An ecological analysis of stink bug and lepidopteran borer complexes associated with pecan and citrus orchards in the Vaalharts region

by

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DECLARATION

I, André van Rooyen, declare that the thesis hereby submitted by me for the Master of Science degree in Entomology at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty. I furthermore concede copyright of the dissertation to the University of the Free State.

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SUMMARY

This study investigates the insect-plant interactions on citrus fruit and pecan nuts in South Africa. The aims of this study were to determine whether lepidopteran borer pests shuttled between adjacent citrus and pecan orchards, and to establish the cultivar preference of the most prevalent stink bug borer species in Vaalharts pecan orchards. Pheromone trap data indicated that there was a significant overlap between the populations of false codling moth (*Thaumatotibia leucotreta*) and carob moth (*Ectomyelois ceratoniae*) between adjacent citrus and pecan orchards. Both species of moth were found within the citrus and pecan orchards. Larval eclosions indicated that false codling moths shuttled between adjacent citrus and pecan orchards; however, the direction of the shuttling could not be established. An abundance of citrus fruit drew false codling moths from an adjacent pecan orchard, which increased infestation in the citrus orchard. No significant evidence of carob moth shuttling was found. Adult false codling moths were found to be active throughout the winter, albeit in decreased numbers. Adult carob moths were almost entirely absent during the winter months, although larvae were still present in pecan nuts and citrus fruit. Grey-brown stink bugs (*Coenomorpha nervosa*) were found to be the most prevalent hemipteran pest on pecan trees in the Vaalharts region. *Coenomorpha nervosa* demonstrated a significant preference for pecan trees of the Wichita cultivar. Adult and nymph grey-brown stink bugs preferred the Wichita cultivar to Choctaw, Barton, and Navaho cultivars. The investigation could not find *C. nervosa* exploiting other viable food sources close to pecan orchards; even potential host plants that were not dead or defoliated during winter. Overall, the study indicates that adjacent citrus and pecan orchards will accommodate shuttling of false codling moths between orchards. Furthermore, grey-brown stink bugs were found to be the dominant hemipteran pest on pecan trees the Vaalharts region. Grey-brown stink bugs prefer the Wichita cultivar and do not exploit food sources near pecan trees of the Wichita cultivar.

Key terms: Lepidopteran borer shuttling, species overlap, false codling moth, carob moth, grey-brown stink bug, host preference, Wichita cultivar, *Thaumatotibia leucotreta, Ectomyelois ceratoniae*, *Coenomorpha nervosa.*

OPSOMMING

Hierdie studie het die insek-plant-interaksies op sitrusvrugte en pekanneute in Suid-Afrika ondersoek. Die hoofdoelwitte van hierdie studie was om te bepaal of lepidoptera-boorderpeste tussen aangrensende sitrus- en pekanboorde migreer; asook om die kultivarvoorkeur van die mees algemene stinkbesieboorderspesie in Vaalharts-pekanboorde te bevestig. Feromoonlokvaldata het daarop gedui dat ’n beduidende oorvleueling tussen die bevolkings van valskodlingmot (*Thaumatotibia leucotreta*) en karobmot (*Ectomyelois ceratoniae*) tussen aangrensende sitrus- en pekanboorde teenwoordig was. Albei motspesies is gevind in die sitrus- en pekanboorde. Die uitbroei data van larwes het aangedui dat valskodlingmotte tussen aangrensende sitrus- en pekanboorde migreer. Die rigting van die migrasie kon egter nie vasgestel word nie. Oortollige hoeveelhede sitrusvrugte lok valskodlingmotte van ’n aangrensende pekanboord, wat lei tot verhoogde besmetting in die sitrusboord. Geen beduidende bewyse van karobmotmigrasie na aangrensende boorde kon gevind word nie. Daar is gevind dat valskodlingmotvolwassenes aktief is in die winter, maar in verminderde getalle. Karobmotvolwassenes was byna heeltemal afwesig gedurende die wintermaande, alhoewel larwes in beide pekanneute en sitrusvrugte teenwoordig was. Grys-bruin stinkbesies (*Coenomorpha nervosa*) was die mees algemene besieplaag op pekanbome in die Vaalhartsstreek. ’n Beduidende voorkeur vir pekanbome van die Wichita-kultivar is deur *C. nervosa* gedemonstreer. Beide volwasse en nimf grys-bruin stinkbesies verkies die Wichita-kultivar bo Choctaw-, Barton-, en Navaho-kultivars. Dit kon nie bewys word dat *C. nervosa* voedselbronne naby pekanboorde gebruik nie, selfs potensiële gasheerplante wat nie in die winter ontbos of vrek nie. Die studie het getoon dat aangrensende sitrus- en pekanboorde migrasie van valskodlingmotte tussen boorde sal akkommodeer. Voorts is gevind dat grys-bruin stinkbesies die dominante besieplaag op pekanbome in die Vaalhartsstreek is. Grys-bruin stinkbesies toon ’n voorkeur vir die Wichita-kultivar en maak nie gebruik van voedselbronne naby pekanbome van die Wichita-kultivar nie.

Sleutelterme: Lepidoptera boordermigrasie, spesiesoorvleueling, valskodlingmot, karobmot, pekan, sitrus, grys-bruin stinkbesie, gasheervoorkeur, Wichita-kultivar, *Thaumatotibia leucotreta*, *Ectomyelois ceratoniae*, *Coenomorpha nervosa*.

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LIST OF ABBREVIATIONS

ARC	Agricultural Research Council
BFAP	Bureau for Food and Agricultural Policy
CM	Carob moth
CRI	Citrus Research International
DAFF	Department of Agriculture, Forestry and Fisheries
EPPO	European and Mediterranean Plant Protection Organization
EU	European Union
FCM	False codling moth
GENMOD	General Linear Model Analysis
IPM	Integrated pest management
SAS	Statistical Analysis Software
SIT	Sterile insect technique
UFS	University of the Free State
USA	United States of America

CHAPTER 1

INTRODUCTION

1.1 Introduction

The pecan nut and citrus industries in South Africa are important primary industries, with exports to the Northern Hemisphere offering significant capacities to absorb South African produce. Export markets to Africa, Asia, and the European Union (EU) all yielded positive trade deficits from 2013 onwards (Bureau for Food and Agricultural Policy (BFAP) 2015). Due to the isolated location and climate of South Africa, agricultural pests that are not historically native to the more temperate regions of the world could be introduced along with export products. Strict evidence-based phytosanitary measures that are the least trade restrictive possible should be employed, and should only be maintained with sufficient scientific evidence (Theyse 2010)

