risso), oleander mealybug (*Paracoccus burnerae* Brian) and the karoo thorn mealybug (*Nipaecoccus viridis* Newstead) (Grout and Moore 2015). Citrus fruit are most susceptible to direct mealybug damage early in the growing season, from petal drop until the fruit reach the size of a golf ball. Mealybug infestation will start under the calyx, then spread to the sides of the fruit and into the navel-end (if a Navel orange). Mealybug produce copious amounts of honeydew on which sooty mould develops (Hattingh *et al.* 1998), which consists of a conglomerate of fungal species (Friend 1965).

Carob moth, *Ectomyelois ceratoniae* Zeller, females are able to differentiate between fungus infected carob pods and uninfected pods, and preferentially oviposit on carob pods infected with *Phomopsis* fungus (Gothilf 1964, Gothilf *et al.* 1975). Serghiou (1983) showed that carob moth and the citrus mealybug exist in a pest complex on grapefruit cultivars in Cyprus within the Mediterranean basin. Carob moth populations will increase within grapefruit orchards if mealybug infestations are not kept under control. In a small scale laboratory study, Serghiou (1983) showed that carob moth larvae are unable to survive and complete development on grapefruit in the absence of mealybug. In southern Africa, this mealybug-carob moth relationship has been noted by various authors (Catling 1970, Honiball and Catling 1998, Moore and Grout 2015), although it has never been quantified.

Where severe mealybug outbreaks occur in grapefruit orchards, carob moth larvae forage on the fruit epidermis, feeding on honeydew and sooty mould. These larvae tunnel into the rind causing physical damage (Fig 4.1A). When this occurs early in the growing season grapefruit will exude copious amounts of gum which generally results in the death of the larvae. However, as the season progresses, gumming becomes less, increasing the fruit's susceptibility to carob moth damage. Carob moth larvae do not penetrate to the flesh of the grapefruit and will pupate in silk sacks on the fruits epidermis (Fig 4.1C). This type of behaviour and damage has also been observed on lemons with heavy mealybug infestation (Fig 4.1B and D).

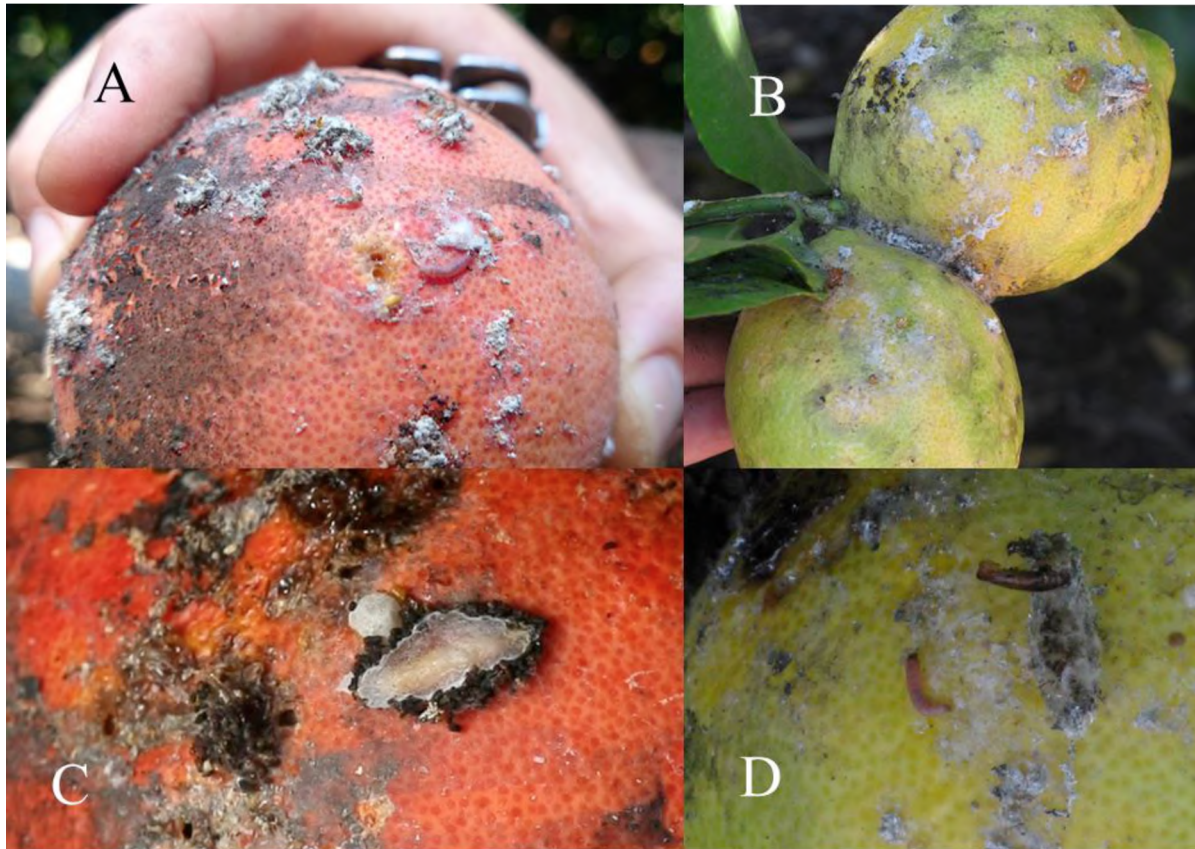


Fig. 4.1 Carob moth larvae and pupae on Star Ruby grapefruit (A, C) and Eureka lemons (B, D).

Recently, the pest status of carob moth on cultivars other than grapefruit, specifically Navel orange cultivars, has been reported to be higher in certain growing regions than previously believed (Moore *et al.* 2014a), possibly due to previous misidentification of larvae of this species as false codling moth (FCM), *Thaumatotibia leucotreta* Meyrick, (Rental 2012, Morland 2015), a real increase of incidence of carob moth in these orchards, or both. In Navel oranges, the behaviour of the carob moth differs to what has been observed in grapefruit and lemons. Larvae are often found within the navel-end of the fruit, and infestations have been reported in the absence of mealybug infestations. Therefore, it is important to establish whether the same relationship between carob moth and mealybug exists in other citrus types in South Africa as in the grapefruit orchards of Cyprus.

The navel-end of oranges is considered to be a safe refuge for mealybug (Hattingh *et al.* 1998) mainly due to the fact that pest control products applied as foliar applications, do not penetrate into the navel-end and do not come into contact with the target species. Pests residing in navel-ends may also be sheltered from natural enemies such as parasitoids. If the navel-end provides a suitable environment for mealybug species, it is possible that carob moth larvae are

able to survive on honeydew produced by these insects within the navel-end or the subsequent sooty mould. Therefore, a reduction in navel-end size may result in a lower proportion of fruit with mealybug residing in the navel-end. This manipulation of navel-end size is possible through the application of 2,4-dichlorophenoxyacetic acid (2,4-D), a plant growth regulator which is used commercially in citrus to increase fruit size (Gaudiola and Garcia-Luís 2000), reduce pre-harvest fruit drop (El-Otmani *et al.* 1990, Anthony and Coggins 1999), and as a post-harvest drench to retain the fruit calyx (Cronjé *et al.* 2005). The application of 2,4-D to Navel orange cultivars close to the time of full petal drop has been shown to reduce the size of navel openings and increase the proportion of closed navel-ends (Verreynne 2008, Mupambi *et al.* 2015). Therefore, the susceptibility of Navel oranges treated with 2,4-D to mealybug infestation in the navel-end could be reduced, which may result in a reduced level of carob moth infestation in these Navel orange orchards.

The aims of this chapter are: (1) to determine the ability of carob moth larvae to survive on Navel oranges under varying levels of mealybug infestation in a laboratory environment; (2) to establish whether carob moth infestation in Navel and Valencia orange orchards is directly related to mealybug infestation; (3) to evaluate the application of 2,4-D and its impact on mealybug infestation, the subsequent carob moth infestation and physical parameters of Navel oranges.

4.2 Materials and methods

4.2.1 Laboratory trial

Experimental design

Fruit with varying levels of mealybug were collected from a Washington Navel orange orchard within the Sundays River Valley in the Eastern Cape of South Africa. These fruit were then separated into three categories: (1) clean (no mealybug or mealybug residues) (Fig 4.2 A), (2) mealybug only (various stages of mealybug life stages but no residues present) (Fig 4.2 B), and lastly, (3) mealybug with residues (a range of mealybug life stages with sooty mould and/or honeydew present) (Fig 4.2 C). Four neonate carob moth larvae were then evenly placed onto the epidermis of the fruit with a number zero paint brush (Fig 2.3), and then left for three weeks at $25 \pm 2^{\circ}\text{C}$ and 16:8 (L: D) hour light cycle. Larvae were obtained from the laboratory culture described in Chapter two. Ten fruit were used for each treatment and the experiment was replicated three times.

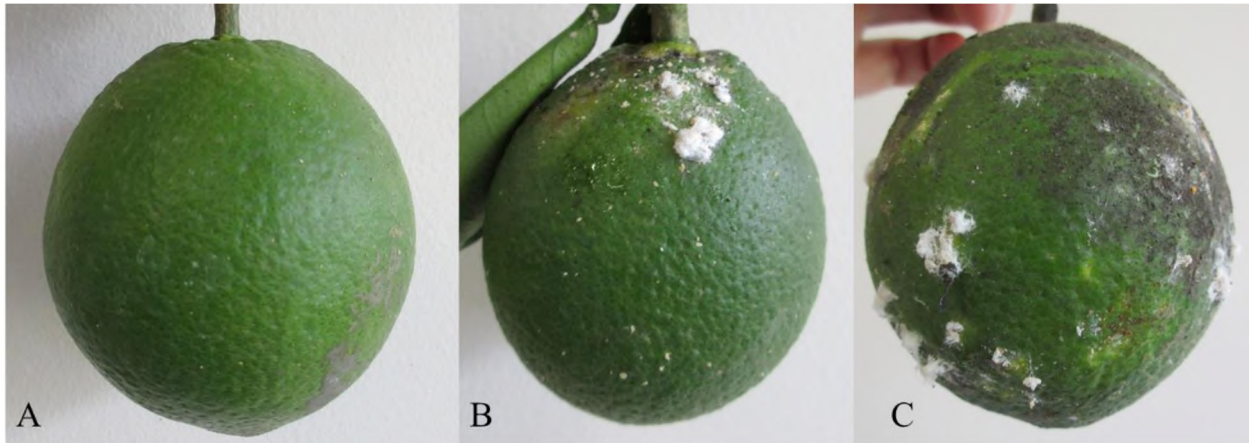


Fig. 4.2 Washington Navel oranges representative of the three different treatments. A - control (free of mealybug), B – Mealybug only (includes any life stage of mealybug but fruit are sooty mould free), C – Mealybug with residues (honeydew and sooty mould present on the fruits epidermis).



Fig. 4.3 A neonate carob moth larva placed onto the fruits' epidermis

Evaluation

After 21 days the fruit were dissected and the number of larvae which were recovered was recorded, along with the penetration point of larvae into the fruit. This was recorded as under the calyx, through the navel-end or through the sides of the fruit.

Statistical analyses

Statistical analyses were conducted in Statistica (Statsoft 2016) and P values of less than 0.05 were considered to denote significant differences. Larval survival was evaluated with a one-way analysis of variance (ANOVA) with the number of larvae surviving on each fruit as the dependent variable and the treatments (clean, mealybug only and mealybug with residue) as the categorical predictor. A factorial ANOVA was used to evaluate statistical differences between the overall percentages of larval penetration points within and between treatments with the number of recovered larvae being the dependant variable and the treatment and entry point being independent variables. Percentages underwent arcsine transformations. A Tukey's HSD post-hoc analysis was undertaken for both the one-way ANOVA and the factorial ANOVA.

4.2.2 Field trial

Experimental design

This field trial was conducted over the 2015-16 growing season at a commercial citrus farm of roughly 500 hectares in the Loskop Valley production area (Limpopo Province, South Africa) (25° 13' 4.75"S 29° 25' 53.45"E), where orchards were monitored for the presence of carob moth and mealybug infestation. A total of eight orchards were monitored and these consisted of five Navel and three Valencia orange orchards (Table 4.1). Five fixed data trees were used in each orchard, with the first data tree being the fifth tree in the fifth row, the next four were positioned diagonally across the orchard in every second row.

Table 4.1 Orchard details for orchards monitored over the 2015-16 growing season for carob moth and mealybug infestation. All orchards were located on one commercial citrus farm of 500 hectares in the Loskop Valley production area (Limpopo Province, South Africa) (25° 13' 4.75"S 29° 25' 53.45"E).

Orchard	Variety	Year Planted	Spacing (m)	Irrigation	Rootstock	Area (ha)
N1	Palmer Navel	1960	7 x 4.3	Drip	GS	7.0
N2	Palmer Navel	1960	7 x 4.3	Micro jet	GS	3.7
N3	Palmer Navel	2005	6 x 3	Drip	Carizzo	10.6

N4	Baihanina Navel	2001	7 x 2	Drip	Carizzo	2.6
N5	Lina Navel	2004	6 x 3	Drip	Carizzo	6.9
V1	Juvelle Valencia	2007	6 x 3	Drip	Carizzo	6.2
V2	Juvelle Valencia	2004	6 x 3	Drip	Carizzo	12.3
V3	Juvelle Valencia	2006	6 x 3	Drip	Carizzo	12.9

Evaluation

Three sampling events took place over the duration of the trial, early in the season (week 50 of 2015), eight weeks later in the middle of the season (week 6 of 2016) and then twelve weeks later in the final stages of the growing season (week 18 of 2016). For each evaluation, 10 fruit were randomly selected on each data tree and inspected for the presence of mealybug, allowing the mean percentage of mealybug infested fruit per tree per orchard to be established. These same data trees were then inspected for carob moth infestation by scouting each tree for 60 seconds and removing all infested fruit from the tree. Fruit were deemed to be infested if they exhibited classic signs of carob moth infestation (early ripening and/or frass/webbing protruding from the navel-end). Fruit were then dissected so that infestation could be confirmed.

Statistical analyses

There was no need to make direct comparisons between orchards due to each orchard representing a true replicate, therefore each orchards carob moth and mealybug infestation for the three sampling events was analysed with only descriptive statistics. The relationship between carob moth and mealybug infestation was subjected to a simple linear regression in Statistica (Statsoft 2016). Data from each citrus type (Navel and Valencia) were combined and three retrospective regression analyses were conducted for each type, which consisted of the following:

1. Week 6 of 2016 carob moth infestation vs week 50 of 2015 mealybug infestation
2. Week 18 of 2016 carob moth infestation vs week 50 of 2015 mealybug infestation
3. Week 18 2016 carob moth infestation vs week 6 2015 mealybug infestation

P values of less than 0.05 were considered to denote a significant relationship between carob moth and mealybug infestations. All percentage data were subjected to arcsine transformation.

4.2.3 Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on carob moth and mealybug infestation

Layout and Application

The effect of 2,4-D on fruit diameter, navel-end size, the percentage of fruit with protruding navels and both mealybug and carob moth infestation were evaluated in a 7ha Palmer Navel orange orchard in the Loskop Valley in Limpopo, South Africa (planted 1960, tree spacing 7 x 4.3m (rows x trees)). A once off application of 2,4-D 500 EC (Dow Agrosciences) was applied on 23 September 2015, roughly two weeks prior to full petal drop, at a rate of 2 ml per 100L and a volume of 4000L per ha with the wetting agent Nu-Film®-17 (active ingredient: 904g/L di-1-p-Menthene; Miller Chemical and Fertilizer Corporation, Pennsylvania, USA). This was applied with a Cima® Blitz 50 trailed sprayer with a T.6M2D spray head. Treatment blocks consisted of 60 trees (3 rows of 20 trees) for both 2,4-D treated blocks and the untreated control blocks with four replicates for each treatment.

Evaluation

Five trees in the centre of the middle row in each block were evaluated on three occasions (week 50 of 2015, week 6 of 2016 and week 18 of 2016) over the 2015-16 growing season. On each data tree, five fruit were randomly selected and picked from the tree and the following data were collected from each fruit:

- mealybug infestation
- mealybug presence in the navel-end
- fruit diameter (mm)
- the navel-end diameter (mm)
- presence of a protruding navel-end
- carob moth infestation was then evaluated on these same five trees by scouting for infested fruit for 60 seconds, picking infested fruit and then dissecting the fruit to confirm infestation.

Statistical analyses

Data from each sampling event were analysed separately. Percentage data were subjected to arcsine transformations and one-way ANOVAs were used to make statistical comparisons of data collected for each data category and sampling event. Post-hoc analyses

were conducted with either Tukey's HSD or Fisher's LSD analysis. All analyses were conducted in Statistica (Statsoft 2016).

4.3 Results

4.3.1 Laboratory trial

There was a significant difference between the number of larvae recovered after three weeks for all treatments ($F_{1,2} = 28.31$, $P = 0.0001$) (Fig. 2.4). Post-hoc analyses showed the highest number of larvae were recovered in the mealybug with residue treatment (1.63 ± 0.23), which was significantly higher than the mealybug only (0.41 ± 0.19) ($P = 0.0001$) and the control (0.1 ± 0.05) ($P = 0.0001$). The number of larvae recovered from the mealybug only fruit was also significantly higher than the control ($P = 0.004$) (Fig. 2.4).

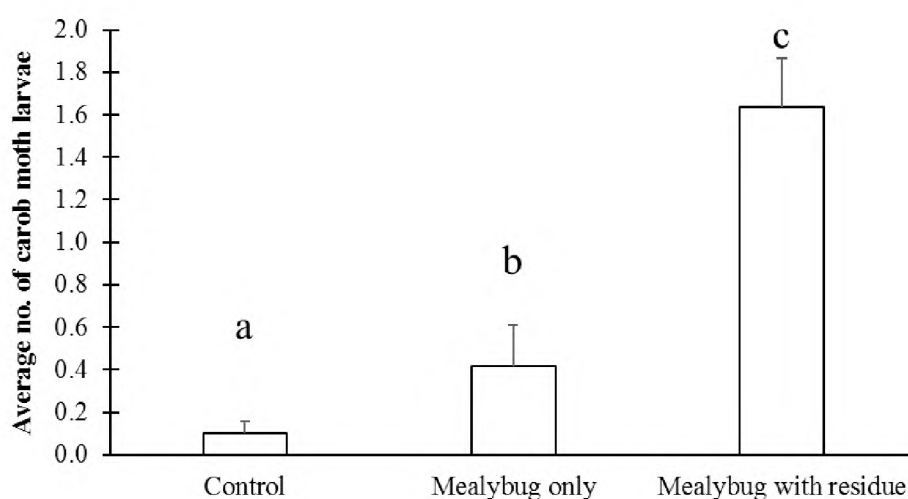


Fig. 4.4 Mean number of carob moth larvae recovered after three weeks on Washington Navel oranges with three different levels of mealybug infestation, a mealybug free control, mealybug only and mealybug with residue such as sooty mould and honeydew. Different letters indicate significant differences ($P > 0.05$) (Fisher's LSD).

The percentage of larval penetration under the calyx, through the navel-end and the side of the fruit differed significantly ($F_{1,2} = 14.29$, $P = 0.000192$) and there was a significant interaction between the different treatments and penetration points ($F_{1,4} = 6.72$, $P = 0.001710$) (Table 2.2). Post-hoc analysis showed significantly higher percentage of larvae penetrated the fruit under the calyx in the mealybug only treatment (93.33 ± 6.66) compared to the control treatment (16.66 ± 16.66) ($P = 0.00559$) and the mealybug with residue treatment (57.93 ± 4.28) ($P = 0.024$). There were no statistically significant differences when comparing the

percentage of larvae penetrating through the side or navel-end of the fruit for all treatments (Table 4.2).

Table 4.2 Mean percentage (\pm SE) of carob moth larval penetration points into Washington Navel oranges at three different densities of mealybug infestation. Different letters in each column indicate significant differences ($P > 0.05$) (Fisher's LSD).