The false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) [Lepidoptera: Tortricidae], is a registered phytosanitary pest for the United States of America (USA) and certain markets in the Far East. It is thus a concern to these export partners; primarily due to the polyphagous capabilities of the larvae and the difficulty of controlling the species with conventional pesticide treatment regimens (Department of Agriculture, Forestry and Fisheries (DAFF) 2016). The efficient and accurate identification of FCM larvae is also of concern. The carob moth (CM), *Ectomyelois ceratoniae* (Zeller) [Lepidoptera: Pyralidae] is an essentially cosmopolitan species that is visually distinct from FCM during the adult stage, which simplifies identification. Differentiation between FCM and CM larvae is more problematic and has the potential for confusion (Rentel *et al*. 2011). Similar larval feeding behaviours result in similar wounding patterns on infested fruit and similar physiological changes to the fruit. This causes potential complications; while it is not so much a threat to the export market as a whole, it might negatively affect individual shipments. It is therefore important to qualify the shuttling of these species between pecan nuts and citrus fruit. The effect of pecan orchards, planted proximally, must be quantified in the short term to ascertain if proximity increases infestation to a significant degree. In the longer term, this information can be used to make informed decisions regarding larger-scale integrated pest management (IPM) programmes.

If increased levels of infestation warrant such measures, farm and orchard layout might also need to be adjusted in order to mitigate the risk of the migrating moths.

The grey-brown stink bug, *Coenomorpha nervosa* Dallas (Hemiptera: Pentatomidae), is a pest of multiple crops in South Africa. In sufficient numbers, these hemipterans can cause substantial crop losses (Cissel *et al.* 2015). Even when larval feeding on the nuts is not sufficient to cause direct losses via nut drop or death, cosmetic damage to the kernel makes the nuts unmarketable. Even nuts sold for decorative purposes cannot be marketed if fed on by the grey-brown stink bug, as the nuts undergo physiological changes after the feeding which alter the external appearance of the nuts. It is thus important to know which cultivars are most at risk of being fed upon in order for measures to be taken to curtail losses for the valuable export market.

1.2 Ecology of false codling moth

1.2.1 History and taxonomy of false codling moth

Fuller described FCM in 1901; samples were discovered infesting citrus fruit in what is known as KwaZulu-Natal today (Schwartz 1981). Fuller named the moth “Natal codling moth” in 1901 and placed it in the genus of *Carpocapsa*. Howard referenced *Enarmonia batrachopa* in 1909 as the “orange codling moth”. In 1914, Kelly referred to *E. batrachopa* as the “false codling moth” which was found in acorns, also in what is today KwaZulu-Natal (Schwartz 1981). From then on, it was referred to as the false codling moth, or FCM. Meyrick, in 1913, described it as *Argyroploce leucotreta*, after which it was transferred to the genus *Cryptophlebia* by Clarke in 1958, and thus renamed *C. leucotreta* (Newton 1998).

In 1999, Komai transferred the moth from genus *Cryptophlebia* to the previously synonymous genus of *Thaumatotibia* (Venette *et al*. 2003). False codling moth can be confused with species such as the codling moth (*Cydia pomonella*), macadamia nut borer (*Thaumatotibia batrachopa*), and the litchi moth (*Cryptophlebia peltastica*) (Timm 2005).

1.2.2 Biology of false codling moth

1.2.2.1 Eggs

The eggs of FCM measure one millimetre in diameter, are hemispherical in shape, and are translucent. Oviposition on the principal citrus host usually occurs within depressions that are present in the rind of the fruit. The size of the eggs and the selection of oviposition sites render the eggs inconspicuous. The eggs are usually laid one by one, unless female moths have direct access to the flesh of the fruit, usually via a break in the rind; at such locations the eggs may be laid in clusters (Stofberg 1954). The eggs can also be laid in aggregate around the navel of navel oranges (US Department of Agriculture 2010). The eggs can be laid in great quantities; some fruit have been observed with up to 100 eggs (Stofberg 1954). The eggs hatch at all times of the day (Newton 1998).

1.2.2.2 Larvae

The first-instar larvae are vulnerable and can suffer high mortality rates due to low humidity under laboratory conditions and low temperatures, particularly during winter conditions in the field (Newton 1998). Intra-specific competition is a factor in the populations of younger larvae, as the larvae may be cannibalistic when concentrations of larvae are very high, or when food resources are not sufficient (Newton 1998). The delicate first-instar larvae rely on pre-existing cracks or wounds in the rind, or the navel ends of navel oranges, to gain access to the interior of the fruit. Only on rare occasions is more than one larva capable of completing their development within one fruit (Catling & Aschenborn 1974). Stotter (2009) found that acorns could in a similar capacity accommodate more than one FCM larva at a time.

The younger larvae have a creamy white body with a brown-black head capsule (Newton 1998). Mature larvae develop a much more noticeable pink body colour and range between 15 and 20 mm in length. The larval development encompasses five instars. Under field conditions, larval development is completed within the range of 25 to 67 days (Stofberg 1954). The time it takes to complete larval development is dependent on prevailing conditions, with temperature being one of the more dependent factors (Stofberg 1954). Food quality was also found to be an important factor in the development of the larvae. Consistent with observations by Newton (1998) found that fifth-instar larvae which leave the fruit, open conspicuous exit holes and drop to the ground to pupate or emerge when the fruit has already dropped from the tree.

1.2.2.3 Pupae

After exiting the fruit, fifth-instar larvae spin a silk cocoon that binds with soil particles and surrounding detritus (Newton 1998). The finished cocoon, which at this point resembles the soil, lies upon the surface of the soil (Stofberg 1954).

Two sub-stages occur within the cocoon: a pre-pupal stage and a pupal stage (Stotter 2009). The pre-pupal stage, which occurs shortly after the spinning of the cocoon, is light beige in colour (Newton 1998). The pupal stage that follows the pre-pupal stage is darker in colour and persists until eclosion occurs (Stofberg 1954). The pupal stage of FCM is completed within 21 to 80 days under field conditions, and the exact duration is dependent on conditions that govern pupal growth, mainly temperature.

1.2.2.4 Adults

Adult FCM are small, inconspicuous moths which tend to be brown-black to grey in colour (Newton 1998). The wingspan is 16 to 20 mm. The anterior wings are mottled and larger and comprise the maximal extent of the wingspan, while the smaller posterior wings are a paler grey colour (Newton 1998).

Male moths are smaller than the females, and are distinguished by densely packed and elongated scales on the hind tibia, an anal tuft of scales, and an olfactory organ near the anal angle of each hind wing (Newton 1998).

Female moths mate within two to three days after eclosion and commence with oviposition as soon as conditions and host availability allow it (Stofberg 1954). Female FCM can produce up to 300 eggs during their lifespan (Stofberg 1939). Temperature has a fundamental impact on the oviposition of FCM, as an average fecundity of 460 eggs per female lifespan is produced at a constant temperature of 25 °C, but only 0.4 eggs per female lifespan at 10 °C (Newton 1998). Oviposition can reach a peak at three to five days of age (Catling & Aschenborn 1974). It has been reported, however, that temperature has a significant effect on the pre-oviposition period of an adult female, varying from one day (at 25 °C) to 22 days (at 10 °C), with 50% of the eggs being laid within six to 23 days after oviposition is initiated (Daiber 1980). The sex ratio of FCM adults has been established at the approximate ratio of 1:2 in favour of the males (Daiber 1980). In wild populations, the sex ratio has been established as closer to 1:1 (Newton 1998).

At a constant temperature and optimal captive conditions, adults can survive for between 13 and 34 days (Newton 1998). Whilst exposed to more fluctuating conditions in the field, adult life expectancy is up to three weeks (Stofberg 1939). As can be expected from an exothermic organism, the total lifespan of FCM decreases as the temperature increases (Stofberg 1954).

The adults are exclusively night fliers (Daiber 1978). Oviposition is assumed to take place only at night, as the moths are not known to be active during the day (Stofberg 1954).

1.2.3 Lifecycle of false codling moth

The complete lifecycle of FCM is 45 to 60 days during the summer months (Stofberg 1954). The lifecycle duration increases during the winter months and lasts between 68 and 100 days. When infesting acorns, the lifecycle lasts an average of 121 days, most of which is taken up by the larval stage (84 to 90 days) (Stofberg 1954).

The incubation period for FCM eggs on citrus is six to eight days in summer and nine to 12 days in winter (Newton 1998). Larval development for FCM on citrus during the summer takes 25 to 35 days and 35 to 67 days in the winter. Pupal development takes 21 to 24 days in summer and 29 to 40 days in winter (Stofberg 1954).

The number of generations per year is dependent on prevailing climatic conditions, particularly temperature. Areas that have moderate temperatures (clearly defined summers and winters) will generally have less than six generations per year. Up to six generations can be expected per year in areas that have higher average temperatures (Stofberg 1954).

In hosts other than citrus, and in particular when food quality is poor and host plants produce drier fruit, no more than three generations per year can be expected (Newton 1998). In Southern Africa, FCM are active throughout the year where a constant supply of host fruit is available (Newton 1998).

1.2.4 Hosts of false codling moth

False codling moths are known to have a broad range of hosts, with potential hosts amongst both wild and cultivated plants (Stotter 2009), yet host specificity for FCM remains uncertain. False codling moths are known as an equatorial pest of cotton in Africa (Newton 1998), a pest of citrus in Southern Africa, particularly in the southern areas (Catling & Aschenborn 1974), and as a macadamia nut borer in Malawi (La Croix & Thindwa 1986).

Citrus damage from FCM is particularly acute in navel orange cultivars (De Villiers & Grové 2006). False codling moths also prefer some varieties of mandarin, satsuma, and star ruby grapefruit (Newton 1998). Valencia oranges and grapefruit cultivars other than star ruby are not generally subjected to heavy infestations (Hofmeyr & Pringle 1998). Lemons have been demonstrated to be non-hosts for FCM when subjected to normal harvesting and packing protocols for export (Moore *et al*. 2015).

The Pest Risk Analysis for *Thaumatotibia leucotreta* report compiled by the European and Mediterranean Plant Protection Organization (EPPO) identified 19 confirmed hosts of particular interest for the European and Mediterranean regions (EPPO 2013). Sampling in South Africa found that litchis and macadamia nuts also act as hosts for FCM (Timm 2005). Similarly, peaches, nectarines, and plums were investigated, and found to be hosts for FCM (Blomefield 1989). False codling moths were reared successfully under laboratory conditions on wild plum and wild almonds, and proved capable of infesting Port Jackson willow galls (Honiball 2004). In an attempt to find possible alternative hosts of FCM, it was found that the moth could penetrate and inhabit the stems of jade (Kirkman & Moore 2007).

1.2.4.1 Cultivated hosts

The following list is a collection of some better-known cultivated plant hosts of FCM (EPPO 2013). It must be stated that not all of the mentioned hosts are capable of supporting FCM populations as effectively as the principal citrus host, but all can act as possible habitats and must therefore be considered.

- Pepper (*Capsicum* spp.)
- Mandarin orange (*Citrus reticulata* & hybrids)
- Orange (*Citrus sinensis* & hybrids)
- Grapefruit (*Citrus paradise*)
- Cotton (*Gossypium* spp.)
- Litchi (*Litchi chinensis*)
- Macadamia (*Macadamia* spp.)
- Mango (*Mangifera indica*)
- Peach (*Prunus persica*)
- Necatrine (*Prunus persica* var. *nucipersica*)
- Avocado (*Persea americana*)
- Guava (*Psidium guajava*)
- Pomegranate (*Punica granatum*)
- Oak (*Quercus robur*)

- Castor bean (*Ricinus communis*)
- Rose (*Rosa* sp.)
- Eggplant (*Solanum melongena*)
- Grape (*Vitis vinifera*)
- Maize (*Zea mays*)

False codling moth larvae encountered on flowers were successfully reared to adults. From this information *Rosa* spp. are considered a host of FCM (EPPO 2013).

1.2.4.2 Damage symptoms

False codling moths lay their eggs on the surface of fruit, and when the larvae hatch, they bore into and feed on the rind of the citrus fruit (Newton 1998). In some cases the larvae bore through the rind and penetrate into the core of the fruit (Stotter 2009). This tunnelling by the larvae in many cases leads to the premature drop of the fruit from the trees. Fruit with exposed flesh are more attractive to gravid FCM females than ripe fruit (Newton 1989). Infested fruit may drop from the trees at the beginning of the growing season (usually November) whilst the fruit is still immature and no more than 15 to 20 mm in diameter (Newton 1989). The rind of citrus fruit infested with FCM takes on a brown to yellow colour around the points of larval entry; these damage symptoms act as possible avenues for further arthropod incursions and fungal infections (Stotter 2009). The overall damage to the citrus industry in 2003 that was attributable to FCM was estimated at R100 million (Moore *et al.* 2004).