Treatment	Penetration point					
	Calyx		Navel-end		Side	
Control	16.6 ^a	± 16.6	50.0 ^a	± 28.8	0.0 ^a	± 0.0
Mealybug only	93.3 ^b	± 6.6	6.66 ^a	± 6.6	0.0 ^a	± 0.0
Mealybug with residues	57.9 ^c	± 4.2	28.74 ^a	± 5.6	13.31 ^a	± 2.4

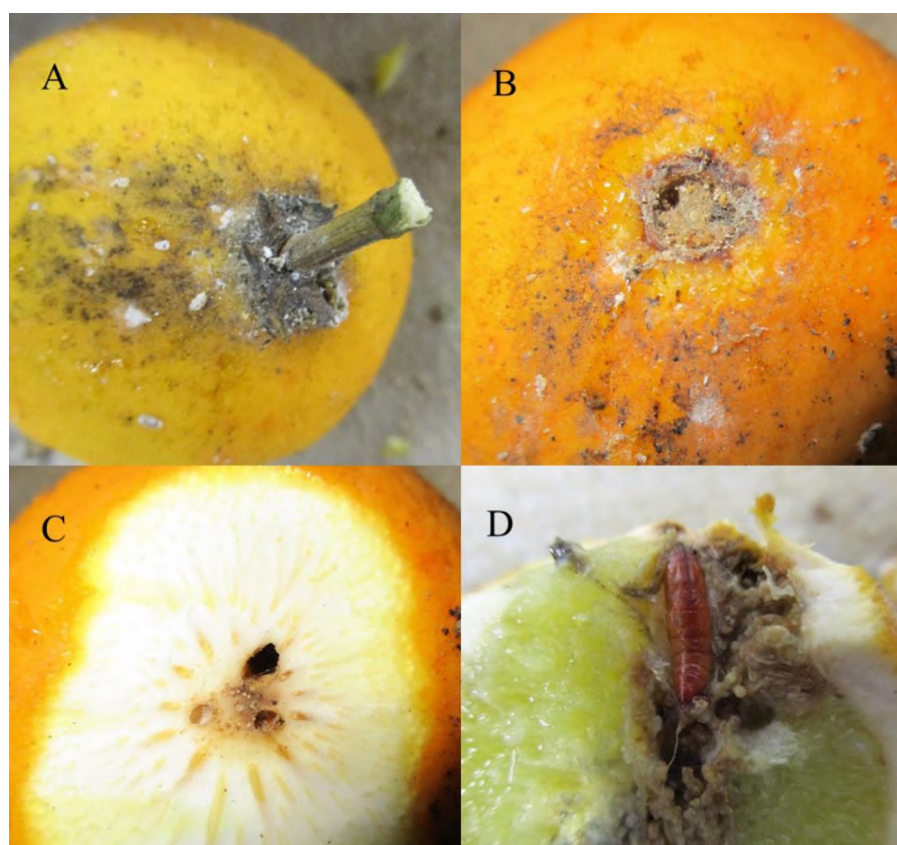


Fig. 4.5 Carob moth infestation on a mealybug only fruit, three weeks after artificial infestation. A: frass surrounding the calyx from larval feeding, B: penetration hole underneath the calyx, C: penetration hole through the rind and albedo of the fruit, D: carob moth pupae within the fruit.

4.3.2 Field Trial

The percentage of fruit infested with mealybug and the number of fruit infested with carob moth per tree were evaluated on three occasions over the 2015-16 growing season (week 50 of 2015, week 6 of 2016 and then week 18 of 2016). Although mealybug infestation levels in week 50 of 2015 were generally low, ranging between 2 % (N3, V3) and 16 % (N1) (Table 4.4), there was a significant relationship between carob moth infestation in week 6 of 2016 and mealybug infestation in week 50 of 2015 for Navel oranges ($R^2 = 0.157$, $P = 0.0498$). However, this was not the case for Valencia oranges ($R^2 = 0.219$, $P = 0.0784$) (Table 4.3). The strongest relationship between carob moth and mealybug infestation was for week 18 of 2016 carob moth and week 6 of 2016 mealybug for both Navel ($R^2 = 0.710$, $P = 0.0023$) and Valencia ($R^2 = 0.524$, $P = 0.0023$) citrus cultivars (Table 4.3). Mealybug levels in week 6 of 2016 had generally increased compared to week 50 of 2015, with the highest level of infestation in an orchard being 24% (N3) (Table 4.4). Week 50 of 2015 mealybug infestation showed no relationship between week 18 of 2016 carob moth infestation in Valencia citrus types ($R^2 = 0.01$, $P = 0.708$). However, there was a significant relationship in Navel citrus cultivars ($R^2 = 0.232$, $P = 0.0147$) (Table 4.3).

Table 4.3 Results for regression analyses for mealybug and carob moth infestation in Navel and Valencia orange orchards.

Citrus Type	Retrospective regression	R^2	Degrees of Freedom	F value	P value
Navel	Week 6 of 2016 carob moth vs Week 50 of 2015 mealybug	0.157	1, 23	4.29	0.0498*
	Week 18 of 2016 carob moth vs Week 6 of 2016 mealybug	0.710	1, 23	56.43	0.0023*
	Week 18 of 2016 carob moth vs Week 50 of 2015 mealybug	0.232	1, 23	6.95	0.0147*
Valencia	Week 6 of 2016 carob moth vs Week 50 of 2015 mealybug	0.219	1, 13	3.65	0.0784
	Week 18 2016 carob moth vs Week 6 2016 mealybug	0.524	1, 13	14.30	0.0023*
	Week 18 of 2016 carob moth vs Week 50 of 2015 mealybug	0.011	1, 13	0.15	0.7080

* Indicates a $P < 0.05$ i.e. a significant relationship

Table 4.4 Mean and standard error of mealybug and carob moth infestation per tree for all Navel and Valencia orange orchards monitored over the season. Evaluation dates were week 50 of 2015, week 6 of 2016 and week 18 of 2016.

Orchard	Cultivar	Infestation type	Infestation			
				Week 50 2015	Week 6 2016	Week 18 2016
N1	Palmer Navel	% mealybug infestation	Mean	16.0	22.0	20.0
			SE	± 14.0	± 2.0	± 4.4
		Carob moth infested fruit/tree	Mean	0.0	1.0	2.6
			SE	± 0.0	± 0.3	± 0.2
N2	Palmer Navel	% mealybug infestation	Mean	6.0	24.0	16.0
			SE	± 6.0	± 7.0	± 6.0
		Carob moth infested fruit/tree	Mean	0.0	0.8	2.0
			SE	± 0.0	± 0.3	± 1.0
N3	Palmer Navel	% mealybug infestation	Mean	2.0	4.0	10.0
			SE	± 2.0	± 2.0	± 6.3
		Carob moth infested fruit/tree	Mean	0.0	0.0	0.6
			SE	± 0.0	± 0.0	± 0.4
N4	Bhahainina Navel	% mealybug infestation	Mean	8.0	14.0	10.0
			SE	± 0.4	± 0.9	± 6.3
		Carob moth infested fruit/tree	Mean	0.0	0.2	1.4
			SE	± 0.0	± 0.2	± 0.9
N5	Lina Navel	% mealybug infestation	Mean	6.0	4.0	14.0
			SE	± 4.0	± 2.4	± 4.9
		Carob moth infested fruit/tree	Mean	0.0	0.0	1.4
			SE	± 0.0	± 0.0	± 0.7
V1	Juvella Valencia	% mealybug infestation	Mean	8.0	14.0	22.0
			SE	± 2.0	± 2.0	± 3.7
		Carob moth infested fruit/tree	Mean	0.0	0.8	1.8
			SE	± 0.0	± 0.4	± 0.3
V2	Juvella Valencia	% mealybug infestation	Mean	10.0	2.0	4.0
			SE	± 4.0	± 2.0	± 2.0
		Carob moth infested fruit/tree	Mean	0.0	0.8	0.0
			SE	± 0.0	± 0.3	± 0.0
V3	Juvella Valencia	% mealybug infestation	Mean	2.0	12.0	14.0
			SE	± 2.0	± 5.0	± 6.0
		Carob moth infested fruit/tree	Mean	0.0	0.0	1.6
			SE	± 0.0	± 0.0	± 0.7

4.3.3 Effect of 2,4-D on carob moth and mealybug infestation

The application of 2,4-D had no significant effect on the fruit diameter when compared to the untreated control for all sampling events ($F_{1,2} = 0.285$, $P = 0.6$) (Table 4.5). However, the diameter of the navel opening was significantly greater in the untreated control than the 2,4-D treated trees ($F_{1,2} = 37.097$, $P = 0.00009$) (Fig. 4.7). Post-hoc analysis revealed that this significant reduction in navel-end diameters in the 2,4-D treated blocks was the case for week 50 of 2015 (65 % reduction) ($P = 0.00216$), week 6 of 2016 (63 % reduction) ($P = 0.002$) and week 18 of 2016 (50 % reduction) ($P = 0.00318$). Overall, there were significantly fewer protruding navels in the untreated control blocks ($F_{1,2} = 47.51$, $P = 0.000002$). However, this was only the case for the second two sampling events when fruit size increased, with 15% of fruit with protruding navels in the untreated control compared to 1% in the 2,4-D blocks in week 6 of 2016 ($P = 0.00001$) and 21% of fruit with protruding navels in the untreated control compared to 7.5% in the 2,4-D treated blocks ($P = 0.00015$).

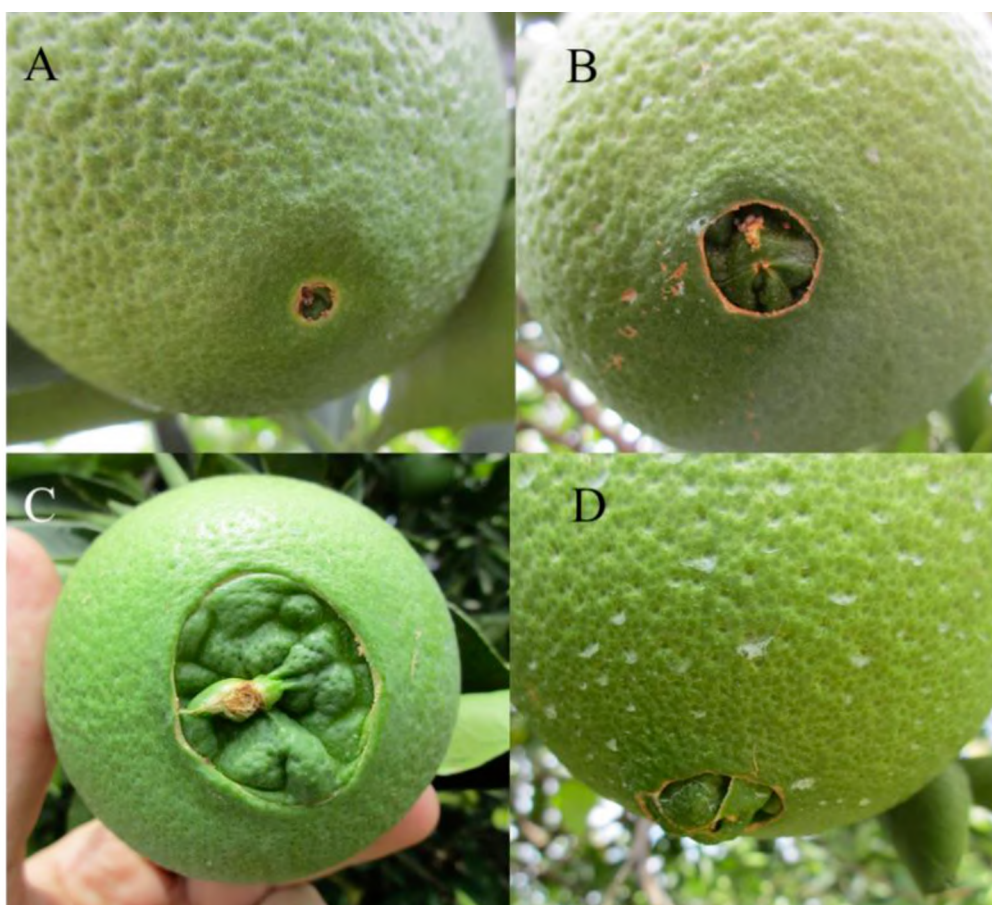


Fig. 4.7 Palmer Navel oranges in week 6 of 2016 and varying range of navel-end diameters (A-C) and protruding navel-end (D).

Table 4.5 Mean fruit dimensions, carob moth and mealybug infestation levels on Palmer Navel oranges treated with 2,4-D and an untreated control. Different letters in each column for each sampling event indicate significant differences between the means ($P < 0.05$) (Tukey's post-hoc test).

Sampling event	Treatment	Fruit diameter (mm)		Navel-end diameter (mm)		Protruding navel-ends (%)		Mealybug infestation (%)		Mealybug in navel-ends (%)		Carob moth infestation fruit/tree	
Week 50 of 2015	2,4-D Ester	47.75 a	± 1.49	2.72 a	± 0.81	1.00 a	± 1.00	12.00 a	± 4.80	45.83 a	± 15.77	0.00 a	± 0.00
	Untreated control	44.47 a	± 1.75	7.75 b	± 1.5	1.00 a	± 1.00	16.00 a	± 8.00	55.00 a	± 21.01	0.00 a	± 0.00
Week 6 of 2016	2,4-D Ester	72.25 a	± 8.02	2.93 a	± 0.73	1.00 a	± 1.00	31.67 a	± 11.78	28.75 a	± 10.87	0.15 a	± 0.05
	Untreated control	66.25 a	± 2.71	7.95 b	± 1.50	15.00 b	± 2.50	29.00 a	± 3.78	61.57 b	± 6.27	0.20 a	± 0.08
Week 18 of 2016	2,4-D Ester	101.25 a	± 3.68	4.86 a	± 0.09	7.50 a	± 0.50	13.00 a	± 1.90	12.50 a	± 7.2	0.10 a	± 0.05
	Untreated control	104.5 a	± 5.04	9.65 b	± 0.32	21.00 b	± 2.51	23.00 a	± 2.51	66.37 b	± 3.65	0.35 b	± 0.09

There was no significant difference in levels of mealybug infestation between the treated and untreated blocks in all sampling events ($F_{1,2} = 0.51$, $P = 0.484147$), although, the percentage of fruit where mealybug was found in the navel-end did differ significantly between the two treatments ($F_{1,2} = 10.05$, $P = 0.00528$). Early in the season (week 50 of 2015), when mealybug infestation was relatively low, there was no significant difference in the percentage of mealybug found within the navel-end ($P = 0.605789$). However, as mealybug infestation increased in the orchards, a significantly higher percentage of mealybug was found in the navel-ends of fruit in the untreated control than the 2,4-D treatment in both week 6 of 2016 ($P = 0.056246$) and week 18 of 2016 ($P = 0.06353$). Although carob moth infestation was low over the season, 2,4-D did have a significant effect on carob moth infestation levels ($F_{1,2} = 4.15$, $P = 0.05$) and there was no significant difference between sampling events ($F_{1,2} = 7.73$, $P = 0.003772$). Carob moth infestation was not significantly different between the two treatments in week 50 of 2015 and week 6 of 2016, however, significantly higher levels of carob moth per tree were recorded in the untreated control (0.35 ± 0.09) when compared to the 2, 4-D treatment (0.1 ± 0.05) in week 18 of 2016 ($P = 0.008721$).

4.4 Discussion

From the experiments conducted above it is clear that carob moth infestation is directly associated with mealybug infestation in citrus types other than grapefruit and lemons. The laboratory trial showed that a small percentage of carob moth larvae were able to survive when placed on Washington Navels in the absence of mealybug, suggesting that under these conditions Navel oranges are not a preferred host for carob moth. As the intensity of mealybug infestation increased, so did the survival of larvae, and it is important to reiterate that preference of larvae was not evaluated, only survival as the fruit were artificially infested albeit topically. The decision of preferable oviposition sites must be made by a gravid female, and this study revealed the direct benefit of carob moth females which choose to oviposit eggs on less favoured hosts that contain additional sources of larval nutrition, improving her offspring's fitness. These results are in line with Gothilf (1969), who illustrated that carob moth larval developmental period on ripe and dry carob pods could take up to three months with very low survival rates, however, where *Phomopsis* sp. fungus was present, larval developmental time and survival increased dramatically. Carob moth larvae entered the fruit significantly more under the calyx in the mealybug only treatment, most likely due to mealybug congregating under the calyx, as reported by Hattingh *et al.* (1998). Larval penetration points were more

evenly distributed in the mealybug with sooty mould treatment than the mealybug only treatment, likely because of the availability of honeydew and sooty mould spread over the fruit surface area, which lead to a smaller proportion of larvae needing to forage under the calyx.

In Navel and Valencia orange orchards, there was a significant relationship between carob moth and mealybug infestation, as Serghiou (1983) showed was the case in grapefruit orchards. No carob moth infestation was observed in week 50 2015, which may be due to the young fruit not being susceptible to larval penetration, or possibly that mealybug infestation so early in the season does not produce enough honeydew (and sooty mould) to sustain carob moth larval survival. Carob moth infestation in week 6 and 18 2016 was significantly related to mealybug infestation 8-12 weeks earlier. This lag time suggests that female moths may only select fruit with more advanced stages of mealybug infestation, due to the increased availability of honeydew and/or sooty mould, which would alter the chemical composition of fruit (Gothilf *et al.* 1975). Therefore, olfactory stimuli may play an important role in mediating carob moth oviposition in citrus orchards, as female moths show an ovipositional preference for hosts infested with fungus (*Phomopsis* sp.) in other hosts (Gothilf *et al.* 1975). Gothilf *et al.* (1975) observed that an extract of the steam distillate of carob pods infected with the fungus *Phomopsis* sp. was more effective in stimulating female moths to oviposit than an extract of uninfected carob pods. Ethyl hexanoate, a volatile compound extracted from fungus infected date fruit, has also been found to stimulate upwind flight of female carob moths (Cosse *et al.* 1994). The teleology of this preference is clear; carob moth females' preferential selection of hosts with fungus has a direct benefit for their offspring, and is most likely a product of natural selection driven by the low availability of suitable hosts in certain areas or at certain times of the year.

Carob moth survival and infestation of sweet citrus (Navel and Valencia oranges) has been shown to be highly dependent on the presence of mealybug residue (honeydew) and subsequent presence of sooty mould. Currently, treatment thresholds state that chemical intervention for the control of mealybug is required if infestation is less than 5% at petal fall, and there is little benefit to chemical intervention between six weeks after petal fall and the end of January, and unless there is an indication of an extensive increase in mealybug infestation over this time period a chemical treatment may be of value (Moore and Hattingh 2012b). However, results of this study indicate that even low mealybug infestation levels in Navel orange orchards in December are an indicator of likely carob moth infestation mid-season and shortly prior to harvest. The strongest indicator of April carob moth infestation is mealybug

infestation levels in February, and according to production guidelines, it is at this time the producer should evaluate the need for chemical intervention to control mealybug based on the level of infestation and presence of biological control (Moore and Hattingh 2012b). If there is a high level of mealybug natural enemies in orchards, it is likely that mealybug populations would be under effective biological control by the time of harvest (Moore and Hattingh 2012b). However, this does not remove the mealybug residues and associated sooty mould from fruit, and is likely to have little effect in reducing late season carob moth infestation. Although this corrective control of mealybug through biological control may have been valid in the past, current citrus production practices incorporate a variety of calendar based plant protection product applications (sprays applied at set time periods to minimise risk of target occurrence) for the control of pests which cause cosmetic damage (eg. citrus thrips, *Scirtothrips aurantii* Faure), physosanitary pests (eg. FCM) and diseases (eg. citrus blackspot, *Phyllosticta citricarpa* McAlpine) due to the majority of southern African citrus being destined for export (CGA 2016). These calendar applications have negative effects on beneficial arthropods and disrupt biological control, resulting in secondary pest outbreaks such as an increase in mealybug infestation levels (Grout and Moore 2015).

The application of 2,4-D was successful in reducing the size of the navel-end opening and the number of protruding navel-ends, which is congruent with other studies which evaluated the impacts of 2,4-D on the physical characteristics of Navel orange cultivars (Krezdon 1969, Verreyne 2008, Mupambi *et al.* 2015). A limited number of studies have considered the benefits of pest control or reduction from manipulating Navel orange fruit parameters with 2,4-D. Moore *et al.* (2014b) showed that with a reduction in the percentage of protruding navel-ends and the size of the navel opening, both mealybug and bud mite infestation was reduced in Navel orange orchards treated with 2,4-D compared to untreated controls. The application of 2,4-D in this trial did not significantly reduce mealybug infestation, however, there was a significant reduction in mealybug found in the navel-end in the middle and towards the end of the growing season. There were significantly higher numbers of carob moth infested fruit in the untreated control compared to the 2,4-D treated trees. These results showed that even though mealybug infestation may be uniform over an orchard, when a higher percentage of mealybug is present in the navel-end of fruits, it results in higher levels of carob moth infestation.

4.5 Conclusion

This study has shown that there is a direct benefit for carob moth females to favour citrus infested with mealybug as larval survival is increased in the presence of honeydew and sooty mould. Carob moth infestation in citrus orchards is directly related to mealybug infestation levels 8-12 weeks prior to the sampling event. It is recommended that current production guidelines for mealybug infestation in citrus orchards should be re-evaluated depending on the desired target market for the fruit and previous carob moth and mealybug infestation levels.