The type of damage that FCM inflict on pecan nuts is similar to the damage caused by CM because the method of pecan husk penetration is similar. The first-instar larvae of FCM are capable of accessing the gap in the husk that occurs due to nut development. The researcher observed that nuts which were completely closed were infested by both CM and FCM, and that the nuts were readily identifiable in both cases due to the development of an oily red sheen on the husk of the nut.

1.2.4.3 Economic significance

South Africa is a major agricultural exporter, with agricultural exports totalling just under R38 billion in 2014 (BFAP 2015). Citrus exports experienced a growth of 25% from 2013 to 2014, which was attributed to an increase in export prices, as the volume stayed the same at

1.74 million tonnes. The net revenue for citrus exports in 2014 was just under R12 billion (BFAP 2015). Iran placed strict phytosanitary standards on all citrus exports from South Africa, which requires much stricter internal control by the producers and cold sterilisation before export (DAFF 2010). Other countries that require cold sterilisation and export control measures for citrus from South Africa are China, the EU countries, Japan, South Korea, Thailand, and the USA (DAFF 2016).

The US Department of Agriculture (2010) reported FCM to be responsible for a loss of up to 30% in macadamia nut production in South Africa and Israel. False codling moths are not known to represent an economic threat to the pecan industry by means of direct damage to crops, but due to the phytosanitary status of the moth, export markets could be in jeopardy if shipments are found to contain FCM.

False codling moths have been found to travel significant distances (1.5 km) to preferred citrus orchards (Stotter 2009). The number of males which shuttled to fynbos, was very low and a significant portion of the FCM population remained in the citrus orchards. False codling moths do not generally shuttle to nearby fynbos vegetation (Stotter 2009); any FCM found within the fynbos were found to be a direct representation of the FCM population in the nearest orchard (Stotter 2009). Guava and acorns were infested within a 1.5 km radius, indicating that nearby potential hosts will initiate FCM shuttling to high densities of such potential hosts (Stotter 2009). Adjacent crops of cotton planted at different times indicated a significant increase of infestation of older cotton situated adjacent to earlier cotton, with up to 60% of the more mature cotton being infested and destroyed by FCM (Reed 1974).

1.2.5 Geographical distribution of false codling moth

False codling moths are endemic and indigenous to the African continent, with a general distribution south of the Sahara Desert, and mostly concentrated in the tropical and sub-tropical regions of the continent (Newton 1998). False codling moth distribution is also evident in Senegal, Côte d’Ivoire, Togo, and Southern Africa. It is also encountered on islands proximate to the continent south of the Sahara in countries such as Madagascar and Mauritius (Newton 1997). The moth is a known pest on citrus in South Africa, Mozambique, Zimbabwe (Stofberg 1954), Swaziland, and Malawi (La Croix & Thindwa 1986). The moth has also been reported in Israel (Wysoki 1986).

1.2.6 Control measures for false codling moth

International market forces are driving a higher demand for pest-free citrus fruit, with limited pesticide use during production. The drivers behind the increase in demand are mainly economic but also social, political, climatic, demographic, and even emotional. To keep citrus production high and increasing in an area that has endemic populations of FCM, without the egregious use of pesticides, is not necessarily an easy proposition. Existing control methods and newly developed ones, used in conjunction, can enable citrus production to remain significant in South Africa. However, keeping domestic production as well as the quality of the citrus competitive in an ever-changing global market is proving challenging. Ever-stricter phytosanitary and quality restrictions upon producers are placing great strain upon established control methods (Government Gazette 2014). It is therefore imperative to develop new strategies to augment the ability to control FCM or to combine existing strategies in order keep the situation under control.

1.2.6.1 Cultural control

One of the more effective control methods to reduce the population of FCM is the cultural practice of removing fallen fruit from orchards. Visibly damaged fruit should also be removed from trees, as these may not only be infested with larvae, but damaged fruit are also known to be attractive to the moths as oviposition sites (Stotter 2009). Orchard sanitation is a vital pest-control measure and the daily removal of damaged fruit will deliver the best possible results. Out-of-season fruit should also be removed from trees as they may also act as possible hosts for FCM (Moore & Kirkman 2009).

Moore and Kirkman (2009) made the following orchard sanitation recommendations for the control of FCM in citrus orchards:

- Remove all out-of-season fruit and unharvested fruit as soon after harvest as possible.
- Orchard sanitation must include removal and destruction of dropped fruit and hanging fruit which appear injured, infested, or decaying.
- Fruit should ideally be destroyed by pulping or burying (30 cm deep and compacted).
- Pulped fruit should be spread on the ground at least 30 m outside orchards.
- Fruit 15 mm in diameter and smaller (during November) can be raked into the inter-rows to bake in the sun; however, removal and destruction are still preferable.

- Orchards should ideally be sanitised twice a week during the hotter months of the year (particularly January to March).

1.2.6.2 Biological control

Biological control of FCM forms an important part of the natural pest control in many citrus-producing areas (Stotter 2009). Twenty-five species of natural enemies of FCM have been listed; all potentially occurring in citrus orchards. Of the 25 species, 12 occur in South Africa (Moore 2002). Hymenopteran parasitoids comprise five of the 12 species, two are dipteran parasitoids, another two are insect predators, with two fungal entomopathogens, and finally a baculovirus (Stotter 2009). False codling moth eggs are susceptible to parasitism by trichogrammatid egg parasitoids for the first three to six days of their lifespan (Schwartz 1981). The augmentative release of FCM egg parasitoids in the form of *Trichogrammatoidea cryptophebiae* has thus been the focus of commercial biological control attempts (Schwartz 1981). The seasonal increase of FCM is followed by an abundance of *T. cryptophebiae* (Catling & Aschenborn 1974). Catling and Aschenborn (1974) recommended augmentative mass releases of *T. cryptophebiae* in the beginning of the citrus season. Schwartz (1981) investigated the augmentative release method and was met with success. It has the potential throughout the season to result in the significant reduction of FCM numbers.

Cryptogran® (River Bioscience (Pty) Ltd, South Africa) is a granulovirus preparation that is applied as a spray formulation to leaf and fruit surfaces. The application of the formulation is regarded as a form of biological control, as the virus is a naturally occurring pathogen of FCM known as *Cryptophlebia leucotreta* granulovirus (Moore *et al*. 2004). Emerging larvae, which attempt to bore into the fruit, ingest the virus, and after they die, large amounts of viral bodies are distributed back onto the plant surface, which may potentially infect other FCM larvae. One of the primary advantages of Cryptogran® is that the product can be used in conjunction with a chemical control programme without lowering the overall effectiveness of the virus itself (Stotter 2009). Also, since the virus is specific to the species of its homologous host, it does not have a detrimental impact on FCM’s natural enemies, is harmless to most other insects and vertebrates (including humans), and does not leave residue on the fruit that can be problematic for markets (Moore *et al.* 2012).

1.2.6.3 Mating disruption

High-density female sex pheromones of FCM in the form of products such as Isomate (Pacific Biocontrol Corporation, USA) disrupt mating over large areas, if distributed homogeneously (Hofmeyr & Pringle 1998). This has the effect of reducing the number of fertilised eggs that are laid on the citrus fruit. The high dosage of female sex pheromones released at one point has the effect of confusing the males as to the location of the females, thus reducing the overall number of successful mating instances (Carde & Minks 1995). Checkmate FCM-F (Suterra LLC, USA), a spray-applied capsule suspension, does not appear to have the same efficacy as Isomate (Moore & Kirkman 2011). The use of Checkmate FCM-F is nonetheless considered viable for low-pressure FCM regions (Moore & Hattingh 2012).

1.2.6.4 Attract and kill

A product such as Last Call FCM® (Insect Science SA, South Africa) combines synthetic pheromones with a permethrin pyrethroid in one simultaneously deployable product at a density of up to 3 000 droplets per hectare. The product comes in pre-calibrated form to provide consistent 50 µl droplets with a hand applicator. When the female sex pheromone succeeds in drawing the male towards the droplet, contact with the droplet results in the death of the male, which leads to lower rates of fertilisation (Stibick *et al.* 2007). The product can be effective in the suppression of light FCM infestations, but without the ability to predict the intensity of an infestation, it makes this labour-intensive product difficult to deploy in efforts to curb an infestation (Hofmeyr & Pringle 1998).

1.2.6.5 Sterile insect technique

Only males are considered important for the sterile insect technique (SIT), and to have an effect, an oversaturation of 1:10 in favour of irradiated males is desired in order to ensure effective control (Hofmeyr & Hofmeyr 2004). The eggs produced by the females which mated with treated males will be non-viable in most instances. If any of the eggs do hatch into the new generation (known as the F1 generation), the members of that generation will be sterile. The treatment of the F1 generation is the large-scale irradiation of male and female FCM that are exposed to a 150 Gγ dose of radiation. The irradiated insects are then released into orchards in order to compete with wild insects of the same sex for mates. This culminates in potentially effective FCM control due to the infusion of significant numbers of sterile individuals into the population. These populations generally produce fewer viable eggs, and, as a consequence, fewer larvae to damage citrus. It remains more important to uphold the oversaturation ratio with new releases into an area to compensate for the deaths of the treated individuals and their very few offspring.

The implementation and eventual full deployment of F1 SIT seem to be a promising avenue for the control of FCM (Schwartz 1975). In 2002, an SIT project was initiated in the Olifants River Valley (Hofmeyr *et al*. 2015). Favourable results of pilot studies led to the development and construction of FCM-specific rearing equipment and mass-rearing facilities (Hofmeyr *et al*. 2015). By 2010, the project had expanded to apply SIT to 4000 hectares, with a tenfold reduction in the wild male population (Hofmeyr *et al*. 2015).

1.2.6.6 Chemical control

Chemical control agents are only economically viable against the egg stages and emerged larvae, with no insecticides registered for use against FCM until the early 1980s. Hofmeyr (1977) demonstrated that synthetic pyrethroids remained effective against FCM for up to 17 weeks. Trials conducted in 1978 and 1983 using cypermethrin and deltamethrin on navel oranges to prevent fruit drop reduced the fruit drop by 90% when applied in one dose, 60 to 90 days before harvest (Hofmeyr 1983).

Trials conducted using the benzoyl-urea chitin synthesis inhibitors, Alsystin® (Triflumuron: Bayer Cropscience, Australia) and Nomolt SC® (Teflubenzurom: BASF Crop Protection, Belgium) indicated that these insecticides had little effect on FCM adults and their larvae (Hofmeyr 1984). Eggs that were laid on spray residue of chitin synthesis inhibitors suffered prolonged suppression. The residues of Alsystin caused up to 85% egg mortality for up to 75 days (Hofmeyr 1984).

The use of Alsystin® and Nomolt SC® as registered FCM products has come under question due to reports of emerging resistance against the effects of the products (Hofmeyr & Pringle 1998). In addition, Alsystin® is known to have detrimental effects on *T. cryptophebiae* (Hattingh & Tate 1997). Two new chemical insecticides were introduced in 2011 – Coragen (Rynaxapyr: Dow Chemical Company, USA) and Delegate (Spinetoram: Dow Chemical Company, USA) – which were found to have comparable efficacy (Moore & Hattingh 2012). Coragen and Delegate have highly favourable eco-toxicology profiles, making them compatible with IPM strategies and suitable for the widespread establishment of acceptable residue tolerances (Moore & Hattingh 2012). In 2013, Runner 240 SC (Methoxyfenozide; Dow Agrosciences SA, South Africa) was introduced as a moult-accelerating compound for the control of FCM on avocados and citrus.

1.3 Ecology of the carob moth

1.3.1 History and taxonomy of the carob moth

The pyralid moth known as *Ectomyelois ceratoniae*, or carob moth, was redescribed by Philipp Christoph Zeller in 1839. Many synonyms were used over the years, but have since fallen out of use; however, the synonym *Apomyelois ceratoniae* was in use recently in some parts of the world (Mehrnejad 1993). It is historically known as a pest of wild fruit around the Mediterranean Sea; however, the exact original host remains unclear. Gothilf described the moth as polyphagous in 1964, and as a consequence of the native environment it can be assumed that the moth evolved as a generalist borer of fruits and pods around the Mediterranean Sea. In addition to being adaptable to new hosts within its original range, the carob moth was able to migrate to new habitats and establish itself as a significant pest in areas outside its original distribution (Gothilf 1964). As a demonstration of the essentially cosmopolitan distribution of carob moth, it has been known since the 1960s that carob moth were present on almonds in Australia (Madge 2012). However, it only became an economically significant pest in the early 21st century due to weather changes and expansion into production areas. Carob moth is also the most prominent pest on dates in the state of California in North America (Madge 2012).