CHAPTER 5: CHEMICAL AND SEMIOCHEMICAL CONTROL OF THE CAROB MOTH IN CITRUS ORCHARDS

5.1 Introduction

Although the carob moth, *Ectomyelois ceratoniae* Zeller, is a pest of concern on citrus in other parts of the world including Cyprus (Orphanides *et al.* 1996), Turkey (Ozturk *et al.* 2011), Israel (Gothilf 1975, 1969) and Egypt (Hashem and El – Halawany 1996), its pest status on citrus in South Africa is regarded as sporadic, with outbreaks usually associated with high levels of mealybug infestation, particularly in grapefruit orchards (Grout and Moore 2015). It was assumed that control methods for other more significant pests of citrus, such as the false codling moth (FCM), *Thaumatotibia leucotreta* Meyrick, would simultaneously control carob moth in citrus orchards (Catling 1970). Therefore, there has not been a need for chemical or semiochemical applications directly for the carob moth in citrus and there are currently no registered products available for its control on citrus in South Africa. A large number of materials from most chemical classifications have been evaluated against carob moth on non-citrus hosts in other regions of the world (eg. Warner *et al.* 1990 and Blumberg 2008). Ampligo® (active ingredient: chlorantranipole) is currently the only product registered for the control carob moth on tree nut crops (almonds, macadamias, pistachios, walnuts and pecans) in South Africa (Agri-intel 2016). In the United States of America, there are three chemical products registered for the control of carob moth on dates: Delegate® (active ingredient: spinetoram), Intrepid® (active ingredient: methoxyfenozide) and malathion applied in a dust formulation (Perring *et al.* 2015).

Recently the pest status of the carob moth on Navel orange cultivars in certain production areas of South Africa has been highlighted (Moore *et al.* 2014a). No region specific research has been conducted on control options for carob moth on citrus in southern Africa, and very little information is available on the efficacy of control methods for carob moth in other citrus growing regions in the world. Thus there was a need to establish whether pesticides registered for the use against other lepidopteran pests which often require chemical control in citrus orchards in southern Africa, such as FCM, would also be effective in controlling carob moth.

Pesticide applications are not always an option for pest control in agriculture, especially with residue level restrictions when commodities are destined for export. Pesticide applications often have non-target effects and disrupt natural enemy complexes, whether these natural enemy complexes are conserved within an agricultural landscape or augmented through mass releases (Roubos *et al.* 2014). An alternative to pesticide application is the use of pest behaviour modification systems through the use of semiochemicals, specifically the use of pheromones, which are by definition species specific (Witzgal *et al.* 2010). Insect pheromones are used to monitor the presence of pests or the population management of pests, of which mating disruption is the most common method (Witzgal *et al.* 2010).

Mating disruption is the use of insect sex pheromones dispensed over an area, which causes disorientation and communication disruption between sexes, and thus delays, reduces or prevents the fertilization of females. When mating disruption was first proposed as a means of pest control, it was assumed that most, if not all, females would need to remain unmated in order for the method to be effective. However, evidence showing that the females' ability to mate merely needs to be impaired, such that their first and second matings are delayed, to provide sufficient population control (Baker 2009 and references therein). The continued long term use of mating disruption products has been shown to reduce pest population levels of the target species over time (Witzgall *et al.* 1999, Varner *et al.* 2001, Weddle *et al.* 2009). This can be attributed to the build-up of beneficial natural enemies and an increase in the efficacy of pheromone products at low population densities (Witzgal *et al.* 2010).

A carob moth mating disruption product, SPLAT® EC (Active ingredient: (Z, E) 7, 9, 11 dodecatrienyl formate; ISCA Technologies, Inc., Riverside, California, USA), which uses a parapheromone of the major component of the carob moth pheromone, (Z, E) 9, 11, 13 tetradecatrienal (Baker 1989, 1991), is currently the only available product for the mating disruption of this species. Todd *et al.* (1992) demonstrated that this parapheromone possessed a more stable release rate in the field than the synthetic blends of carob moth pheromone, which were not able to outcompete calling females due to the decomposition of the highly labile triene major component of the pheromone (Millar 1990). SPLAT® EC has been evaluated in date gardens in California, USA, where it was found to produce control levels comparable to pesticide applications (Mafra-Neto *et al.* 2013). The pheromone mimic has been registered as an organic use product in dates by the US Environmental Protection Agency (EPA) and is currently marketed as SPLAT® EC (Perring *et al.* 2014). Mamay and Dag *et al.* 2016 showed that the product was also effective in reducing damage to pomegranates in Turkey.

Many factors could influence the efficacy of this product against carob moth in South Africa, such as geographic variations in sex pheromone (Huang *et al.* 1998, Mc Elfresh and Millar 1999) and the landscape in which the product is applied (citrus orchards are structurally different from date gardens). Thus, there was a need to evaluate the ability of SPLAT® EC to control carob moth infestations in citrus orchards.

There are two aims for this chapter: (1) to determine the field efficacy of available foliar applied products, which are registered for the control of other lepidopterans on citrus in South Africa, against the carob moth; and (2) to evaluate the ability of SPLAT® EC to cause carob moth trap catch shutdown and reduce infestation of citrus fruit in the field.

5.2 Materials and methods

5.2.1 Spray trial

In both the 2014-15 and 2015-16 growing seasons, a spray trial was conducted in a seven hectare (ha) commercial Palmer Navel orange orchard in the Loskop Valley production area (Limpopo, South Africa) (GPS coordinates 25°11'46.755"S, 29°25'15.292"E), which has shown high levels of carob moth infestation in previous seasons. This orchard was planted in 1960 with a spacing of 6 x 3 m (333 trees/ha) under drip irrigation.

Application and layout

Seven treatments were applied in the form of a randomised complete block design (Petersen 1994), with ten single tree replicates (Moore *et al.* 2015). The trial was conducted in two separate seasons; the application date in the 2014-15 season was 23rd of April 2015 and 14th of December 2015 for the 2015-16 season. Treatments were applied as medium film cover sprays until the point of run-off at concentrations registered for lepidopteran control on citrus in South Africa (Table 5.1). A Janisch spray machine with a Honda 250 cc motor was used with hand held spray guns set at 20 bar pressure with a 2 mm diameter nozzle. Certain products used are ultraviolet (UV) sensitive (Broadband® and Dipel®) and these were applied in the last two hours of daylight to ensure that UV breakdown was minimal. Additionally, trees must be dry before product application, in order to avoid product run-off or dilution. Both of these factors were considered and managed for in both trial applications.

Table 5.1 Chemicals applied to single tree replicates in a complete randomised block design in a Palmer Navel orchard in Limpopo Province, South Africa (25°11'46.755"S, 29°25'15.292"E).

Treatment	Active compound	Concentration per 100L	litres/tree	
			23/3/2015	14/12/2015
Delegate®	Spinetoram	20g	30.5	28
Coragen®	Chlorantraniliprole	17.5ml	28.5	29
Runner®	Methoxyfenozide	60ml	33	32
Cypermethrin®	Cypermethrin	25ml	33	32
Dipel®	<i>Bacillus thuringiensis</i>	12.5g	31	25
Broadband® +				
Breakthru ¹	<i>Beauveria bassiana</i>	50ml + 5ml	30	30
Untreated control	*	*	*	*

¹ Breakthru® (active ingredient: polyether–polymethylsiloxane–copolymer 1000 g/l) (Evonik Industries, Germany)

Evaluation

2014-15 season

For the 2014-15 season trial, it was assumed that the behaviour of carob moth was similar to FCM and therefore the physiological response of the fruit should also be similar i.e. 100% of infested fruit will be abscised by the tree. This resulted in the use of the standard method used to determine FCM infestation in citrus orchards to evaluate infestation levels of carob moth (Moore *et al.* 2015). This entailed initiation of evaluations only three weeks after application of treatments, allowing all fruit that may have been infested before treatments were applied to drop from the trees. Additionally, it is recognised that FCM-infested fruit take a minimum of three weeks to drop off the tree. These dropped fruit were then cleared under all data trees three weeks after application of treatments, indicating that any fruit drop over the coming weeks would be from infestation that occurred after the treatment application. For eight consecutive weeks, on a set day of the week, all dropped fruit were collected and inspected for the presence of larval infestation. Any larvae that were recovered were placed into 70% ethanol and kept for identification. Larvae were distinguished between carob moth and FCM according to Rental (2012) and Morland (2015) (Fig. 3.4). The total number of larvae collected each week

were then divided by the number of data trees, allowing infestation to be reported as number of infested fruit per tree per week (Moore *et al.* 2015).

A once-off evaluation was then performed 11 weeks after application of treatments, which consisted of using a novel scouting method (picked fruit method). Each data tree was scouted for sixty seconds, searching for fruit that showed typical signs of carob moth infestation, such as premature ripening along with frass and webbing protruding from the navel-end (Fig. 3.6), first circling the tree and then moving under the canopy. All fruit exhibiting these symptoms or which appeared suspicious were picked from the tree and then dissected. Due to the morphological similarity between carob moth and FCM larvae, all larvae and pupae were placed into 70% ethanol and identification was confirmed in the laboratory according to Rental (2012) and Morland (2015) (Fig. 3.4). Pupae found in fruit were considered to be carob moth as FCM will pupate in the soil (Newton 1998, Love *et al.* 2014). The mean number of fruit infested with carob moth per tree per week over a predetermined time period was determined.

2015-16 season

As monitoring weekly fruit drop was not considered sufficiently accurate in the 2014-15 season, only the picked fruit method, described above, was used for evaluating the performance of the relative treatments. Evaluations took place 11 weeks (10th of February 2016) and 22 weeks (18th April 2016) after application of the products on the 12th of December 2015.

Statistical analyses

Results from each season and sampling event were analysed separately with the use of a General Linear Model (GLM) in Statistica (Statsoft 2016). Tukey's HSD test was used as post-hoc analyses and P values less than 0.05 were considered to indicate significant differences.



Fig. 5.1 Typical signs of carob moth infestation in Palmer Navel orange. **A:** infested fruit exhibiting the physiological response of premature ripening due to larval infestation. **B:** frass and webbing protruding from the navel-end. **C:** carob moth infestation with frass, webbing and mealybug residue.

5.2.2 Mating disruption – SPLAT® EC

SPLAT® EC evaluation trials were conducted in the Loskop Valley production area over 2014-15 and 2015-16 growing seasons. The trial site for the 2014-15 season was a 10.5ha Palmer navel orange orchard which was planted in 1984 with a spacing of 6.5 x 4m (385 trees per ha) (25°2'9.999''S, 29°23'39.119E). The 2015-6 study site consisted of a 9ha commercial Palmer navel orchard which was planted in 2005 with a spacing of 3 x 6m (555 trees per ha) (25°12'1.293''S, 29°25'43.179''E).

Application and layout

In the 2014-2015 growing season SPLAT® EC was applied on the 20th of September 2014 and 28th of January 2015 at 310g per ha using a grease gun calibrated to 1.3g per leaver

pull (Fig. 5.2 A). Each pull produced a 3-5cm long glob of the product which was applied to undamaged leaves in the top third of trees from the back of a vehicle (Fig. 5.2 B and C). Due to a limited amount of available product, a single 3 ha treated block and a single 3 ha untreated control were compared (Fig. 5.3).

For the 2015-2016 growing season the product was applied on 22nd of October 2015 and 12th of February 2016 at 310g per ha. However, the consistency of SPLAT® EC had changed and the grease gun was calibrated to 1.6g per leaver pull for the 2015-2016 growing season. SPLAT® EC was applied to three 1.3ha blocks which were separated by three 2.5ha untreated control blocks (Fig. 5.4).



Fig. 5.2 Application of SPLAT® EC. **A:** application with grease gun from the back of vehicle. **B:** SPLAT® EC applied to leaf surface. **C:** close up of SPLAT® EC on leaf immediately after application. **D:** splat droplet in week 25 of 2016 that was applied in week 46 of 2015.

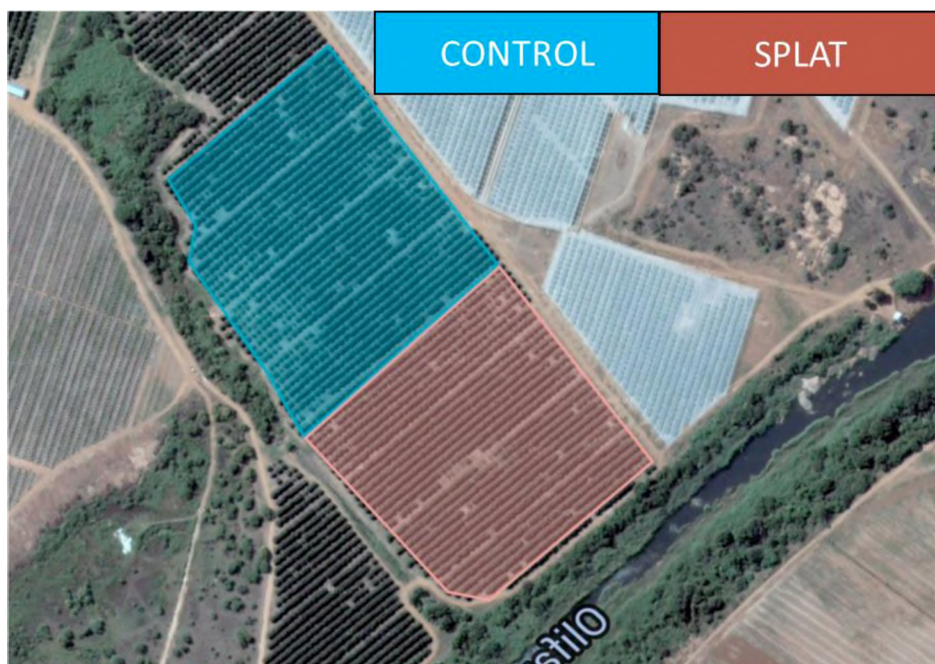


Fig. 5.3 Layout of SPLAT® EC trial site for the 2014-15 season in the Loskop Valley production area, Limpopo Province, South Africa (GPS 25°2'9.999''S, 29°23'39.119E).



Fig. 5.4 Layout of SPLAT® EC trial in the 2015-16 season in the Loskop Valley production area, Limpopo Province, South Africa (GPS 25°12'1.293''S, 29°25'43.179''E).

Evaluation

2014-2015 season

Due to the relatively small treatment area (mating disruption is most effective over large areas), incomplete permeation of the product may increase the edge effect (Milli *et al.* 1997). One yellow delta trap (Insect Science, Tzaneen, South Africa) was placed in the centre of each treatment block and this was baited with a carob moth male lure (active ingredient: Z, E-7,9,11 dodecatrien-ol formate). This was replaced every six weeks and monitored weekly throughout the experiment. In order to counter the possibility of gravid females immigrating into treated plots from untreated blocks (i.e. the edge effect), fruit infestation was monitored in the centre of the orchard by collecting dropped fruit from 10 data trees (five trees on each side of the delta trap). This evaluation took place from week 42 of 2014 until the week before harvest (week 14 of 2015).

In addition to monitoring infestation in dropped fruit, the picked fruit sampling method was used in a once-off sampling event in week 14 of 2015 to evaluate the presence of carob moth infestation between the treated and untreated block using the described scouting method with a total of 30 trees scouted in each block. These trees were located in the centre of each orchard (ten trees over three rows).

2015-2016 season

One yellow delta trap was placed in the centre of each treatment block and this was baited with a carob moth male lure (Insect Science). This was replaced every six weeks and monitored weekly throughout the experiment. The picked fruit method was used for evaluation of SPLAT® EC efficacy against carob moth and was compared to the untreated control. Evaluations took place in week 49 of 2015, week 7 of 2016 and week 18 of 2016 (two weeks before harvest). For each evaluation 20 trees were scouted in the dead centre of each replicate for all treatments (six trees in four rows).

Statistical analyses

Each season's data was treated separately. Trap catches were compared using an Analysis of Variance (ANOVA). Weekly infestation and destructive sampling results were analysed using a General Linear Model (GLM). All statistical tests underwent post-hoc analyses in the form of Tukey's HSD test. P values less than 0.05 were considered to indicate significant differences. All analyses were conducted using Statistica (Statsoft 2016).

5.3 Results

5.3.1 Spray trial

2014-15 season

Weekly fruit drop over a five-week period showed no trend in levels of infestation between treatments (Fig. 5.5). There were no significant differences in the number of infested fruit observed among the treatments ($F_{12, 684} = 0.64$, $P = 0.262$). However, there were statistical differences in levels of infestation between weeks ($F_{12, 684} = 0.64$, $P = 0.044$). The picked fruit sampling method, undertaken 11 weeks after treatment applications, showed that some treatments significantly reduced levels of carob moth infestation ($F_{1, 6} = 3.003$, $P = 0.012$) (Fig. 5.6). Delegate® and Runner® treated trees showed a mean infestation of $0.2 (\pm 0.2)$ fruit per tree with both significantly reducing infestation ($P = 0.043$) by 89% compared to the untreated control with a mean of $1.9 (\pm 0.5)$ infested fruit per tree. Coragen® (0.3 ± 0.21) reduced infestation by 84% compared to the untreated control, however, this difference was not statistically significant ($P = 0.069$). Cypermethrin® (0.6 ± 1.4) and Dipel® (0.8 ± 0.39) reduced infestation by 68.4% and 57.8% respectively, and infestation for Broadband® was $1.5 (\pm 0.62)$ infested fruit per tree, only 21% lower than the untreated control.

2015-16 season

Infestation 11 weeks after application ranged from 0.3 to 1.0 fruit per tree with significant differences recorded among the treatments ($F_{1, 6} = 2.52$, $P = 0.03$) (Fig. 5.7). Post-hoc analyses showed that Delegate® was the only treatment that was significantly different from the untreated control ($P = 0.039$), lowering infestation by 88.9%. Coragen®, Runner®, Cypermethrin® and Dipel® did not significantly reduce infestation ($P = 0.321$, $P = 0.162$, $P = 0.321$, $P = 0.162$). However, these all reduced infestation by 60% or more. Broadband® was only able to reduce infestation by 20%, which was also not statistically different from the untreated control ($P = 0.99$).

Evaluation 22 weeks after the initial treatment application showed that control with Delegate®, Coragen®, Runner® and Cypermethrin® was still being maintained. With infestation being 33%, 30%, 50% and 30% respectively lower than the untreated control. However, these differences were not statistically significant when compared to the untreated control ($F_{1, 6} = 0.625$, $P = 0.257$) (Fig. 5.8).

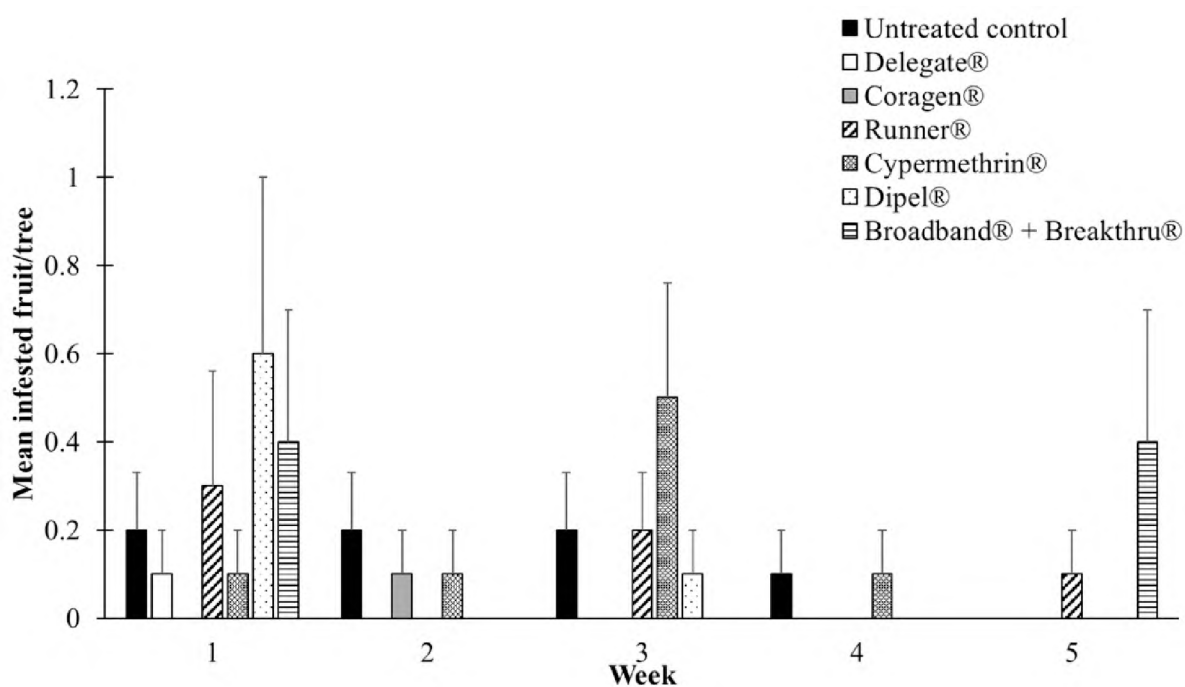


Fig. 5.5 Weekly carob moth infestation in dropped fruit starting four weeks after application for the 2014-15 growing season. Error bars show standard error from the mean.

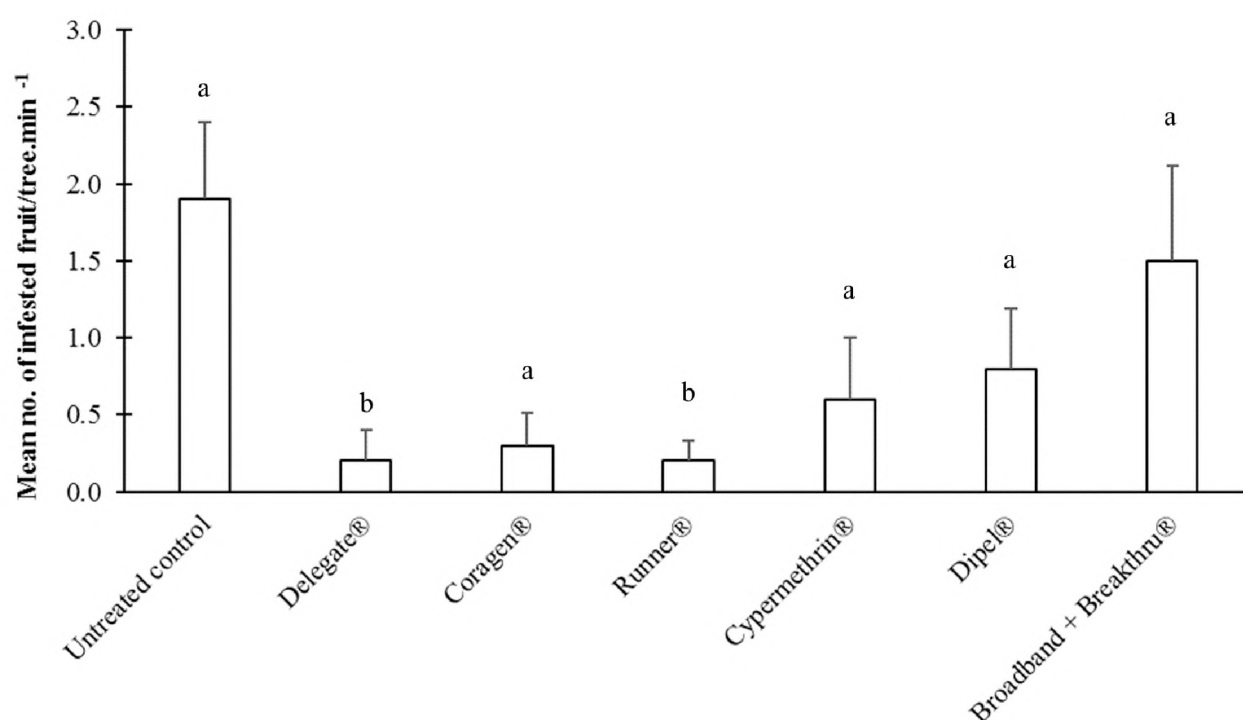


Fig. 5.6 Mean carob moth infestation per tree recorded in one minute at 11 weeks after treatment application in the 2014-15 growing season. Error bars show standard error from the mean. Different letters indicate significant differences ($P > 0.05$) (Tukey's post-hoc test).

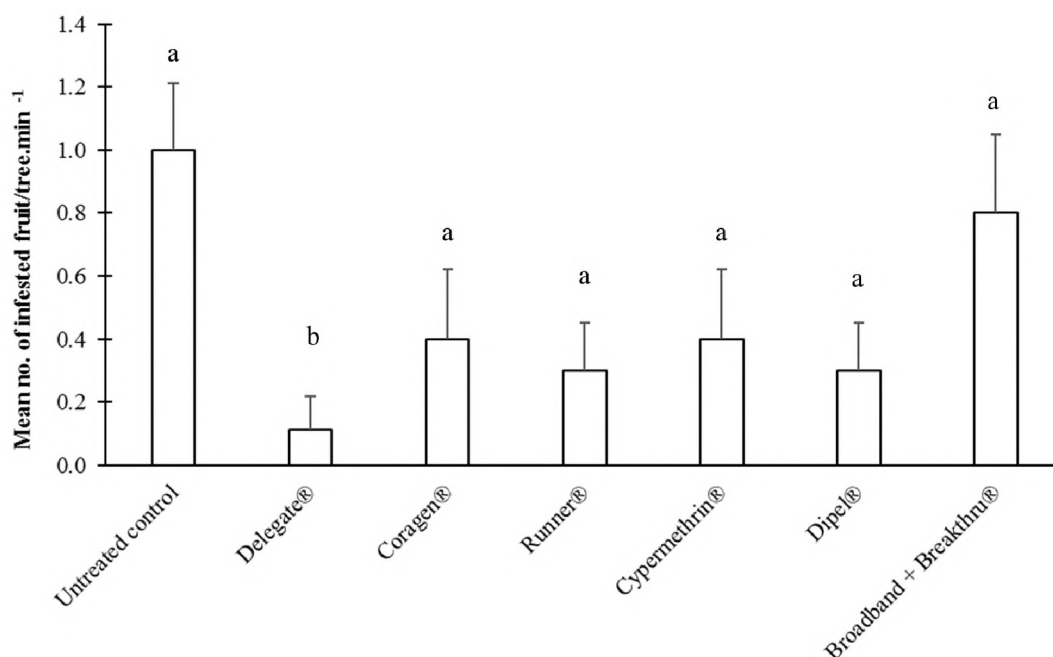


Fig. 5.7 Mean carob moth infestation per tree recorded in one minute at 11 weeks after product application in the 2015-16 growing season. Error bars show standard error from the mean. Different letters indicate significant differences ($P > 0.05$) (Tukey's post-hoc test).

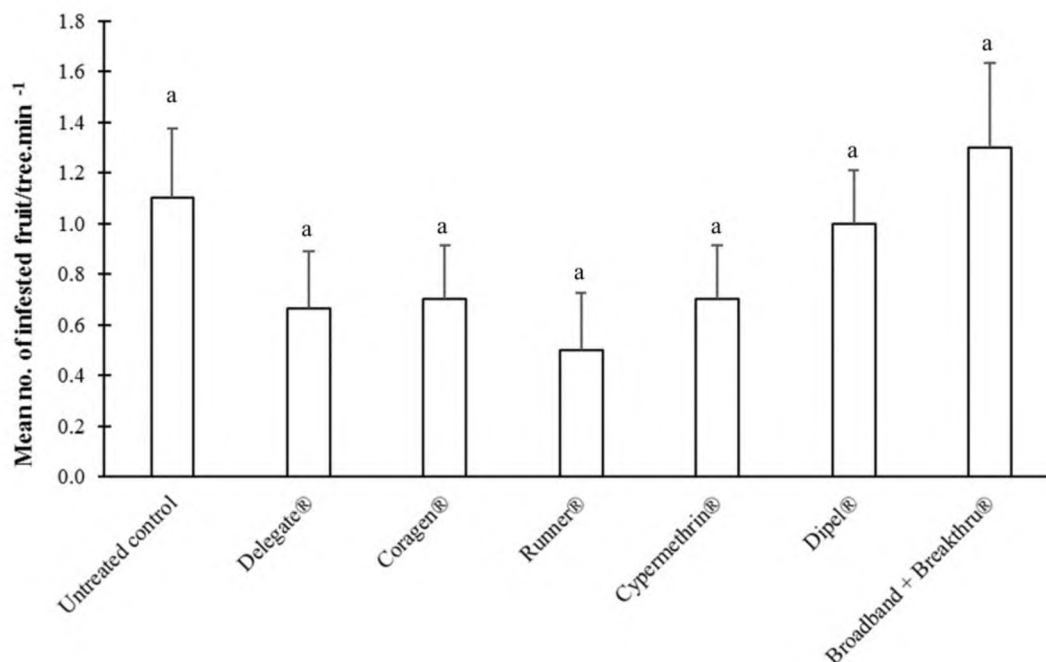


Fig. 5.8 Mean carob moth infestation per tree recorded in one minute at 22 weeks after treatment application in the 2015-16 growing season. Error bars show standard error from the mean. Different letters indicate significant differences ($P > 0.05$) (Tukey's post-hoc test).

5.3.2 Mating disruption – SPLAT® EC

2014-15 season

Twenty moths were caught in the untreated control block over the season, which was significantly higher than the zero moths caught in the SPLAT® EC treated block ($F_{1, 52} = 5.26$, $P = 0.0258$) (Fig. 4.9). Two flight peaks were observed over the monitoring period, the first occurring between weeks 39-40 of 2014, with the second taking place over weeks 1-5 in 2016. No moths were caught from week 6 onwards for the remainder of the trapping period.

Infestation was monitored weekly over the season from week 44 in 2014 to week 14 in 2015 (Fig. 5.10). A total of three infested fruit were collected from control data trees while only one infested fruit was recovered in the SPLAT® EC treated block. There was no statistically significant difference between infestation in the two treatments ($F_{1, 46} = 0.003$, $P = 0.306$).

A once off sampling event took place in week 14 (Fig. 5.11). Mean (\pm SE) infestation in the control block was $0.5 (\pm 0.15)$ fruit per tree and $0.33 (\pm 0.12)$ fruit per tree in the SPLAT® EC block, which is a 34% reduction of infestation. However, this was not statistically significant ($F_{1, 58} = 0.41$, $P = 0.403$).

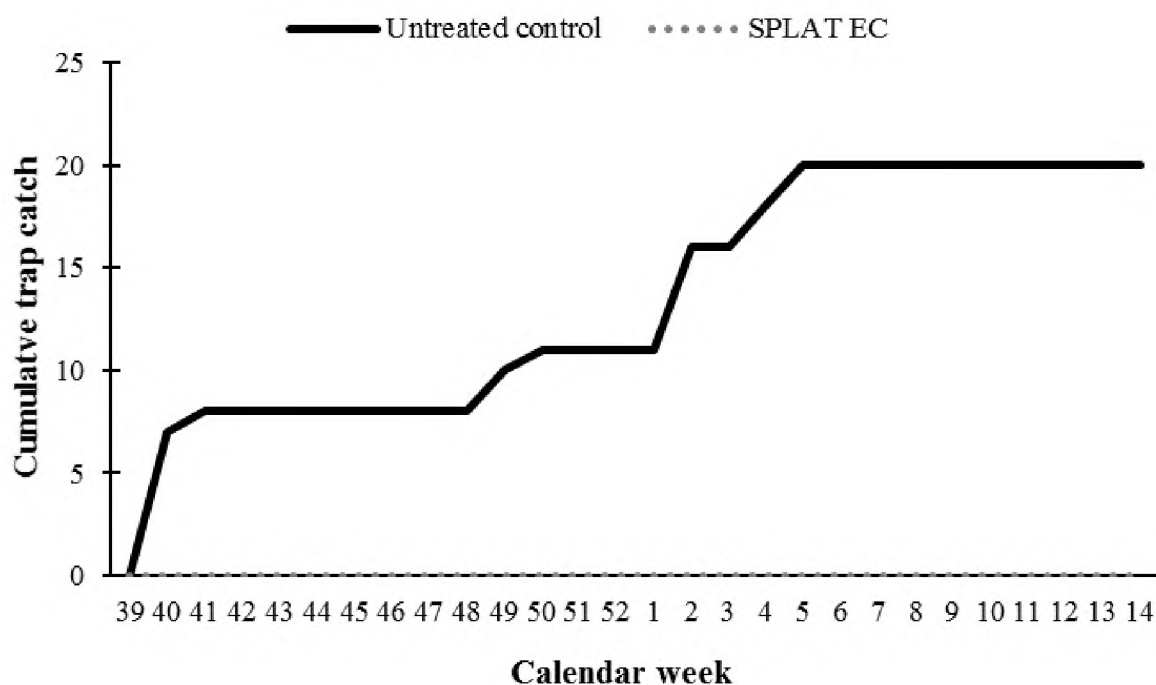


Fig. 5.9 Cumulative trap catches per week in the SPLAT® EC and untreated control treatments for the 2014-15 growing season.

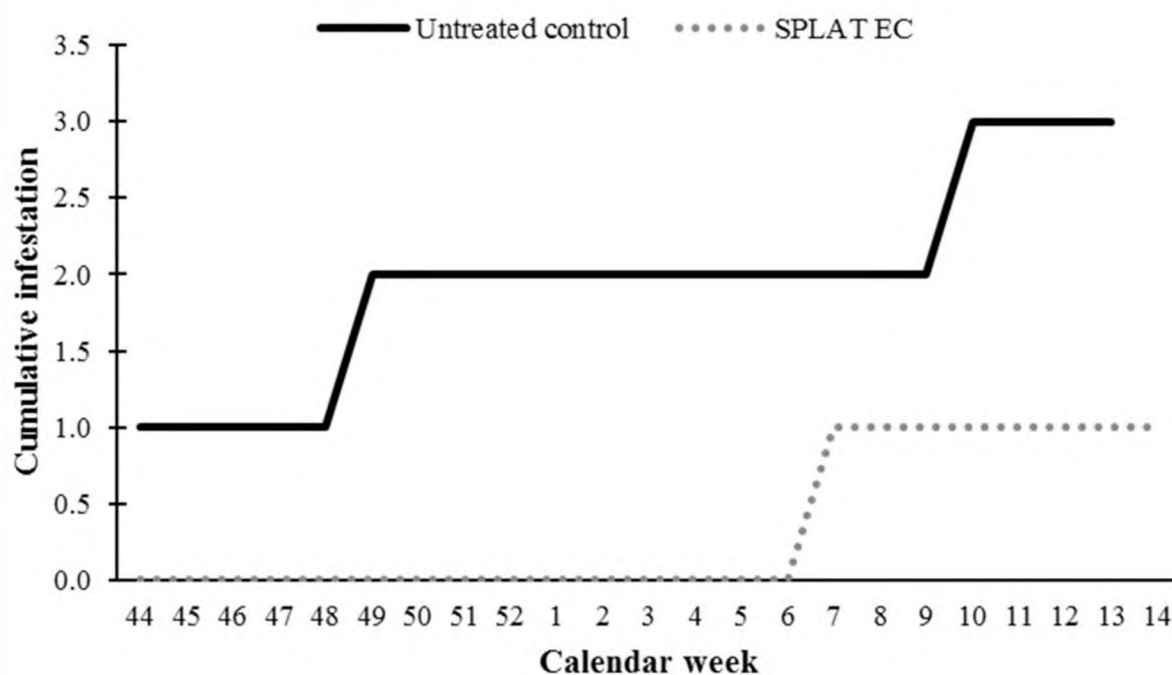


Fig. 5.10 Cumulative carob moth infested fruit per week in the SPLAT® EC and untreated control treatments for the 2014-15 growing season.

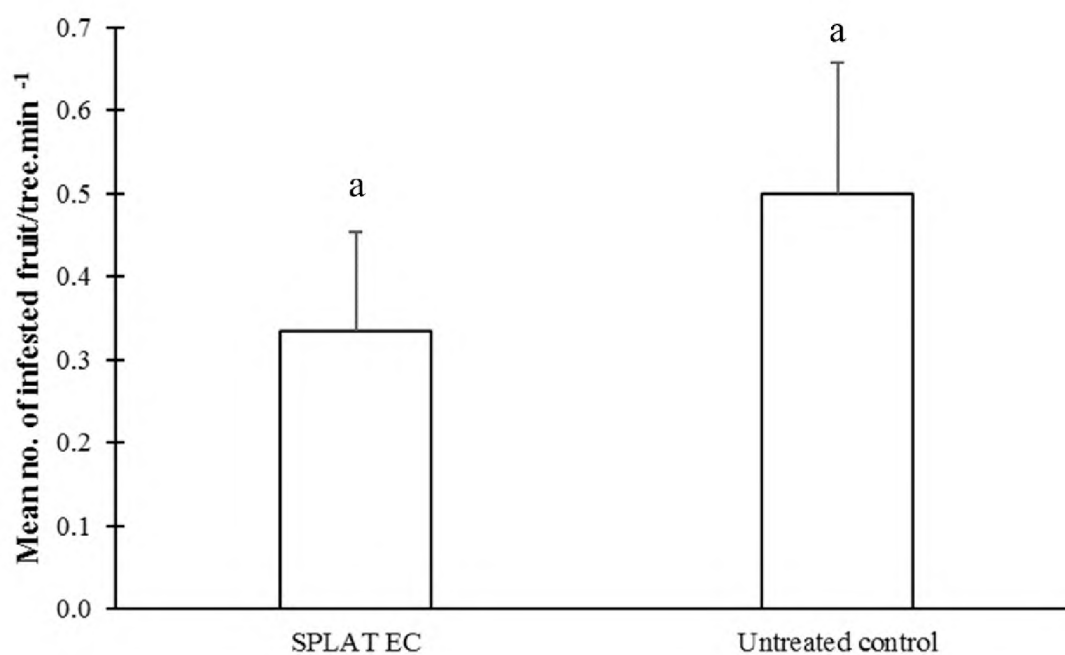


Fig. 5.11 Mean number of carob moth infested fruit in the SPLAT® EC and untreated control treatments in a once-off sampling event in week 14 (2015). Error bars show standard error of the mean. Different letters indicate significant differences ($P > 0.05$) (Tukey's post-hoc test).

2015-16 season

Combined carob moth trap catches in the control blocks reached a total of 23 individuals over the season, which was significantly higher than the 3 carob moth caught in the SPLAT® EC treated blocks ($F_{1, 160} = 14.25$, $P = 0.000224$) (Fig. 5.12). However, unlike the 2014-15 trap catches, where male moths were caught during two distinct flight peaks, male moths were caught continuously from week 45 in 2015 to week 18 in 2016.

The destructive sampling evaluation in week 51 of 2015 yielded no results with not a single carob moth infested fruit recorded in all control and SPLAT® EC treated blocks. In the next sampling event, week 10 of 2016, there was carob moth infestation within the orchard, with mean infestation in the control blocks of $0.4 (\pm 0.07)$ fruit per tree and $0.15 (\pm 0.05)$ infested fruit per tree in the SPLAT® EC treated blocks (Fig. 5.13). This was a statistically significant reduction of infestation by 62.5% ($F_{1, 118} = 6.13$, $P = 0.014652$).

Scouting for carob moth infestation in week 18 of 2016 showed similar results to the previous sampling effort, with infestation increasing slightly (Fig. 5.14). The mean control block infestation was $0.7 (\pm 0.07)$ fruit per tree while the SPLAT® EC blocks showed a significant reduction of carob moth infestation of $0.21 (\pm 0.044)$ fruit per tree ($F_{1, 118} = 16.95$, $P = 0.000071$), which is a 70% reduction of infestation in comparison to the control blocks.

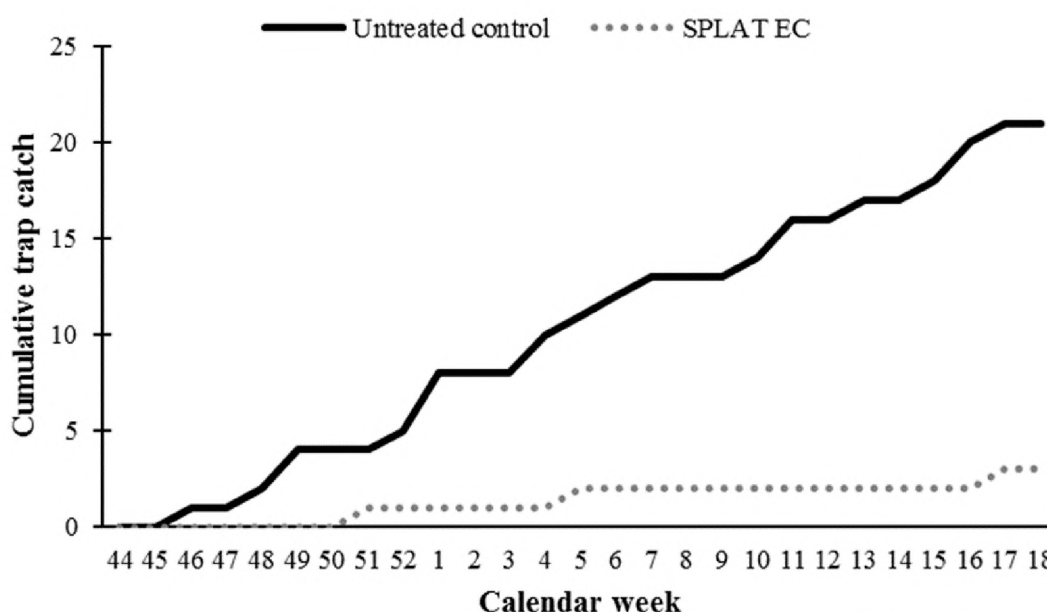


Fig. 5.12 Cumulative trap catches in the SPLAT® EC and untreated control treatments for the 2015-16 growing season.