1.3.2 Biology of the carob moth

1.3.2.1 Eggs

Carob moth eggs were found to hatch in 3.05 days under laboratory conditions (Mediouni & Dhouibi 2007). Female CM that were kept in mass-rearing conditions produced significantly fewer eggs in comparison to wild females. The difference in egg production was attributed to the lack of space available for copulation (Mediouni & Dhouibi 2007). Mediouni & Dhouibi 2007 observed that when female carob moth were mass-reared, they produced an average of 115.6 eggs per female, of which 95.9 were fertile. Female carob moth reared in single-pair situations produced, on average, 182.5 eggs, of which 140 were fertile. Carob moths are known to show preference for oviposition onto already damaged fruit, which increases the survivability of the vulnerable first-instar larvae (Hashemi-Fesharaki *et al.* 2011).

1.3.2.2 Larvae

Carob moth larvae hatch from the eggs after an average of three days. At temperatures of 28 ± 1 °C, a photoperiod of 14:9 (Light:Dark), and 45 ± 5% relative humidity, the first and most vulnerable larval instar also takes the longest to develop; measured at five days under laboratory conditions (Mediouni & Dhouibi 2007). Under similar conditions it takes the second- and third-instar larvae 4.5 days each to develop, the fourth-instar larvae develop in 4.6 days, and the fifth-instar larvae take 4.8 days to develop to the point where pupation takes place (Mediouni & Dhouibi 2007).

1.3.2.3 Pupae

The CM larvae leave the confines of the host nut or fruit to pupate. The pre-pupating larvae might bore a new tunnel and thus create an extra wound on the surface of the fruit in order to accomplish this, or simply exit via the first tunnel. Pupation may take place in the soil and the larvae spin a cocoon similar to that of FCM, to aggregate substrates into the silk and make the cocoon less visible to potential predators and parasitoids. There are also reports of larvae pupating in the nuts (Mehrnejad 1993). The pupal stage of CM is completed within 6 to 10 days under field conditions, which depends on the conditions that govern pupal growth – mainly temperature (Madge 2012). This was confirmed when a comparison between mass rearing (7.33 days) and single-pair rearing (7.01 days) under laboratory conditions was conducted (Mediouni & Dhouibi 2007). This study observed that larvae will spin a cocoon on the surface of a fruit if no suitable substrate (such as sand) is available for the larvae to utilise. It was also observed that the larvae may make use of the confines of a pecan shell or a pecan husk to spin a cocoon.

1.3.2.4 Adults

Adult CM are inconspicuous moths and have a pale brown pattern on the anterior wings, with plain white posterior wings. Adult CM emerge after a six- to ten-day pupal period, and if conditions are suitable, they will mate and start oviposition immediately (Madge 2012). Under field conditions, the adults have a short lifespan – usually no more than two weeks; however, colder temperatures allow the adults to survive longer (Madge 2012). By observing adult lifespans under laboratory conditions, Mediouni and Dhouibi (2007) found that male CM survived on average six days, whilst females survived on average 8.7 days. Under laboratory conditions, female CM are both larger and heavier than the males, with an average adult weight of 24.7 mg; the average male weight was 16.6 mg (Mediouni & Dhouibi 2007).

1.3.3 Lifecycle of the carob moth

Carob moth eggs are usually deposited individually on fruit (Gothilf 1969). The physical condition of the fruit may lead to clusters of eggs being laid in order to take advantage of exposed flesh on the fruit (Hashemi-Fesharaki *et al.* 2011). Mozaffarian *et al*. (2007) observed that CM emerge from early May (late spring) in Iran. Pomegranates are attacked first as the conditions on the fruit are deemed most suitable for oviposition (Mozaffarian *et al*. 2007). The pomegranate (*Punica granatum*), particularly the sour cultivar, is prone to skin cracking, which increases the prevalence of CM infestation (Hashemi-Fesharaki *et al.* 2011). Madge (2012) observed that CM in Australia preferred nuts from the previous season still present on the trees as hosts for renewed oviposition. The range of the lifecycle from egg to adult is 34 to 61 days (Reuther *et al.* 1989). The length of each stage depends on both the climatic conditions as well as the quality of food available for the growth of the larvae. During most years in the USA, four complete generations and a partial fifth generation occur on a yearly basis (Reuther *et al.* 1989).

1.3.4 Hosts of the carob moth

Some of the better-known hosts of CM have been identified as *Ceratonia siliqua*, *Acacia farnesiana*, *Ficus carica*, and citrus varieties (Gothilf 1964). The pest is considered polyphagous as it is capable of attacking both pomegranates and pistachio nuts, which is of considerable concern in Iran (Hashemi-Fesharaki *et al.* 2011). After completing some generations on pomegranates, as soon as other host plants provide suitable conditions for laying eggs, such as the grooves and tracks that occur on pistachios, CM will infest pistachio nuts as well (Mehrnejad 1993).

Gothilf (1964) found that the presence of fungal infections of *Phomopsis* spp. on carob pods leads to increased CM oviposition. In addition, gravid CM females, given the opportunity, will prefer to oviposit on nuts infested with *Phomopsis* spp. (Gothilf 1964). A series of trials conducted in Cyprus demonstrated that in order for grapefruit to be infested by CM, an established citrus mealybug (*Planococcus citri*) infestation was a prerequisite (Serghiou 1983). If no *P. citri* infestation was present on the surface of the grapefruit, gum exuded by the fruit was able to kill the first-instar CM larvae before they managed to cause significant damage to the fruit (Serghiou 1983).

1.3.4.1 Cultivated hosts

The following list is a collection of the more economically important crop plants which are attacked by the CM.

- Almond (*Prunus amygdalus*)
- Carob (*Ceratonia siliqua*)
- Castor oil (*Ricinus communis*)
- Orange (*Citrus sinensis*)
- Date (*Phoenix dactylifera*)
- Fig (*Ficus carica*)
- Grapefruit (*Citrus paradisi*)
- Macadamia (*Macadamia integrifolia*)
- Pecan (*Carya illinoinensis*)
- Pistachio (*Pistacia vera*)
- Pomegranate (*P. granatum*)
- Walnut (*Juglans regia*)

1.3.4.2 Damage symptoms

The physical condition of the pomegranate neck, which protects the eggs and leads larvae to the inside of the fruit, has an influence on where oviposition takes place (Mozaffarian *et al*. 2007). Due to this, CM larvae are capable of boring through weak plant tissue that is present in this area and penetrating into the fruit (Mozaffarian *et al*. 2007). The feeding behaviour of CM presents an opportunity for secondary infestations by fungal and bacterial pathogens (Hashemi-Fesharaki *et al*. 2011).