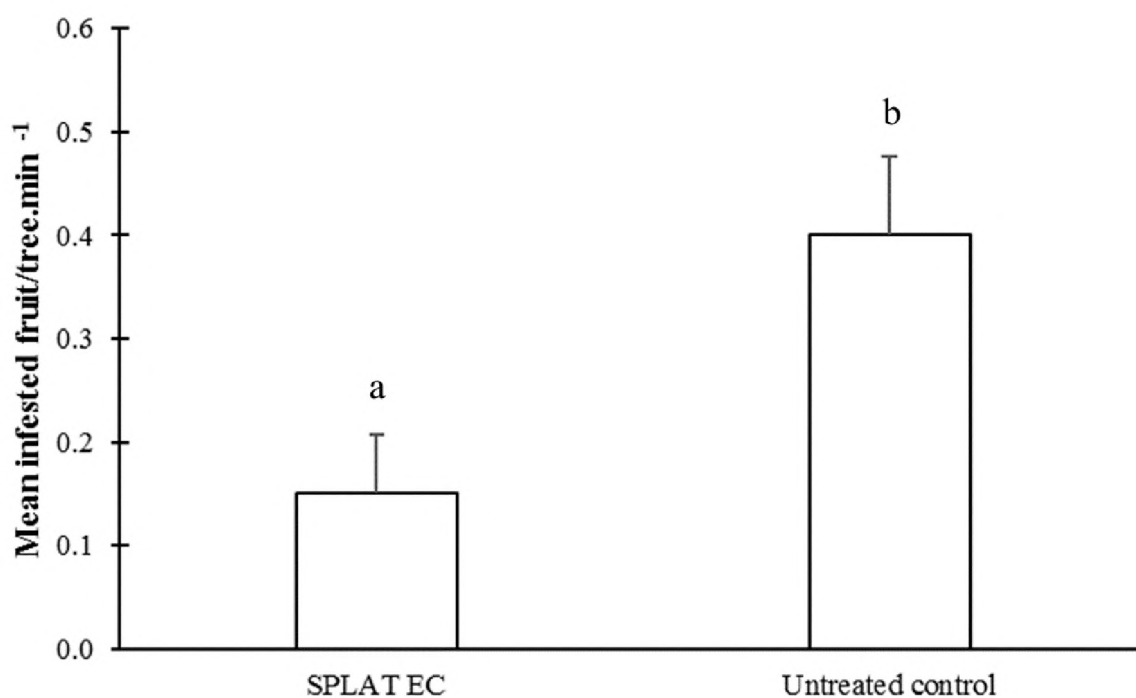


Fig. 5.13 Mean number of carob moth infested fruit in the SPLAT® EC and untreated control treatments in week 10 of 2016. Error bars show standard error from the mean. Different letters indicate significant differences ($P > 0.05$) (Tukey's post-hoc test).

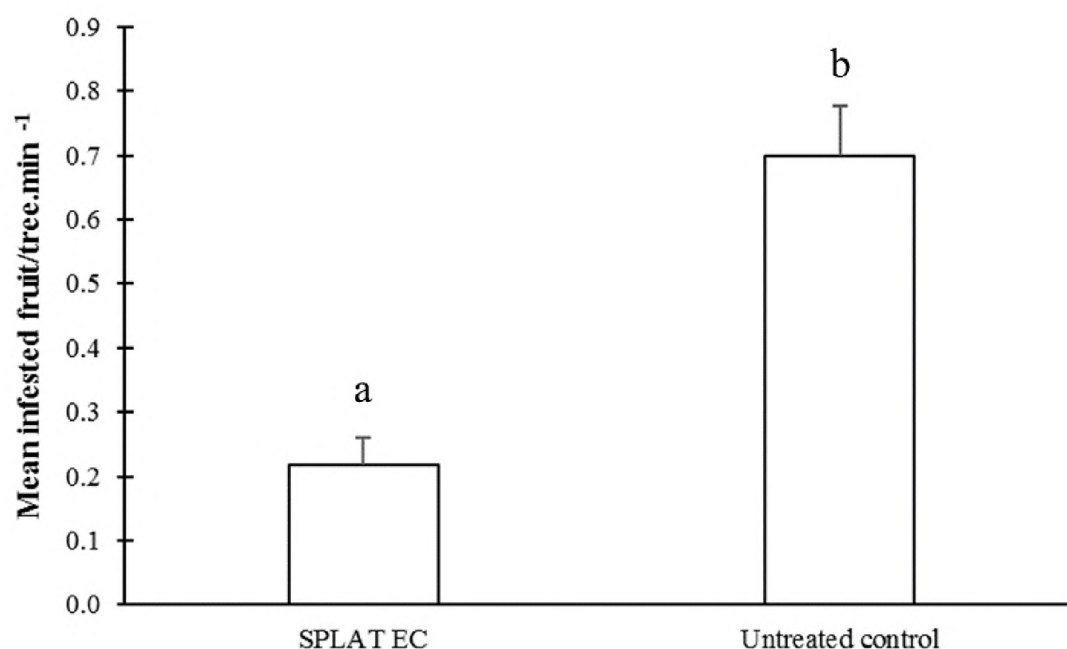


Fig. 5.14 Mean number of carob moth infested fruit in SPLAT® EC and untreated control in a once-off sampling event in week eighteen (2016). Error bars show standard error from the mean. Different letters indicate significant differences ($P > 0.05$) (Tukey's post-hoc test).

5.4 Discussion

5.4.1 Spray trials

Spray trial results showed that chemical control of carob moth is possible in citrus orchards with most products tested showing some form of reduction in infestation compared to the untreated control. Runner® significantly reduced infestation by 89% in the 2014-15 season, while Delegate® was the only product that showed significant reduction in infestation, by almost 90%, for both the 2014-15 and 2015-16 seasons. Delegate® and the active agent in Runner® (methoxyfenozide) are registered for the control of carob moth in date gardens in the United States (Perring *et al.* 2014). The efficacy of the various products tested against carob moth in this study are very similar to their efficacy against FCM in citrus orchards when applied as late season control options, where both Runner® and Delegate® reduced weekly infestation significantly (Kirkman and Moore 2012; Moore and Kirkman 2014, Moore *et al.* 2015). The poor performance of Broadband® in both seasons could be attributed to a single application, as the label stipulates that weekly sprays should be conducted for a period of four weeks to achieve the best results. However, similar reduction of FCM infestation was observed by Moore and Kirkman (2014) with multiple applications. The active ingredient of Dipel® (Bt), has been shown to be effective in controlling carob moth in various laboratory (Mnif *et al.* 2013, Boukedi *et al.* 2015) and field trials (Davarci 1996, Mediouni and Dhouibi 2007, Ozkan *et al.* 2001). Harpaz and Wysoki (1984) documented high 95% mortality of fourth instar larvae in laboratory tests using a high rate of bacterial spores but found that mortality was lower at rates than would be practical for field use. The best field efficacy reported was 82% reduction in infestation, achieved after four applications in pomegranates (Alrubeai 1988). Reduction of carob moth infestation levels of up to 95% as a result of late season Bt applications to citrus have been recorded in Turkey, however, this consisted of multiple applications at 20 day intervals (Davarci 1996). This study has shown that a once-off application has not produced comparable control levels to Davarci (1996) but did still show some promise in the 2015-16 season. The mediocre performance of Coragen® was unexpected as the active ingredient, chlorantriple, is registered for the control of carob moth in nut trees in South Africa (Agri-intel 2016).

Unfortunately, unlike when monitoring weekly infestation, the sampling method used in this study does not enable one to evaluate the residual efficacy of products tested against carob moth. However, the sampling event in April 2016 suggests that the chemical products

tested have longer residual efficacy than the two biological products (Dipel® and Broadband®). Pest management within citrus production in South Africa is orientated towards integrated pest management (IPM) and growers are encouraged to consider the potential negative effects of chemical applications, such as the disruption of natural enemy complexes. Grout *et al.* (2011) produced a database outlining the non-target effects of various active ingredients against five key natural enemies of important pests on citrus. Of the products evaluated for the control of carob moth, Cypermethrin® is by far the most disruptive product when compared to other treatments. It would be important for producers to consider potential non-target effects, which would be influenced by the timing of application during the growing season; applications in the middle of the season may result in secondary outbreaks of pest species, which in the absence of a disruptive spray application, would otherwise be under good biological control (Michaud and Grant 2003).

The levels of carob moth infestation between the two seasons did differ, with infestation in the untreated control in the 2014-15 almost double what was observed in the 2015-16 season. This is most likely due to the higher levels of mealybug observed, but not recorded, in the orchard in the 2014-15 season. It is well known in other citrus growing regions, where carob moth is a pest, that there is a strong relationship between the presence of mealybug and carob moth outbreaks (Serghiou 1983).

5.4.2 Mating disruption – SPLAT® EC

Assessing trap capture reduction provides a robust, graded data set from these continuously emitting sources, allowing the disruption formulation to be challenged throughout the attraction period, evaluating its potential to continuously suppress the ability of males to locate females (Baker 2009). SPLAT® EC was effective in causing trap shut down for both seasons' trials, which is indicative of successful mating disruption as if males are not able to locate lures then it is highly unlikely they will be able to locate calling females. However, there was no difference in the infestation for the 2014-15 growing season in treated and untreated blocks. SPLAT® EC reduced carob moth infestation by over 60% for sampling events in weeks 7 and 18 (2016) for the 2015-16 growing season. Results obtained in this study show similar results to Mafra-Neto *et al.* (2013) where SPLAT® EC reduced carob moth infestation and caused trap shut down. However, trap catches were much higher in their study suggesting that the pest pressure was greater than what was observed in our study. One of the prerequisites for successful mating disruption is low pest pressure (Baker 2009, Witzgal 2010). Therefore,

it is uncertain how SPLAT® EC would perform against increased carob moth populations within citrus orchards.

Although these results only show the efficacy of SPLAT® EC against carob moth in citrus orchards, there are certain deductions that can be made from this field trial that are of significant value. Firstly, the trap shut down recorded in treated blocks over two seasons suggests that the synthetic pheromone mimic in SPLAT® EC was effective in disrupting the location of the male carob moth pheromone lure, which has similar chemistry to SPLAT® EC. The reduced infestation in treated blocks in the 2015-16 season shows that there were lower numbers of successful mating in treated blocks, which shows that even if there is geographic variation in the carob moth sex pheromones, this did not affect the efficacy of the product. This also clarifies any speculation that large geographic distances between populations where SPLAT® EC had previously been evaluated, could decrease the product's efficacy due to variation in sex pheromones. However, Mozffarian *et al.* (2007, 2008) showed that although carob moth morphology can vary between populations, this is not due to restrictions in gene flow but as a result of host nutrition.

The area to which SPLAT® EC was applied was small relative to the area over which mating disruption products should ideally be applied to for optimal efficacy. The significant reduction in infestation achieved in the 2015-16 season can be attributed to the performance of the product and also to the low levels of gravid females overflowing into the treated blocks from the untreated blocks. From this we can conclude that carob moth flight lengths in citrus orchards do not cover long distances, which corresponds with Dhouibi *et al.* (2002) who found that the average dispersal distance of carob moth in pomegranate orchards was less than 100m.

Although SPLAT® EC was effective in reducing carob moth infestation in citrus orchards, it was labour intensive and therefore unlikely to be adopted by growers. Future research should evaluate the efficacy of SPLAT® EC applied at the same rate, but with a reduced number of point sources. If a reduced number of point sources resulted in good control, growers may be encouraged to use the product due to the reduced labour costs. An example of where this has been effective is with Codlemone (Suttera, U.S.A) for the mating disruption of the codling moth (*Cydia pomonella* Linnaeus) in apple orchards, where puffers have been used to reduce the number of point sources and also optimise the time of pheromone release to coincide with moth flight activity within orchards (Shorey and Gerber 1996). A factor that is of more concern than the lack of ease of application is the cost of the product itself, which is

currently R14 300 per kg (ISCA Technologies, CA, USA), this equates to a cost of R9 330.00 per hectare with two applications per season, this cost does not include application. As mating disruption needs to be dispensed in orchards before pest populations start to reach levels of economic significance, and because carob moth seems to be a sporadic pest in citrus as a consequence of mealybug infestation. It is highly unlikely that conventional citrus growers (as opposed to organic) would choose this method over a corrective chemical application, which would control other lepidopteran pests and come at a greatly reduced cost.

5.5 Conclusion

The spray trial produced results which showed that there are certain products currently registered for the control of lepidopteran pests in citrus that are effective in controlling carob moth. Of the products evaluated in this study, Delegate® and Runner® proved to be most effective. The mating disruption of carob moth with SPLAT® EC produced trap shut down and lowered infestation, suggesting that pheromone mimics derived from carob moth populations in the USA are effective in disrupting carob moth mate location in South Africa. Additionally, it could be concluded that carob moth adults do not undergo long range dispersal in citrus orchards in South Africa and that the method of SPLAT® EC application was effective.

CHAPTER 6: A NEW SPECIES OF *PHANEROTOMA* WESMAEL (HYMENOPTERA: BRACONIDAE: CHELONINAE) REARED FROM CAROB MOTH IN SOUTH AFRICA

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Abstract

A new species, *Phanerotoma carobivora* van Achterberg and Thackeray, **sp. nov.** is described from South Africa. It is a common endoparasitoid of the carob moth (*Ectomyelois ceratoniae* Zeller (Pyrilidae)) on pecan (*Carya illinoensis* (Wangenh.) K. Koch) and citrus fruits in South Africa. Levels of parasitism varied considerably between hosts and sampled localities.

Key words: *Phanerotoma*, new species, South Africa, carob moth, *Ectomyelois ceratoniae*, pecan, *Carya illinoensis*, citrus.

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6.1 Introduction

The carob moth (*Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae)) is highly polyphagous and in many regions of the world, a serious pest of many high-value nut and fruit commodities (Perring *et al.* 2015). The larvae are concealed feeders and cause economic loss through direct feeding damage resulting in fruit drop in the orchards, post-harvest decay due to larval infestation or impeding the export of susceptible hosts to certain target markets due to phytosanitary restrictions. The carob moth (Gothilf 1968) is also known as the locust bean moth (Goater 1986), the carob bean moth (Gonzalez and Cepeda 1999), the pomegranate fruit moth

(Krasil'nikova 1964, Moawad 1979), and the pomegranate fruit worm (Al-Jamali 2006). In South Africa, *E. ceratoniae* is considered a minor pest on pomegranates, *Punica granatum* L. (Giliomee and Barnes 2015), macadamia, *Macadamia integrifolia* Gross and Weston (de Villiers 2001, Schoeman and de Villiers 2015), pecans (Fig. 1A), *Carya illinoensis* Koch, and citrus (Fig. 1B), *Citrus* sp., where infestation is associated with high levels of honeydew producing insects such as mealybugs (Catling 1970, Grout and Moore 2015).

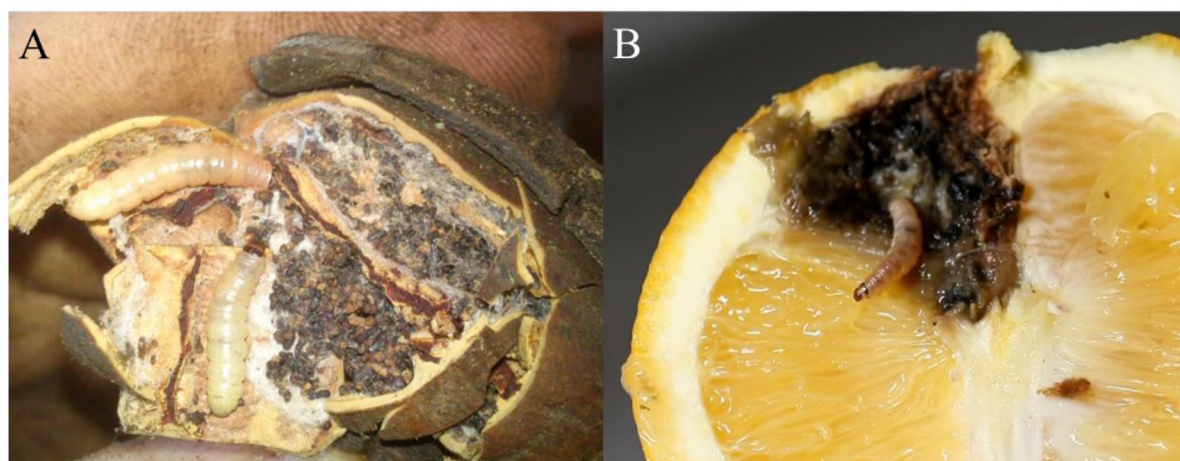


Fig. 6.1 *Ectomyelois ceratoniae* larvae infesting an out of season pecan nut (A) and a citrus fruit, as a result of high levels of mealybug infestation (B).

There are a number of natural enemies reported of *E. ceratoniae* including egg parasitoids, larval parasitoids, pupal parasitoids and other predacious species (Perring *et al.* 2015 and references therein). In South Africa only one species of parasitoid has been recorded: *Phanerotoma ornatulopsis* de Saeger, 1942 (Hymenoptera: Braconidae), which was reared from infested acorns in the Citrusdal area of the Western Cape (Honiball and Catling 1998). Several braconid parasitoids are known to parasitise *E. ceratoniae* (Yu *et al.* 2016), including three species of the cosmopolitan genus *Phanerotoma* Wesmael, 1838 (Braconidae: Cheloninae: Phanerotomini). The oldest reports (Thompson 1946, Lepigre 1963 and Aubert 1966) list *Phanerotoma dentata* (Panzer 1805), which is most likely a misidentification because it is a European species and it has been almost universally misidentified in the past (van Achterberg 1990). Other species (viz., the South Palaearctic and Afrotropical *P. leucobasis* Kriechbaumer, 1894 (Gothilf 1969b, Mesbah *et al.* 1998 and Bouka *et al.* 2001), *P. ornatulopsis* from Central Africa and *P. myeloisae* Fullaway, 1956, from Hawaii) are reported more recently as parasitoids of the carob moth. Other names of *Phanerotoma* spp. used for parasitoids of *E. ceratoniae* include *Phanerotoma ocularis* Kohl, 1906 (Khoualdia *et al.* 1996, Bouka *et al.* 2001) and *P. flavitestacea* Fischer, 1959 (Gothilf 1969a, 1969b, Biliotti and

Daumal 1970, Daumal *et al.* 1973, Madkouri 1978). *Phanerotoma flavitestacea* is a junior synonym of *P. leucobasis* (van Achterberg 1990) and *P. ornatulopsis* is probably a synonym of *P. ocularis*.

The species of *Phanerotoma* reared from the carob moth in South Africa by the junior author lacks the semicircular third metasomal tergite of all listed species and has an apical triangular lobe at the female hypopygium (Figs 15–16). This feature separates it from these species and no named other species could be found having this combination. Therefore, we describe it as a new species, together with notes on its biology. The terminology used follows van Achterberg (1990, 1993). The aim of this study was to evaluate the levels of parasitism by *Phanerotoma* spp. and other parasitoids occurring in carob moth populations in the two prominent citrus production areas of South Africa.



Fig. 6.2 *Phanerotoma carobivora* **sp. nov.** (Holotype, female) **2.** Habitus, lateral aspect; **3.** Id., dorsal aspect.

6.2 Materials and methods

Ectomyelois ceratoniae larvae were collected in 2015 and 2016 in the Loskop Valley (Mpumalanga, South Africa) and the Vaalharts (Northern Cape, South Africa). The number of *E. ceratoniae* larvae collected, along with the sampling date and locality are outlined in Table 6.1. Citrus and pecan nuts infested with *E. ceratoniae* larvae were collected from the aforementioned locations, brought back to the laboratory and placed onto an artificial diet of soy flour, sucrose and distilled water (25:25:49.8) (Cox 1979) with the addition of 0.1% sorbic acid and 0.1% nipagin to reduce fungal contamination. All larvae were reared singularly in size eight Polytop glass vials (Bonpak, South Africa) at 25°C (17:8 L:D) and left until either a parasitoid emerged, an adult *E. ceratoniae* eclosed or the larva died. The parasitism rate of *E. ceratoniae* by *Phanerotoma* between regions in citrus was compared, along with a comparison between hosts (citrus and pecans) in the Vaalharts production area. Percentage data was subjected to arcsine transformation and means were compared with an analysis of variance (ANOVA) in Statistica (Statsoft 2016).

Photographs were made with an Olympus SZX12 motorized stereomicroscope with AnalySIS Extended Focal Imaging Software and an Olympus SZ40 stereozoom microscope was used for the descriptions and measurements. The type series is deposited in the Naturalis Biodiversity Center, Leiden, the Netherlands (RMNH) and in the Albany Museum, Grahamstown, South Africa (AMG).

6.3 Results and discussion

An overall mean (\pm SE) survival of 78.8% (\pm 2.71) of larvae placed onto artificial diet was observed. When making the comparison of parasitism levels in citrus hosts between the two production areas, the lowest level of parasitism was observed in the Loskop Valley (2.16% \pm 1.16) which was significantly lower than parasitism observed in citrus orchards in the Vaalharts (29.67% \pm 1.55) ($F_{1, 11} = 55.44$, $P = 0.000013$). However, when comparing levels of parasitism between pecans and citrus in the Vaalharts, there was no significant difference ($F_{1, 3} = 0.066$, $P = 0.813$) (Table 2). These results are similar to those of Gothilf (1969), who found that levels of parasitism of *E. ceratoniae* in citrus orchards were generally low, and that levels of parasitism varied between locations, host and the type of surrounding vegetation.