The means by which CM penetrate citrus fruit is very similar to the mechanism by which FCM gain access. That is to say, the larvae hatch, bore into, and feed on the rind of the citrus fruit

(Newton 1998). In some cases, the larvae bore through the rind and penetrate into the core of the fruit (Stotter 2009). The feeding of CM larvae results in premature discolouration of the fruit and to fruit eventually dropping (Reuther *et al.* 1989). The similar progression of discolouration with the eventual result of fruit drop in citrus indicates that it is a physiological reaction of the plant to the feeding of CM and FCM larvae which results in the symptoms. Thus, many of the damage effects described above concerning the action of FCM on citrus may be similar to the damage that CM inflict on citrus.

The penetration of the nut and the subsequent damage to the nut caused by the feeding action render the nut unsuitable for human consumption (Madge 2012). The penetration of nut crops (as with pomegranates and citrus) generates concern regarding the potential increase in risk for fungal infections (Madge 2012). The first-instar larvae of CM are capable of accessing the gap in the husk that occurs due to nut development. Also, it is assumed that CM are capable of accessing closed nuts by penetrating through the weak point that exists at the base of the stem attachment to the nut. The researcher observed that entirely closed nuts were infested by both CM and FCM (however, never at the same time), and that the nuts were readily identifiable in both cases due to the development of an oily red sheen on the husk of the nut.

1.3.4.3 Economic significance

Carob moths were found to cause significant damage to the fruit of the carob tree in the Mediterranean region (Wood 1963). Wood 1963 noticed in the early 1960s that the CM was starting to establish itself as a citrus pest in the Mediterranean. After investigations during harvest in a carob plantation, Gothilf (1964) found that the magnitude of CM infestation depends on the proportion of cracked fruit in each plantation. Only eggs and young CM larvae were present on grapefruit and this was very seldom (Gothilf 1969). Carob moth eggs are laid individually on citrus fruit and very few were found during the monthly samplings; therefore the damage of CM to grapefruit is considered minimal (Gothilf 1969). The recent expansion of the almond industry, combined with higher-than-average rainfall in Australia, has prompted the rise of CM as a significant pest of an emerging industry (Madge 2012). Similarly, the conditions that led to an increase of CM on almond nuts in Australia now threaten the date (*Phoenix dactylifera*) industry as well (Madge 2012).

In Iran, CM are considered one of the most important factors responsible for the quantitative and qualitative reduction of pomegranate yield throughout the pomegranate cultivation regions of the country. Carob moths also attack pistachio orchards in Iran (Hashemi-Fesharaki *et al.* 2011). Appropriate weather conditions and the suitable physical properties of the fruit (early maturity and sensitivity to skin cracking) are considered the two main reasons for an increase in infestation (Hashemi-Fesharaki *et al.* 2011). In most cases where secondary infestations are not significant, loss of fruit quality due to moisture loss and feeding damage lowers the marketability of the produce (Hashemi-Fesharaki *et al.* 2011). Shakeri (2004) found that within the primary pomegranate production areas of Iran, CM could infest and thus render up to 80% of the crop unmarketable during the fruiting season and at harvest. Carob moth infestations have been found to be notably high in pistachio orchards adjacent to pomegranate plantations. It was found that CM attack pistachio nuts during the early summer, hence the requirement for an alternative host in the spring. In the absence of an alternative host, CM are not able to establish in pistachio orchards (Mehrnejad 1993; 1995).

1.3.5 Geographical distribution of the carob moth

While CM are a well-known pest in the Mediterranean, they are also present in other parts of the world, including Hawaii, the USA, as well as the tropical and subtropical regions of the Americas (Gothilf 1969). Heinrich (1956) postulated that CM were introduced to the New World directly from the Mediterranean. In the Americas they are widely distributed throughout the USA and are present in Argentina and the West Indies (Reuther *et al.* 1989). Carob moths also occur in Australia, where they cause significant damage to the almond industry.

1.3.6 Control measures of carob moth

1.3.6.1 Cultural control

Gothilf (1964) observed that CM develop mostly in cracked carob pods. Of the pods investigated at harvest, 70% to 90% of all cracked pods were found to be infested by CM (Gothilf 1964). The removal of carob pods from the previous season was strongly encouraged, as infestation of the pods increases as the season progresses (Gothilf 1969).

For the control of CM in almonds, the removal of almonds still present from the previous season proved to be effective to reduce the pest population (Madge 2012). The previous seasons’ nuts were found to be preferred habitats, which housed up to six CM larvae per nut; in contrast to nuts from the current season, which only harboured an average of two. The manual removal of nuts from the previous season was strongly encouraged (Madge 2012).

Al-Izzi *et al.* (1985) reported that orchards in Iran that demonstrated poor sanitation procedures had the highest incidence of CM. Sanitation procedures such as the removal and destruction of dropped pomegranate fruit are encouraged in order to remove potential additional hosts for future populations of CM to reproduce (Al-Izzi *et al.* 1985).

Infected pomegranates might not be the only overwintering sites for CM (Mozaffarian *et al.* 2007). This suggests that the removal of pomegranates left after harvest may only reduce, rather than prevent the overwintering of CM (Mozaffarian *et al.* 2007). The same study found that high levels of CM infestation on host plants other than pomegranates were not present. Mozaffarian *et al.* (2007) suggested that cultural control practices should not only focus on the sanitation of pomegranates but also on other potential overwintering hosts could become significant factors in the future, such as pistachio nuts.

1.3.6.2 Biological control

In 1946, only one species of braconid wasp, *Habrobracon brevicornis*, was recognised as a natural enemy of the CM (Thompson 1946).

In Israel it was found that CM were parasitised in carob tree plantations. The parasitism was mainly attributed to the braconid *Phanerotoma flavitestacea* and the tachinid *Clausicella suturata* (Gothilf 1969). Carob moth infestation of carob pods can reach a maximum of 56% in carob plantations (Gothilf 1969). Similarly, the incidence of parasitised insects seemed to be linked more to the location of the carob plantation rather than to the carob variety, and seemed to vary from year to year. Gothilf (1969) also observed that parasitism of CM increased as the carob season ended, with the incidence of parasitism averaging 20% to 50% during August. The incidence of CM egg parasitism was found to be low, with only isolated instances of parasitism by *Trichogramma* spp. being recorded (Gothilf 1969).