Table 6.1 The localities where *E. ceratoniae* larval samples were collected, the relevant hosts, collection dates and the overall number of larvae placed onto artificial diet.

Production area	GPS co-ordinates	Host	Date collected	number placed onto diet
Loskop Valley, Limpopo	25° 13' 4.75"S 29° 25' 53.45"E	Citrus	March 15	120
			April 15	65
			Feb 16	44
			April 16	58
	25° 22' 34.65"S 29° 22' 35.1"E	Citrus	Feb 16	35
			April 16	41
	25° 11' 23.54"S 29° 24' 32.8"E	Citrus	Feb 16	28
			April 16	36
Vaalharts, Northern Cape	27° 52' 40.54"S 24° 47' 15.2"E	Pecans	July 16	80
	27° 48' 31.36"S 24° 52' 1.33"E		July 16	80
	27° 49' 53"S 24° 50' 11.2"E		July 16	80
	28° 17' 40.9"S 24° 35' 13.4"E	Citrus	Feb 16	26
			March 16	21

Table 6.2 Mean percentage of *E. ceratoniae* parasitised by *Phanerotoma* spp. in the Loskop Valley (Limpopo, South Africa) and the Vaalharts (Northern Cape, South Africa) collected from citrus and pecan orchards. Different letters in the same column indicate significant differences $P < 0.05$.

Region	Host	Mean % parasitism	Standard error
Loskop Valley, Limpopo	Citrus	2.16a	± 1.16
Vaalharts, Northern Cape	Citrus	29.67b	± 1.55
	Pecans	27.92b	± 8.02

6.4 Species description

Phanerotoma carobivora van Achterberg and Thackeray, sp. nov.

(Figs 2–22)

Type material. Holotype, ♀ (RMNH), “South Africa: N. Cape, Vaalharts, 28°00'60"S, 24°42'60"E, ex *Ectomyelois ceratoniae* Z., on *Carya illinoensis*, coll. 9-15.vii.2016, S. Thackeray, RMNH”. Paratypes: 16 ♀ + 8 ♂ with same data as holotype (RMNH); 5 ♀ + 5 ♂, id. (AMG); 3 ♀ + 1 ♂ with same data, but collected 6.v.2015 (RMNH).

Holotype ♀: body length (excluding ovipositor) 4.4 mm; antenna 3.3 mm; fore wing 3.2 mm; visible part of ovipositor sheath 0.5 mm.

Head: Width 1.5 times median length in anterior view and part of head above eye in lateral view 0.3 times height of eye (Fig. 12); antenna with 23 segments and slightly longer than fore wing, rather abruptly narrowed subapically, with 5 moniliform apical segments (Figs 13–14), third, fourth and penultimate segments 2.8, 2.6 and 1.3 times as long as wide, respectively; area of stemmaticum coriaceous; OOL: diameter of posterior ocellus: POL= 13: 5: 3; length of eye 2.3 times temple in dorsal view (Fig. 11); frons largely rugose and only anteriorly with median carina; vertex reticulate-rugose with fine coriaceous background sculpture, setose; temple (gena) densely rugulose and rather dull; face with oblique rugae and without distinct median ridge; clypeus punctulate but largely smooth and shiny, with 3 minute teeth ventrally (Fig. 10); eye short in lateral view (Fig. 12), in anterior view 0.5 times minimum width of face; upper condylus of mandible distinctly above lower level of eyes (Fig. 10); malar space with few curved striae and mainly coriaceous, 0.5 times as basal width of mandible; lower tooth of mandible 0.3 times as long as apical tooth (Fig. 18).

Mesosoma: 1.7 times as long as wide in lateral view (Fig. 2); side of pronotum rugose, but largely smooth ventrally and dorsally; mesoscutum reticulate-rugulose with granulate background, setose; notauli not differentiated; scutellar sulcus medium-sized and with 9 short crenulae (Fig. 5); scutellum mainly granulate with some fine rugulae, posteriorly smooth and shiny; metanotum with short median carina anteriorly and small tooth posteriorly; propodeum coarsely reticulate rugose with distinct transverse carina behind anterior areola (Fig. 5), median carina absent and slightly tuberculate laterally.

Wings: Fore wing 2.6 times longer than its maximum width; length of 1-R1 1.3 times pterostigma; r issued much beyond middle of pterostigma and 0.2 times 3-SR; 2-SR distinctly

bent and basally nearly parallel with posterior margin of pterostigma (Fig. 4); SR1 strongly curved; 2-SR+M longitudinal and m-cu narrowly postfurcal; parastigma large and yellow dorsally but ventrally and surroundings dark brown pigmented; 1-CU1 0.4 times as long as vein 2-CU1; r:3-SR:SR1 = 3:20:46; 2-SR:3-SR:r-m = 26:20:7; r-m reclivous; 2-M distinctly curved (Fig. 4).

Hind wing: M+CU:1-M:1r-m = 26:25:10.

Legs: Hind femur 3.2 times as long as wide; middle tibia with ivory blister (Fig. 9); inner spur of middle tibia 0.5 times its basitarsus; hind coxa with satin sheen and superficially coriaceous.

Metasoma (Figs 6–7, 15): Cylindrical in dorsal view and 2.2 times as long as wide and 1.2 times as long as mesosoma; first–second tergites with interconnected longitudinal spaced rugae; third tergite 1.9 times longer than second tergite, mainly densely and finely reticulate-rugulose and truncate medio-posteriorly (Fig. 6), lateral lamella not protruding latero-apically and medium-sized, nearly straight medio-apically (Fig. 17); setose part of ovipositor sheath 0.02 times as long as fore ng and visible part of ovipositor sheath 0.15 times as long as fore wing and 0.3 times metasomal carapace; hypopygium with apically rounded triangular lobe (Fig. 16; secondarily slanted inwards in holotype (Fig. 7) as in several paratypes).

Colour: Yellowish brown; head dorsally largely and laterally, clypeus, mandible (except dark brown teeth), tegulae, notaulic area and medio-posterior part of mesoscutum and tarsi pale yellow or ivory; first tergite (except basally), second tergite medially, palpi, pronotum, coxae, fore and middle legs (except tarsi), hind trochanter, trochantellus, basal third of hind femur, basal two-thirds of hind tibia (but with brown subbasal band) and metasoma ventro-basally white; apical third of hind tibia brown; apical 6 antennal segments and humeral plate next to tegulae dark brown; stemmaticum black; pterostigma dark brown with distinct pale yellowish basal spot and apex (Fig. 4); wing membrane below dark part of pterostigma and near vein 1-CU1 infuscate; vein 1-M brownish yellow; veins 1-CU1, cu-a, surroundings of parastigma, parastigma ventrally (but dorsally yellow), r, 2-SR (except posteriorly), 3-SR and 2-M dark brown, remainder of veins (including 1-R1) pale yellow.

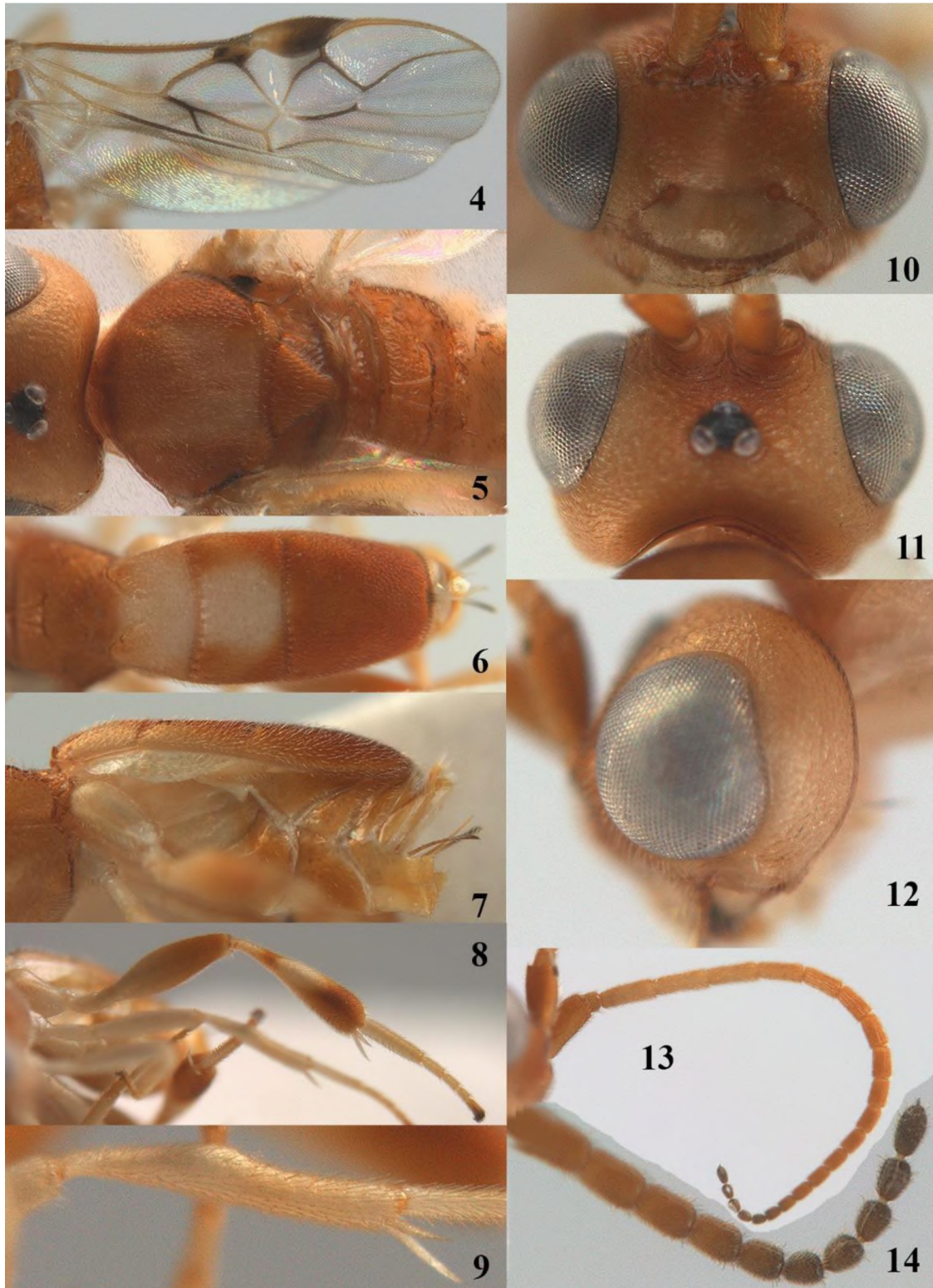


Fig. 6.3 *Phanerotoma carobivora* **sp. nov.** (Holotype, female) **4.** Wings; **5.** Mesosoma, dorsal aspect; **6.** Metasoma, dorsal aspect; **7.** Metasoma, lateral aspect; **8.** Hind leg, lateral aspect; **9.** Middle tibia, lateral aspect; **10.** Head, anterior aspect; **11.** Head, dorsal aspect; **12.** Head, lateral aspect; **13.** Antenna; **14.** Apical segments of antenna.

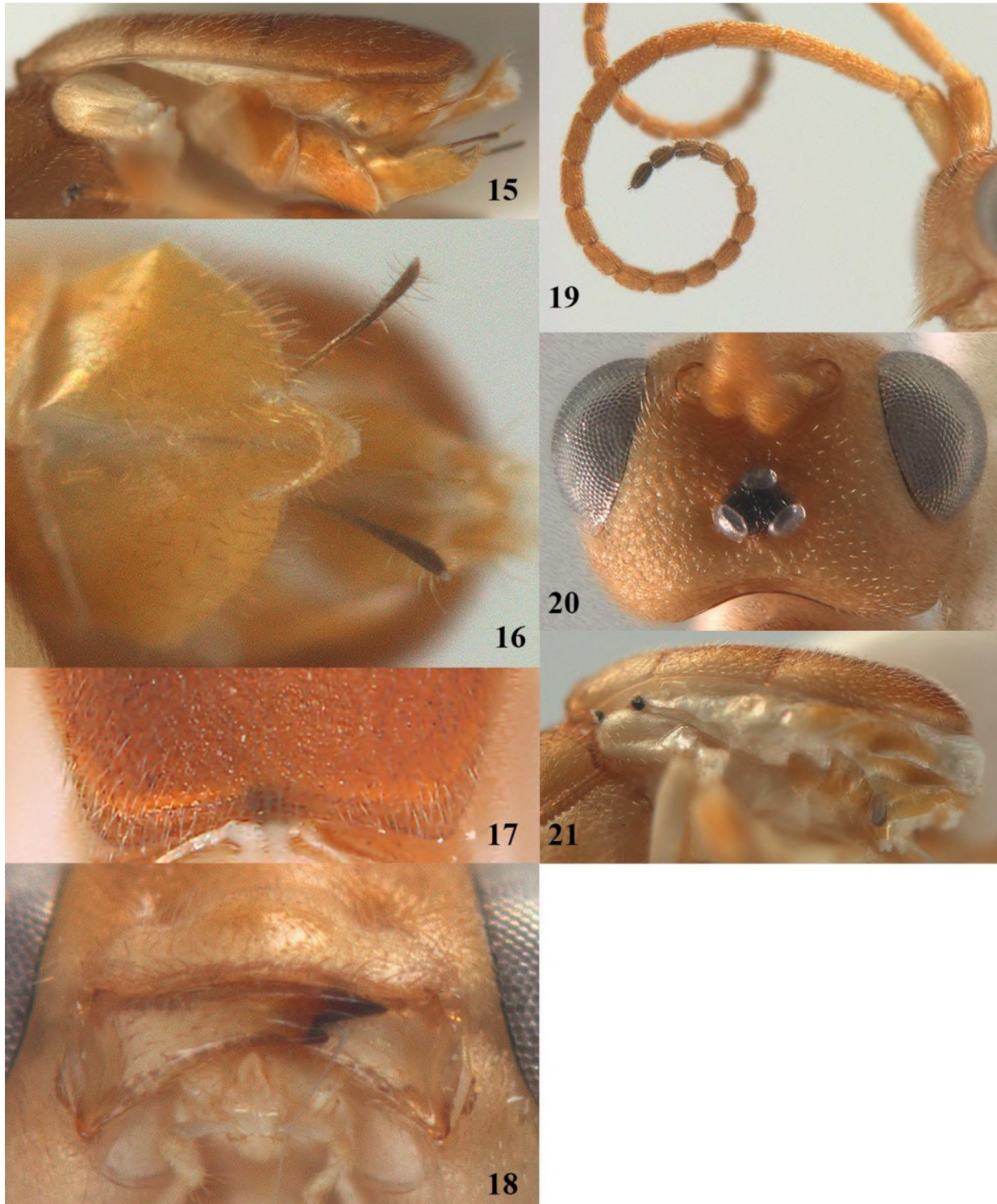


Fig. 6.4 *Phanerotoma carobivora* **sp. nov.** (Paratype, female: **15–16**; holotype, female: **17–18**; paratype, male: **19–22**)- **15, 21**. Metasoma, lateral aspect; **16**. Hypopygium, ventral aspect; **17**. Apex of third tergite, dorso-apical aspect; **18**. Mandible, ventral aspect; **19**. Antenna, lateral aspect; **20**. Head, dorsal aspect; **22**. Genitalia, ventral aspect.

Male (Figs 19–22): Very similar to female but apical antennal segments gradually narrowed, non-moniliform (Fig. 19) and third metasomal tergite more or less oval; genitalia: Fig. 22.

Variation: Length of fore wing 2.7–3.4 mm, of body 3.6–4.7 mm; parastigma dorsally brownish yellow or brown; third tergite 1.7–1.9 times as long as second tergite; length of carapace 1.7–2.2 times as long as wide; subbasal brown band of hind tibia sometimes with small dark brown patch; scutellum brownish yellow to partly dark brown subposteriorly.

Biology: Koinobiont endoparasitoid of *Ectomyelois ceratoniae* (Pyrilidae) larvae in pecan nuts and citrus fruits.

Notes: The new species differs from all described Afrotropical and South Palaearctic species by the combination of the elongate third metasomal tergite (1.7–1.9 times as long as second tergite and truncate posteriorly) and the apically rounded triangular lobe of the female hypopygium posteriorly (Fig. 16). The new species differs from the species reported from the carob moth by having the third metasomal tergite parallel-sided (or nearly so) and 1.7–1.9 times as long second tergite (hemicircular and up to 1.4 times in *P. leucobasis*, *P. ocularis* and *P. myeloisae* (van Achterberg 1990, Zettel 1990), the hypopygium of female with an apical lobe (absent), the upper condyli of mandibles above lower level of eyes (below), vein m-cu of fore wing narrowly postfurcal (subinterstitial) and the third tergite truncate medio-posteriorly (slightly concave).

Etymology: Named after the first part of the popular name of its host (“carob moth”) and “voro” (Latin for “devour”), because the new species devours the larvae of the carob moth.

CHAPTER 7: COMPARING THE COLD SUSCEPTIBILITY OF CAROB MOTH COMPARED TO FALSE CODLING MOTH

7.1 Introduction

The South African citrus industry is the second largest global exporter of citrus (CGA 2016). Recently China became a new market for South African citrus. The protocol of phytosanitary requirements for the export of citrus from South Africa to China, signed between the governments of the two countries in 2006, specifies several quarantine pests. All citrus is required to undergo mandatory cold disinfestation through a cold treatment of fruit pulp temperature of -0.6°C for 22 days (SA-DAFF 2016). This mandatory treatment can be considered a generic quarantine treatment (USDA APHIS PPQ 2004) towards fruit flies and the false codling moth (FCM). This was experimentally shown to have been effective at a very high level (Myburg 1963, 1965, Myburg and Bass 1969) and its effectiveness has also been confirmed through commercial practice over many years (to the USA and elsewhere). Although this treatment has not been shown to have Probit 9 efficacy against FCM, as often required (Follet and Neven 2006), in the International Standards for Phytosanitary Measures (ISPM) number eight (2008) there is no mention of Probit 9, and therefore the demonstration of Probit 9 efficacy may be unnecessary (Follet and Neven 2006). The protocol also lists carob moth as a quarantine pest and states that the mere presence of carob moth in an orchard, packhouse or during phytosanitary inspections, will lead to the expulsion of the relevant orchard from the Chinese export programme for the duration of the season (SA-DAFF 2016). The protocol states that if any carob moth infestation of fruit is recorded on inspection in China (larva alive or dead), then the consignment will be returned or destroyed and the relevant orchard and packhouse suspended (SA-DAFF 2016). This is only the case for FCM and fruit flies if the larva found is alive, due to the demonstrated efficacy of various cold treatment trials (Myburg 1965, Grout *et al.* 2011, Moore *et al.* 2016a, 2016b, 2016c). As there is no data to show the effect of cold disinfestation on carob moth, China cannot assume that the cold treatment schedule for FCM is adequate for carob moth, and consequently the finding of a larva, whether alive or dead, is simply interpreted as a sign of the presence of the pest in the consignment and thus provides reason for rejection.

Moore *et al.* (2014a) and outcomes of this study (Chapter 3) showed that levels of carob moth infestation may be significantly higher in certain citrus production areas than originally

thought, which increases the chance of an interception at a receiving port (Follet and Neven 2006). Therefore, there is a need to establish whether the generic cold treatment implemented when exporting citrus to China will be effective against carob moth. Grout *et al.* (2011) outlines four phases in developing a robust cold treatment disinfestation; phase one: establish growth rates of pest species within hosts; phase two: determine the least cold susceptible life stage; phase three: determine the most effective temperature and dose and phase four: undertake large scale studies to obtain Probit 9 efficacy. However, due to the low prevalence of carob moth in all citrus types, a Probit 9 standard may not be necessary (Landolt *et al.* 1984). Another option to validate that current generic treatments efficacy against carob moth is through equivalence studies (FAO 2005, Follet and Neven 2006).

Preliminary data generated by Moore *et al.* (2014a) provided a strong indication that carob moth is substantially more cold susceptible than FCM and that the FCM cold sterilisation protocol will indeed be adequate for carob moth. However, this was conducted as a single replicate with a small data set. Therefore, the aims of this study were to apply Grout *et al.*'s (2011) cold treatment development protocol and (1) to determine size categories of head-capsules for carob moth instars; (2) to determine the larval instar(s) with the highest cold tolerance; and (3) to determine whether these are at least as cold susceptible as the FCM larval stages with the highest cold tolerance.

7.2 Materials and methods

7.2.1 Source of Insects

Pecan nuts and Navel oranges infested with carob moth and FCM were collected between the 11th and 15th of June 2016 from Vaalharts (27° 52 '40.54"S 24° 47') in the Northern Cape of South Africa. Pecan nuts were collected from four different farms at sorting tables and Navel oranges from two different orchards. All collected material was kept separately in order to ensure that each collection was an independent sample and therefore a true replicate. Therefore, four true replicates existed of roughly 55kg of pecans in each replicate. False codling moth larvae in artificial diet were obtained from River Bioscience (Hermitage, Eastern Cape Province, South Africa), where FCM had been reared for numerous generations (Moore *et al.* 2014b). Sixty jars were obtained from two separate cohorts, providing two true replicates, when larvae were in the intended life stage. in this case fourth and fifth instar which are the most cold tolerant (Moore *et al.* 2016a).

7.2.2 Cold room

A 5m by 4m by 2.5m polyurethane cold room with a galvanised floor was used for the experiment at Citrus Research International in Port Elizabeth, South Africa. Temperatures were monitored with a Brainchild VR18 temperature logger with 16 PT100 probes (WIKA Technologies, Port Elizabeth, South Africa). Probes were calibrated before the experiment using the freezing point method where the probes were immersed in melting ice and the temperature recorded when they reached equilibrium. A certified calibrated thermometer (SANAS Calibration Laboratory, Pretoria, South Africa) immersed in the melting ice was used to confirm the temperature (Grout *et al.* 2011, Moore *et al.* 2016a). The cold room was set at -0.8°C in order for product temperature to be as close to -0.55°C as possible. This temperature was used as it is the current standard cold treatment temperature when exporting citrus from South Africa to China (SA-DAFF 2016). Probes were inserted into pecan nuts (seven probes), oranges (four probes) and artificial diet (four probes) in order to monitor product temperature and not air temperature. Individual probes measured air temperature at the inflow and outflow of the cold room. Temperatures were recorded at ten minute intervals for the full duration of the study; hourly mean, maxima and minima were calculated.

7.2.3 Mortality induced through cold treatment

After each product had reached a mean temperature of -0.5°C, the cold treatment was initiated. Cartons of Navel oranges, pecan nuts and artificial diet were removed from the cold room at 3, 6, 9, 12, 15 and 18 days. These were then kept at $25 \pm 2^{\circ}\text{C}$, 30% RH, and a photoperiod of 16:8 (L: D) for 24h to enable any surviving larvae to become active (Moore *et al.* 2016a, 2016b, 2016c). Immediately thereafter, pecan nuts, Navel oranges and artificial diet were dissected and numbers of live and dead larvae (and instar) were recorded for each species. Larvae were identified according to Rental (2012). Larvae were considered alive if colouration was normal and moved after prodding (Moore *et al.* 2016a). Samples (at least 25 individuals per species) of each instar were kept in 70% ethanol, and estimated life stage was verified and numbers of larvae corrected by measurement of head-capsule size (Table 7.1).

Table 7.1 Head-capsule width for carob moth and false codling moth.

Species	Instar and head-capsule size (mm)				
	1	2	3	4	5
Carob moth ¹	0.0-0.34	0.35-0.64	0.65-0.94	0.95-0.14	1.15-wider
FCM ²	0.0-0.28	0.29-0.46	0.47-0.77	0.78-1.16	1.17-wider

¹ Established in Chapter 2
² Hofmeyr *et al.* 2016

7.2.4 Larvae surviving cold treatment

To determine the fate of carob moth larvae that survive the cold treatment, all surviving individuals found after the 18-day treatment were placed onto artificial diet individually in 30 ml capacity Poly Top glass vials (Bonpak, Johannesburg, South Africa) and kept at 25 ±2°C, 30% RH, and a photoperiod of 16:8 (L: D). These larvae were monitored for survival and ability to reach the adult life stage. This was compared to a control survival where 80 carob moth larvae (20 from each replicate) were dissected from pecans and placed onto artificial diet and monitored until adult eclosion or the larva died.

7.2.5 Statistical analyses

All statistical analyses were conducted with Statistica (Statsoft 2016) unless stated otherwise. To determine whether the mortality of FCM and/or carob moth was comparable in the different products (pecan nuts, oranges and artificial diet), comparisons of mean hourly temperature over the 18-day treatment period were made with an Analysis of Variance (ANOVA). Tukey's post-hoc analysis was used to determine where significant differences occurred.

Percentage mortality data were corrected for control mortality (Abbot 1925) and data underwent arcsine transformation. Carob moth mean survival of each instar in pecans and oranges were compared separately for each time period with a factorial ANOVA to determine instars with the highest levels of cold tolerance. Tukey's post-hoc analysis was used to determine where significant differences occurred.

A comparison of mean mortality of the least cold susceptible carob moth instar against the combined mortality of FCM fourth and fifth instars at all time periods was conducted with a General Linear Model (GLM) factorial ANOVA and Tukey's post-hoc analysis was used to determine where significant differences occurred. Regression analysis was used to determine

the functional relationship between log time period of cold treatment and Probit of mortality of fifth instar carob moth along with fourth and fifth instar FCM larvae using PROBAN at a test level of $P < 0.05$ (Van Ark 1995).

7.3 Results

Temperatures within products were monitored over the duration of the study (Fig. 7.1) and comparison of hourly means showed that there was a significant difference in product temperature over the 18-day period ($F_{1, 2} = 23.26$, $P = 0.00$) (Table 7.2). Therefore, direct comparisons could not be made on mortality of FCM and carob moth between Navel oranges, pecan nuts and artificial diet.

Table 7.2 Mean hourly internal product temperature over 18 days. Different letters denote significant difference between means established through Tukey's post-hoc analysis ($P < 0.05$).

Product	Mean temperature (°C)	Standard error
Pecan nuts	-0.36a	± 0.0021
Navel oranges	-0.53b	± 0.0028
Artificial diet	-0.20c	± 0.0022

Carob moth mortality in pecan nuts over the duration of the cold treatment was significantly different between larval instars ($F_{1, 3} = 998.5$, $P = 0.000028$). Post-hoc analysis showed that carob moth fifth instar was the least cold susceptible with 94.6% mortality after 18 days which was significantly lower than second instar (100%, $P = 0.00016$), third instar (98.4%, $P = 0.049$) and fourth instar (99.1%, $P = 0.00182$) (Table 7.3). In Navel oranges, 100% mortality was observed for all instars over the 18-day treatment. There were no significant differences between cold susceptibility of instars for the shorter durations ($F_{1, 3} = 1.65$, $P = 0.924$), due to the low number of treated individuals.

A total 35 carob moth larvae survived the 18-day treatment, however, none of these larvae were able to pupate, compared to the control where 78.8% (± 2.71) were able to reach adulthood. Due to the small number of carob moth larvae recovered in Navel oranges, only corrected mortality data generated from pecan nuts was used to compare species cold susceptibility. Combined mean mortality of FCM fourth and fifth instars (Table 7.5) were significantly different to carob moth fifth instar ($F_{1,1} = 1645.4$, $P = 0.000$). There was a significant interaction between species and cold treatment duration ($F_{1, 6} = 243.5$, $P =$

0.000068). For all time periods, carob moth mortality was higher than FCM, however, post-hoc analysis showed that this was only significantly so at 9 ($P = 0.0000142$) and 12 days ($P = 0.000138$). After 18 days, carob moth and FCM mortality was 95.4% and 88.7% respectively, but this was not significantly different ($P = 0.947$) (Fig. 5.4).

Table 7.3 Corrected mean (\pm SE) percentage mortality of carob moth second to fifth instars for four replicates at -0.36°C for seven different time treatments in pecan nuts.

Treatment (days)	Combined n	Larval instar corrected mortality (%)			
		2	3	4	5
Control	450	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$
3	1120	$19.4 \pm 12.4a$	$25.7 \pm 11.4a$	$20.4 \pm 2.7a$	$8.9 \pm 1.7a$
6	1184	$37.9 \pm 14.6a$	$32.2 \pm 4.81a$	$29.65 \pm 5.3a$	$20.9 \pm 1.7b$
9	826	$91.2 \pm 5.9a$	$56.9 \pm 4.5b$	$72.4 \pm 5.6b$	$51.8 \pm 4.8b$
12	930	$93.8 \pm 7.2a$	$84.3 \pm 7.7a$	$89.4 \pm 3.7a$	$78.7 \pm 2.3b$
15	611	$100.0 \pm 0.0a$	$95.2 \pm 4.0a, b$	$98.3 \pm 1.9a$	$90.6 \pm 3.6b$
18	1032	$100.0 \pm 0.0a$	$98.4 \pm 1.8a$	$99.1 \pm 0.6a$	$94.6 \pm 1.8b$

Table 7.4 Corrected mean (\pm SE) percentage mortality of carob moth second to fifth instars at -0.53°C for seven different time treatments in Navel oranges.

Treatment (days)	Combined n	Larval instar corrected mortality (%)			
		2	3	4	5
Control	8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
3	9	-	-	0.0 ± 0.0	0.0 ± 0.0
6	21	75.0 ± 35.4	87.5 ± 17.8	75.0 ± 35.4	33.3 ± 47.2
9	14	50.0 ± 0.0	100.0 ± 0.0	50.0 ± 0.0	50.0 ± 0.0
12	15	100.0 ± 0.0	100.0 ± 0.0	50.0 ± 0.0	50.0 ± 0.0
15	15	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
18	14	-	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Regression analysis of empirical Probit values was conducted and residual variances were homogenous ($F_{4, 4} = 1.213$, $P < 0.01$), lines were parallel ($X^2 = 0.567$, $P < 0.05$), and the comparison of elevations of adjusted means was found to be significantly different ($F_{1, 9} = 6.027$, $P < 0.05$) (Fig. 7.3). Empirical Probit values at log 18 days was 6.7 for carob moth and 6.1 for FCM. The expected time period to reach LD_{50} was 10.5 days for FCM and 8.5 days for carob moth; and LD_{90} value for carob moth was 16.5 days and 22 days for FCM. The LD_{90} value for FCM may be unreliable due to the range between fiducial limits ($UF = 66.72$, $LF = 15.91$).

Table 7.5 Combined fourth and fifth instar false codling moth corrected mortality in different products. Different letters in each column indicate significant differences ($P < 0.05$) determined with Tukey's post-hoc analysis.

Treatment	Corrected mortality (%)								
	Pecan nuts			Navel oranges			Artificial diet		
	n	Mean	SE	n	Mean	SE	n	Mean	SE
Control	85	0.00a	± 0.00	10	0.00a	± 0.00	3143	0.00a	± 0.00
3	176	5.51b	± 2.26	15	0.00a	± 0.00	2066	6.27b	± 1.35
6	183	19.19c	± 5.89	9	25.00ab	± 25.00	1332	30.28c	± 7.39
9	240	24.30c	± 5.30	13	48.75b	± 15.10	1333	95.78d	± 1.98
12	197	54.00d	± 4.40	13	100.00c	± 0.00	2124	100.00e	± 0.00
15	228	79.28e	± 3.74	18	100.00c	± 0.00	1560	100.00e	± 0.00
18	279	87.83e	± 9.94	20	100.00c	± 0.00	1892	100.00e	± 0.00

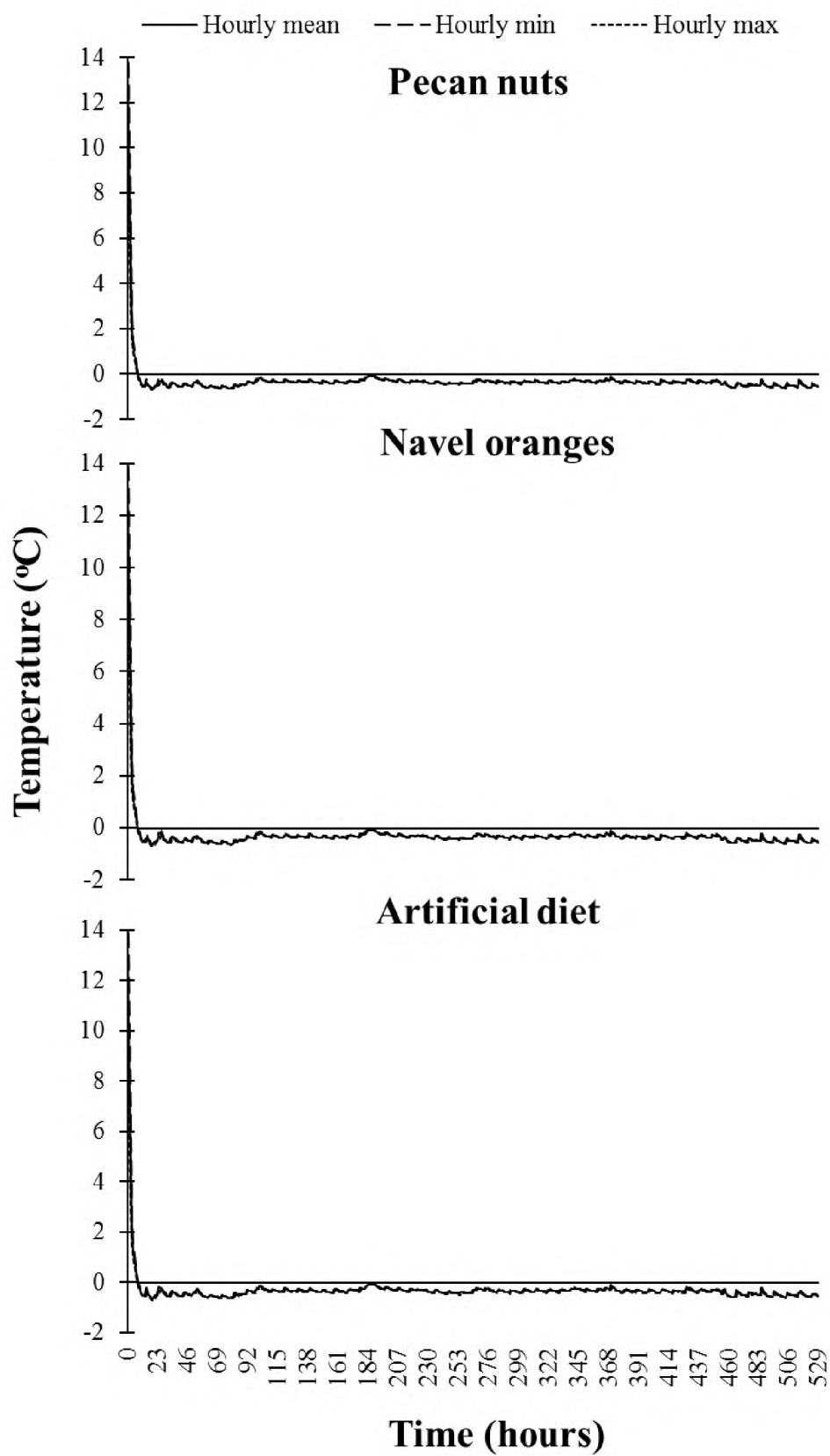
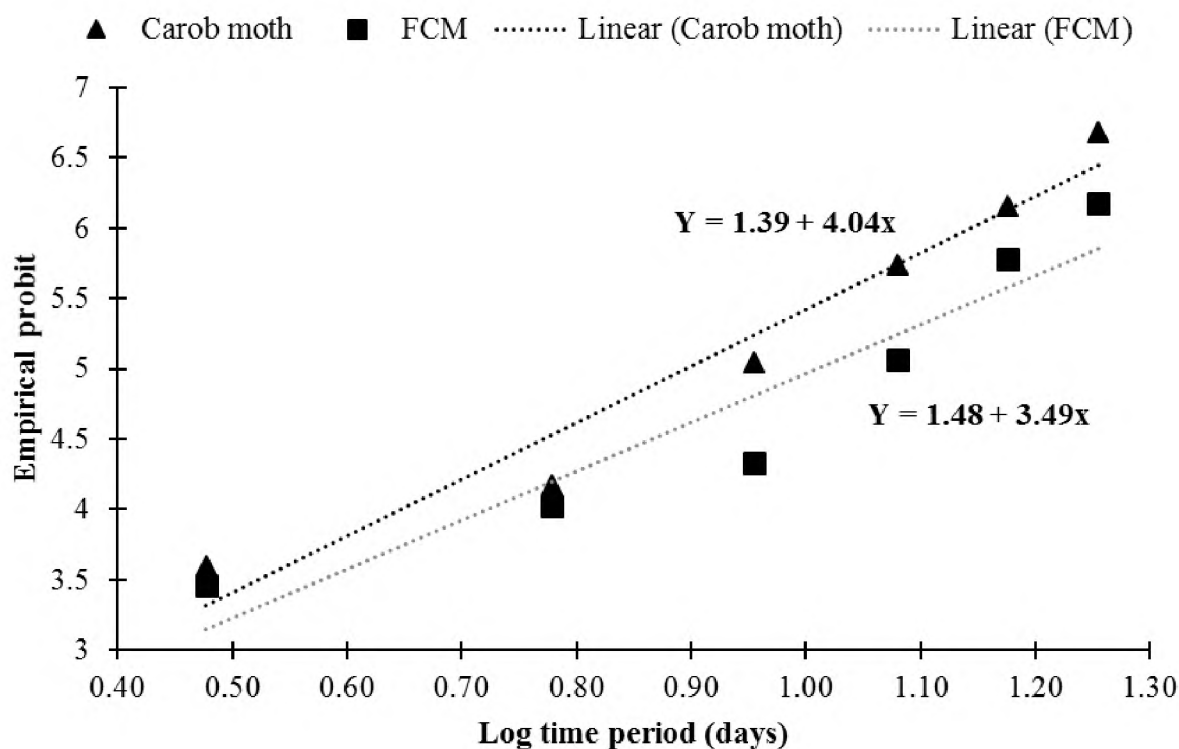


Fig. 7.1 Hourly mean temperature for products over the full 18 day treatment period including cooling down.



Time period (days)	Log time	Number of individuals exposed	
		Carob moth	FCM
3	0.48	625	176
6	0.78	666	183
9	0.95	580	240
12	1.08	617	197
15	1.18	397	228
18	1.26	669	279

Fig. 7.2 Regression of empirical Probit values against the log time period in days for carob moth and false codling moth in pecan nuts with the combined number of individuals exposed to each time period in four true replicates.

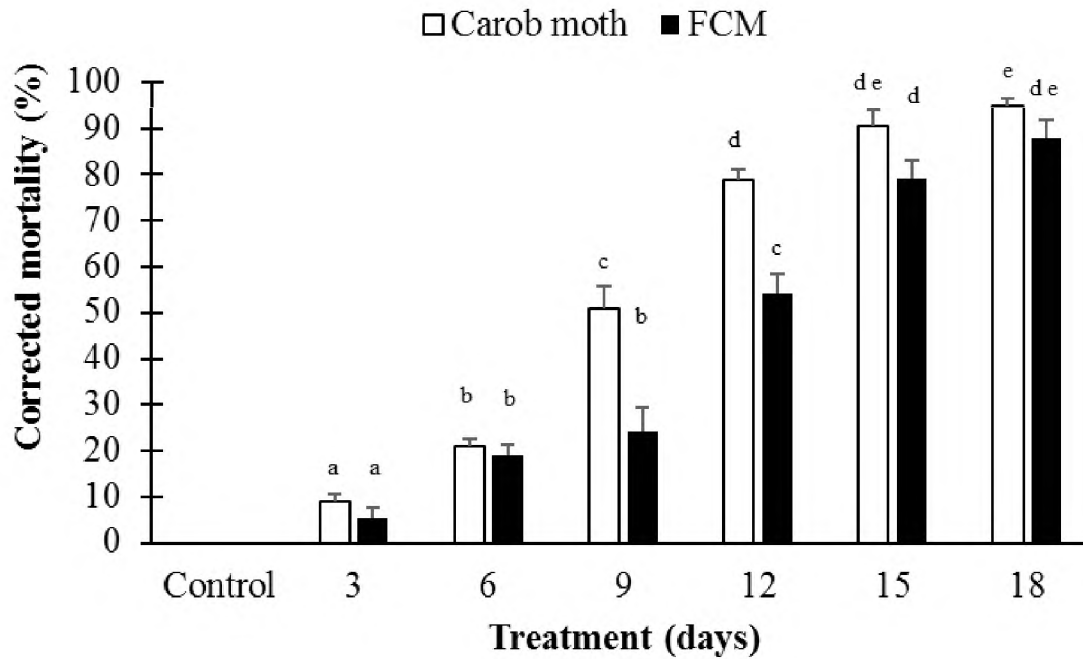


Fig. 7.3 Mean corrected survival of carob moth and false codling moth in pecan nuts at -0.34°C . Letters above bars indicate significant differences between means determined with Tukey's post-hoc analyses ($P < 0.05$).

7.4 Discussion

Moore *et al.* (2016a) showed that FCM larvae reared in artificial diet were suitable for demonstration of cold tolerance for post-harvest treatments due to their cold susceptibility being comparable to that in oranges. In this study, internal temperatures of products were significantly different and therefore not comparable. These differences in temperatures may have been a result of probe placement and the positioning of products within the cold room or internal quality of the products.

In lepidopteran larvae, the most cold-tolerant instars have been found to be either the final or the final two instars (Daiber 1979, Neven 2004, Moore *et al.* 2016a). The most cold-tolerant larval stage of carob moth was the fifth instar. The corrected mortality of carob moth (oranges) and FCM (oranges and artificial diet) reached 100% after 12 days. Although there is no literature on the cold treatment of carob moth, Moore *et al.* (2016c) recorded 100% mortality of FCM in artificial diet after 16 days at -0.5°C . The observed reduction in time to reach 100% mortality in this study was most likely due to the small sample size.

The reduced efficacy of the 18-day cold treatment on corrected mortality of both species in pecan nuts compared to Navel oranges or artificial diet can be attributed to multiple variables. Acclimatization is the modification of an organism's physiology in response to natural environmental change (Follet and Neven 2006). In the field, thermal fluctuations are common and both insect and host commodity modify their physiology in response to these fluctuations (Follet and Nevan 2006). Infested pecan nuts used in this study often only consisted of the outer shell due to larval feeding within the nut, and thus Navel oranges provided a higher level of insulation. This may have altered the range of temperatures field collected larvae experienced prior to the cold treatment. Night time temperatures in Vaalharts often fall below freezing, allowing larvae in a less insulated environment (pecans) to potentially acclimatize to sub-zero temperatures, increasing cold-hardiness.

The cold-hardiness of insects can be estimated through super cooling points (SCP) (Baust and Rojas 1985, Khani and Moharramipour 2010). The SCP is established through the measuring of the point at which body fluids undergo a phase change from liquid to solid as represented by the onset of the latent heat of fusion (Baust and Rojas 1985). The SCP of individual species can vary depending on whether the larva is overwintering or in diapause (Baust and Rojas 1985). Salt (1936, 1963) determined that surface moisture can affect SCP capacity dramatically, while water consumption can decrease SCP capacity (Cannon *et al.* 1985). Super cooling points of both FCM (Boardman *et al.* 2012) and carob moth (Heydari and Izadi 2014) have been established. In both species it was found that with an increase of water content, a subsequent increase in SCP was recorded and larvae were less cold-tolerant than their counterparts with low water content. The water content of pecans nuts is negligible and the tissue which larvae consume is 60-80% oil (Beuchat 1978), while water content of Navel oranges at the growth stage used in this study was between 70-80% (Pers comm Z. Zondi, Citrus Research International Cultivar Evaluator). The low water content with the proposed acclimatization of larvae infesting pecans are both likely to contribute to lower SCPs in carob moth and FCM.

7.5 Conclusion

Results demonstrated that carob moth fifth instar was the most cold-tolerant instar. Which was more cold susceptible than the most cold-tolerant FCM instars. Therefore, all cold treatments effective against FCM will be as effective, if not more effective, against carob moth.

CHAPTER 8: DISCUSSION

8.1 Introduction

Honiball and Catling (1998) outlined key research priorities to develop management techniques for carob moth when attacking citrus in South Africa. These were: (1) develop a monitoring system, (2) establish a reliable artificial medium for rearing carob moths to facilitate mass rearing of larval and egg parasitoids, and (3) establish whether carob moth can be controlled with products registered for control of other Lepidoptera on citrus. This thesis has addressed all of these aspects and has generated an understanding of the ecology of carob moth in citrus orchards. This includes establishing a reliable monitoring method, evaluation of chemical and semiochemical control options, devising a reliable protocol for rearing carob moth in the laboratory, evaluation of levels of parasitism, and investigation of whether the cold treatment protocols for false codling moth (FCM), *Thaumatotibia leucotreta*, would be effective against carob moth.

The discussions provided at the end of each chapter have aimed to critically interpret the results of each of these experimental chapters and to discuss how these results relate to similar studies. Little attention was given in these chapter discussions as to how the results obtained would impact management practices for citrus growers in South Africa. In contrast, the aim of this chapter is to discuss the practical implications of results obtained throughout this study and make recommendations to how industry stakeholders could incorporate these findings into current management practises.

8.2 Carob moth infestation - a consequence of pest resurgence?

In Cyprus there is a strong relationship between levels of carob moth infestation and the presence of mealybug in grapefruit orchards (Serghiou 1983). Similarly, this study has demonstrated that the presence of mealybug in Navel oranges is a prerequisite for increased levels of carob moth infestation. Mealybug is known to have a very effective biocontrol complex (Hattingh *et al.* 1998, Grout and Moore 2015). In recent growing seasons there have been numerous reports of mealybug outbreaks throughout the country and it has been suggested that these may be due to repercussions as a result of careless use of pesticides (Moore and Grout 2015). This can be considered a secondary pest resurgence, a replacement of a primary pest with a secondary pest (i.e. mealybug), due to an unintended consequence of a pesticide

treatment (Dutcher 2007). Examples of this include secondary pest resurgence of the white apple leafhopper, spotted tentiform leafminer and European red mite as a result of pesticide applications targeted at the codling moth (*Cydia pomonella*) (primary pest) in apples (Howitt 1993).

The primary pest in this particular case is citrus thrips, *Scirtothrips aurantii*, which is a major cosmetic pest of citrus in southern Africa (Gilbert and Bedford 1998). Although citrus thrips does often require chemical interventions to knock down populations below damage thresholds, these populations are able to be maintained below thresholds when predatory mites *Euseius citri* or *E. addoensis* are present in abundance (Grout and Richard 1992). However, in conventional and organic orchards the control of citrus black spot (CBS) *Guignardia citricarpa*, a disease of major phytosanitary concern, is achieved through application of mancozeb, copper and other chemicals, which along with the mineral oil used as an adjuvant, are highly detrimental to predatory mite populations (Grout 1998), often resulting in the exclusion of these predatory mites from citrus orchards (Thackeray *et al.* 2015). Consequently, to maintain citrus thrips population levels under damage thresholds, multiple applications of thripicides are required. These thripicides often have non-target effects on natural enemies (Mgocheki and Addison 2009, Moore and Grout 2015), and can result in secondary pest outbreaks such as increased levels of mealybug infestations. Therefore, a possible explanation for an increase in carob moth infestation in citrus orchards as a result of high mealybug levels may be a secondary effect of an increased number of thripicide applications, as a result of the non-target effects of CBS control measures. This illustrates how complex the potential mechanisms responsible for pest outbreaks can be.

8.3 Management of mealybug to limit carob moth infestation

The relationship observed between carob moth and mealybug infestation in grapefruit orchards by Serghiou (1983) was confirmed in this study. The Citrus Research International Production Guidelines outline appropriate control measures with recommended thresholds for mealybug attacking citrus in South Africa (Moore and Hattingh 2012b), however, these do not take into account the possible secondary outbreak of carob moth as a result of mealybug infestation. If citrus producers are hoping to export fruit to markets where carob moth has phytosanitary restrictions associated with it, production guidelines for the management of mealybug in orchards may need to be amended. Especially in orchards which have a history of

both mealybug and carob moth infestation. Therefore, the following recommendations are proposed:

- 1) Orchards which have been susceptible to mealybug and carob moth infestation in previous seasons should be subjected to an application of a registered product for mealybug control in spring as a preventative treatment. There does seem to be a fairly good correlation between petal fall and movement of mealybug crawlers, therefore a well-timed application could target these crawlers when they are more susceptible and exposed to insecticides. In this thesis it was determined that a mealybug infestation of 6% of fruit per tree in December (Chapter 3). Current guidelines state that severe mid-season infestations will often become under control by harvest if good biological control takes place, and 20% infestation six weeks after petal fall indicates the need for chemical application (Moore and Hattingh 2012b). However, both of these scenarios allow infestation levels of mealybug to reach high enough levels to result in high levels of carob moth infestation at the time of harvest. Therefore, scouting for mealybug should take place in the first week of December, and if more than 5% of fruit are infested, an additional corrective chemical intervention should take place, preferably using a product with a short residual efficacy to minimise adverse impacts on natural enemy complexes within orchards. These recommendations may be unrealistic for growers who choose to use augmentative releases of biological control agents such as *Cryptolaemus montrouzieri*, (predatory beetle) or *Coccidoxenoides perminutus* (parasitoid) for mealybug, for two reasons. First, the use of a corrective chemical application would be detrimental to augmented biological control as a result of the effect on these released agents. Second, a corrective application is likely to reduce levels of natural enemies of other key pests, which could lead to secondary pest outbreaks.
- 2) The presence of sooty mould increased the survival of carob moth larvae (Chapter 3) and fungus acts as an oviposition stimulus for gravid females (Gothilf *et al.* 1975). Mealybug infestation in December and February were strong predictors of the levels of carob moth infestation towards harvest. Producers relying on natural enemies to control mealybug infestation generally only see a reduction in infestation levels in the second half of the season. Once mealybug has established in the sheltered navel-end and sooty mould has developed on the fruit, this provides favourable conditions for carob moth infestation to establish in an orchard. Therefore, a correctly timed application of 2,4-D

prior to petal drop can alter the position of mealybug infestation on the surface of the fruit, subsequently reducing carob moth infestation. The reduced presence of mealybug in the navel-end will also likely increase the efficacy of control measures targeted at mealybug. It is important that producers actively monitor the physical parameters of the fruit to ensure that the application of 2,4-D has resulted in the desired outcomes.

It has been observed that the area of citrus being produced under netting in South Africa is increasing. Two of the main purposes of netting are to reduce hail damage and sunburn of fruit. In the Loskop Valley, where the highest levels of carob moth infestation were recorded, three separate growers have each erected roughly 350 ha of netting over a 24-month period over various citrus types. Netting changes the microclimate of an orchard; relative humidity is increased and wind, light intensity and temperature range is reduced (Prins *et al.* 2016). This will most likely result in a complete shift in the pest complex in these new microclimates. Although there is no scientific literature available on how a pest complex may change in citrus under netting, popular articles suggest mealybug and citrus thrips occur at higher densities under netting (Ferriera 2016). Personal observations also indicate that mealybug infestations do increase under netting. This has obvious consequences for carob moth infestation.

8.4 The pest status of carob moth on citrus

The pest status of carob moth will vary depending on the potential economic impact on a particular farm. A reliable monitoring system has been established (Chapter three), which will allow growers to monitor for the presence of carob moth in orchards and estimate levels of infestation, enabling producers to make economic decisions as to whether control measures should be implemented and what control methods are most suitable for the specific case.

8.4.1 Carob moth as a production pest

Incidence of carob moth infestation in citrus orchards is higher in the northern than southern production areas and is strongly related to the presence of mealybug within an orchard. When considering carob moth as a production pest, this study has shown that the highest recorded infestation of carob moth over a season resulted in an economic loss in production of R3718.00 per hectare. If one interprets weekly trap catches as an indication of population size and consequently pest pressure, trap catches from orchards with the highest loss in production are relatively low when compared to trap catches from other studies. Madge *et al.* (2013) recorded mean weekly trap catches of over 70 moths in periods of peak flight activity in Almonds in Australia. Marfra-Neto *et al.* (2013) report mean trap catches of over 80

moths in date gardens in weeks of high moth activity, while Mennan and Baspinar (2015) and Mamay and Dag (2016) reported mean weekly trap catches of between 1 and 7 moths over a growing season in pomegranate orchards in Turkey. In this thesis, the highest mean weekly trap catch recorded was 2.5 moths, suggesting that the population sizes of carob moth within citrus orchards are not as high as in other crops in other parts of the world, or possibly that pheromone lures are not as attractive to South African carob moth populations.

In most cases the production loss through carob moth infestation was similar to that of FCM, a major production and phytosanitary pest of citrus. It is important to note that in all orchards monitored in this study, stringent control measures including granulovirus applications, chemical applications and in some cases SIT and mating disruption were used to reduce FCM infestation. When making comparisons between carob moth and FCM as production pests, one must consider that the levels of FCM infestation would be much higher without these control measures. This may lead to a comparative overestimate of the actual status of carob moth as a production pest.

When deciding whether the application of a plant protection product makes economic sense, one needs to consider whether the cost of the control measure exceeds the economic loss through damage or reduced production (Gullan and Cranston 2010). The cost of control products evaluated in Chapter 5 are outlined in Table 8.1 and are represented as cost of only the product per hectare and exclude labour, fuel, water and wear and tear of machinery. For products applied through foliar spray applications, a volume of 10 000 L per hectare was used in the calculations, as this would be a realistic industry standard for full cover sprays (van Zyl *et al.* 2013). This may differ slightly depending on the number of trees per hectare, canopy density or tree size. Only the cost of Cypermethrin®, Broadband® and Dipel®, as once off applications, are less expensive than the highest production loss through carob moth over the season. However, these products showed the lowest efficacy against carob moth in field trials. Therefore, the producer would need to consider this in calculating whether applying these products would be justified. If a producer is applying a treatment targeted at both FCM and carob moth, a product with not only higher efficacy, but which is effective against both species, such as Runner®, Coragen® or Delegate®, would be more cost effective.

Table 8.1 The cost of plant protection products tested against carob moth on citrus, per application per hectare, at rates registered for control of other Lepidoptera. The cost of foliar applications was established at a volume of 10 000 litres per hectare, to reflect a full cover spray until the point of runoff. (R14.50 = 1 USD)

Foliar applications	
Product	Cost/application/ha^a
Coragen® (Chlorantraniliprole)	R 7 500.00
Runner® (Methoxyfenozide)	R 8 400.00
Delegate® (Spinetoram)	R 7 070.00
Cypermethrin® (Cypermethrin)	R 250.00
Broadband® (<i>Beauveria bassiana</i>)	R 1 500.00
Dipel® (<i>Bacillus thuringiensis</i>)	R 975.00
Semiochemical products	
Product	Cost of control from petal drop to harvest/ha^b
SPLAT EC	R 9 330.00
Mass trapping (20 units/ha) ^c	R 8000.00

^a Prices are representative of actual cost to growers (Pers. Comms. Jacques Fouche, Nexus AgChem)

^b Prices provided by River Bioscience (Port Elizabeth, South Africa)

^c Number of traps per hectare the same as Mamay and Dag (2016) and include the costs of delta traps and sticky floors

8.4.2 Carob moth as a phytosanitary pest

Although implementing control measures specifically targeting carob moth as a production pest may sometimes not make economic sense, if a producer is hoping to export to China, and considers the current legislation regarding the phytosanitary status of carob moth, economic consequences of an interception must be considered. The reason for legislation being so strict, is because there is no quantitative data to exhibit that current compulsory post-harvest cold treatments, targeted against FCM, are effective against carob moth larvae infesting citrus. However, in Chapter 7 it was demonstrated that cold treatments aimed at FCM would be as or more effective against the most cold-tolerant carob moth larval instars.

However, a recently publication by Ren and Yang (2016), aimed at describing a new species of *Ectomyelois* in China, states that “*Ectomyelois* was only represented by the common carob moth in China before this study”. The authors go on to list multiple museum specimens that have been collected throughout China. Due to the proven efficacy of cold treatments

against carob moth along with it already being established in China, the current phytosanitary status of the carob moth in citrus when exporting to China should not be so stringent and needs to be changed. If legislation is altered to stipulate that a consignment will only be rejected if a live larva is intercepted, the economic risk of growers is reduced significantly. This reduced risk would most likely result in producers reconsidering the need for implementing control measures specifically aimed towards reducing the possibility of a carob moth interception at a receiving port.

The International Standards for Phytosanitary Measures (ISPM) number four outlines the requirements for the establishment of a Pest Free Area (PFA). A PFA is defined as “an area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained” (ISPM No. 4). A PFA can exist for an entire country, an uninfested part of a country in which a limited area is present or an uninfested part of a country situated within a generally infested area (ISPM No. 4). In order to obtain a PFA, a sound pest risk assessment combined with strong evidence of effective surveillance and exclusion measures to maintain the areas pest free is required (Follet and Neven 2006). In order to determine a production area a PFA a reliable method for monitoring for the presence and infestation of the pest is required (ISPM no. 10). This thesis has established such a reliable method through evaluating the efficacy of available pheromone lures and determining a method to evaluate infestation in orchards. It has also been determined that in the Eastern and Western Cape Provinces, carob moth infestation was not observed over two seasons. Suggesting that production areas within these two provinces could pursue the establishment of PFA’s for carob moth when exporting to China.

8.5 Risk mitigation of carob moth and FCM through corrective chemical applications

There are a number of control products registered for FCM on citrus in South Africa (Moore and Hattingh 2012a). Some of these are highly species specific such as granuloviruses, mating disruption, attract and kill and the sterile insect technique. However, certain broad spectrum chemicals registered for FCM also show efficacy against carob moth (Chapter 5). With the multitude of choices in crop protection products available, very seldom do producers follow identical spray programmes. However, due to the phytosanitary status of FCM, it is very common for producers to apply a late season control measure in the form of a virus or chemical application, consequently reducing the chances of FCM infesting fruit in the weeks leading up

to harvest. If a producer is concerned that carob moth infestation may lead to possible interceptions when exporting to sensitive markets (i.e. China or other FCM sensitive markets, as carob moth may be misidentified as FCM) a late season application targeting both FCM and carob moth may reduce this risk.

Oranges infested with FCM will abscise from the tree 3-4 weeks after egg lay (Newton 1998). However, carob moth infestation is less damaging to fruit and the period from egg lay to abscission is likely to be longer than 3-4 weeks; personal observation suggests 5-6 weeks. Therefore, to reduce the risk of both carob moth and FCM infested fruit entering the packhouse, producers should apply these corrective applications 6-7 weeks before harvest. Therefore, virtually all fruit which were infested before the treatment was applied should have been abscised by the tree. However, any infested fruit which might still be hanging at the time of harvest, should be infested with late instars and will therefore be easily detectable and can thus be excluded at harvest or even during the packhouse grading process. Any infested fruit that escapes detection will in any case be subjected to cold treatment in transit to market, which will kill any larvae. If the corrective spray is applied earlier than seven weeks before harvest, it is possible that the product may no longer be effective for some time before harvest, increasing the likelihood of fruit infested with early instars entering the packhouse, defeating the point of risk mitigation.

8.6 Future research

Although this study has generated an understanding of carob moth ecology, monitoring methods and control in citrus orchards it has also provided a platform for future research. These areas of proposed future research are outlined below:

Citrus pest complexes under permanent netting

There is a lack of understanding of the citrus pest complex when cultivated under netting. However, early observations indicate that mealybug and thrips may become more important. With the change of microclimate, it is highly likely that this will influence the number of generations of pest species over a season, alter the efficacy of control products and impact current augmentative biological control programmes. This will influence many current management practices, especially the timing of chemical applications and the residual efficacy of products at reduced ultraviolet radiation. Although there is a strong relationship between carob moth and mealybug, the recommendations made in this study are a result of a good

understanding of current management practices, tree phenology and pest biology. Therefore, base line studies should be initiated on mealybug ecology under netting, allowing management practices to be developed, resulting in limiting carob moth infestation.

Female attractant

The use of female attractants through volatile organic compounds (VOCs) has become a valuable monitoring technique (Knight 2002). A method which enables females to be trapped may provide a more appropriate and accurate method for timing spray applications, as peak periods of female activity should coincide with high levels of oviposition. Carob moth females are attracted to short chain alcohols emitted by certain fungus species, these compounds have been identified and their stimulus proven (Gothilf *et al.* 1975, Cosse *et al.* 1994). However, there are no published studies that have evaluated the use of these compounds as lures in the field. If these compounds show efficacy in the field, they could then be developed further into female attract and kill stations. Examples where kairomones have been used successfully to trap female moths for monitoring purposes include the codling moth (*Cydia pomonella*) and oriental fruit moth (*Grapholita molesta*) (Knight *et al.* 2014).

Bioprospecting for microbial control products

A method which enabled the establishment of a laboratory culture of carob moth in South Africa was described in this study and a microsporidian infection was identified. Various microsporidia have been researched for their potential use of biopesticides, to this point only one is registered as a microbial insecticide; *Nosema iocustae* for grass hopper control (Solter and Maddox 1998). However, other potentials include *N. pyrausta* for European corn borer and *N. lymantrae* or *Vairimorpha disparis* for gypsy moth (Solter and Hajek 2009, Solter *et al.* 2012). There are numerous insect viruses which have been discovered in laboratory cultures and subsequently developed into commercial products. Carob moth is a major pest of many agricultural commodities and although the South African market for such product would be relatively small, this may be of extreme value to other industries and localities where carob moth is a serious pest.

8.7 Conclusions

This chapter has discussed considerations to be made by various stakeholders in citrus production in South Africa. Control measures aimed at reducing carob moth as a production pest do not make economic sense unless these applications are simultaneously targeted at other

pests, such as FCM. Late season chemical applications to mitigate risk of carob moth infested fruit entering the packhouse should take place 6-7 weeks before harvest. The phytosanitary status of carob moth when exporting should be revised due to the reported presence of carob moth in China, the demonstrated efficacy of existing post-harvest cold treatments, and the option of PFAs should be included in legislation. Future research should be focused on developing a female attractant, bioprospecting for microbial agents for the development of biopesticides and generating an understanding of how the pest profile of mealybug will change when citrus is cultivated under permanent netting.

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