When infesting carob plantations, only small numbers of parasitoids of CM other than *P. flavitestacea* and *C. suturata* were collected (Gothilf 1969). The other parasitoids noted were as follows: *Baronia brevicornis*, *Apanteles lacteus*, *Apanteles* spp., *Anisopteromalus mollis, Pristomerus vulnerator, Horogenes* spp., *Gelis* spp., *Brachymeris aegyptiaca* (possibly a hyperparasite), *Antocephalus mitys*, and *Perilampus tristis* (Gothilf 1969). Other species also collected were a number of hyperparasites such as *Gelis* spp., *B. aegyptiaca*, and *P. tristis*, with *P. tristis* being specifically identified as a hyperparasite of *P. flavitestacea* (Gothilf 1969).

Many of the abovementioned species were recorded for the first time as parasitoids of CM on carob, in particular members of the genus *Apanteles* in 2012 (Kishani Farahani *et al.* 2012).

A recent study found that female *Apanteles myeloenta* typically preferred to parasitise second-instar CM over third- or first-instar CM (Kishani-Farahani *et al.* 2012). The oviposition activity of *A. myeloenta* on the CM larvae peaks on the seventh and eighth days following the emergence of the adult parasitoids from pupae (Kishani-Farahani *et al.* 2012). Parasitism of CM by *A. myeloenta* was noted to be particularly high, with up to 30% of larvae and up to 25% of the overwintering larvae being parasitised during cultivation (Kishani-Farahani *et al.* 2012). Kishani-Farahani *et al.* (2012) found that the sex ratio of *A. myeloenta* was of profound importance for the parasitoid to act as a potential biological control agent. The larval stages parasitised was found to have the greatest influence on the sex ratio, therefore second-instar CM larvae were the best option to maximise the overall number of female *A. myeloenta* that emerged (Kishani-Farahani *et al.* 2012). The availability of carbohydrates for adult feeding of *A. myeloenta* was also found to increase longevity and fecundity, both of which are important for the agent to act as efficiently as possible for as long as possible (Kishani-Farahani *et al.* 2012).

1.3.6.3 Mating disruption

Various forms of pheromone dispensers for the disruption of CM mating have been developed. The function of the dispenser is to confuse male CM as to the location of potential females (Carob Moth UC IPM Fact Sheet, UC Davis College of Agricultural & Environmental Sciences 2012). The half-life and pheromone release rate of traps vary to a great degree; female sex pheromones are utilised in this capacity. The chemicals used by each company are usually proprietary in nature and results between traps tend to vary accordingly. There have also been suggestions that large-scale deployment of a selected pheromone solely for the purpose of mating disruption could be a viable option. This can be done by dispersing large quantities of sex pheromone within an area to confuse the male CM (Madge 2012).

The oil extracts of Asant (*Ferula asafoetida*) have shown to be efficient in repelling CM (Peyrovi *et al.* 2011). The application of a 4 ml solution of *F. asafoetida* oil and ethanol (in a 50/50 proportion) onto a section of fabric, inserted into a polycarbonate tube, 20 tubes per hectare, achieved the best results (Peyrovi *et al.* 2011). To ensure optimal potency, the solution should be reapplied on a monthly basis. A suspension of the Asant solution was also shown to be a potential oviposition disruptor for CM (Peyrovi *et al.* 2011).

1.3.6.5 Sterile insect technique

The development of an SIT programme for CM was last reported to be in the pilot phase (Simmons *et al.* 2009). Only limited releases of CM took place, with efforts still focused on formulating an artificial diet that could increase the fertility and fecundity of adult CM (Mediouni & Dhouibi 2007). It is essential that irradiated males are capable of competing with wild males (Mediouni & Dhouibi 2007). Irradiated males were found to be able to compete with wild males after limited releases and sex pheromone trap captures indicated statistically insignificant discrepancies between wild and irradiated males recovered from the traps. It was concluded that irradiated CM males still respond to virgin female pheromone dispersal. Mediouni and Dhouibi (2007) stressed that more research needed to be conducted regarding the formulation of the artificial diet. Mediouni and Dhouibi (2007) also indicated during the progression of their study that CM were highly resistant to radiation, requiring more than 400 Gγ in order to ensure complete sterility in the females but only partial sterility in the males.

1.3.6.6 Chemical control

The developmental biology and behaviour of CM larvae are not amenable to the widespread use of chemical insecticides for control (Kishani Farahani *et al.* 2012).

An effective measure of chemical control over CM was achieved with the application of Trichlorfon (Organophosphate: Miles, Inc., USA) or fenthion (Organophosphate: Bayer CropScience, South Africa) over a period of 10 days (Reuther *et al.* 1989). Mecarbam (Methylcarbamate: Sigma-Aldrich, USA), originally formulated as a scalicide, was tested in Cyprus as an effective means of control for CM (Serghiou 1983). The combined application of chlorpyriphos (Organophosphate: DowElanco, USA), mecarbam, methomyl (Carbamate: DuPont Agricultural Products, USA), and methidathion (Organophosphate: Ciba-Geigy Corporation, USA) were effective in controlling both citrus mealybug and CM infestation when they occured together as a complex (Serghiou 1983).

It has been suggested that the use of products such as Naturalyte® (Spinosad: Dow AgroSciences LLC; USA) is the best option for the control of CM within an IPM programme (Carob Moth UC IPM Fact Sheet 2012). As an insect growth inhibitor, Fenoxycarb

(Carbamate: Syngenta Crop Protection (Pty) Ltd, South Africa) has been suggested for use within an IPM programme (Carob Moth UC IPM Fact Sheet 2012). It is recommended that insecticides should be applied within a week of the first trap captures of CM in order to ensure the maximum chance of treating the exposed first-instar larvae before they move into the crop (Carob Moth UC IPM Fact Sheet 2012).

1.4 Ecology of the grey-brown stink bug

1.4.1 History and taxonomy of the grey-brown stink bug

Dallas described the grey-brown stink bug as *Coenomorpha nervosa* in 1851. As a member of the family Pentatomidae (Hemiptera), the grey-brown stink bug shares many biological traits with other Pentatomidae, specifically with species that indicate a convergence of preferences for inhabiting similar environments and damaging the same plants. *Coenomorpha nervosa* is a pest of avocado. Other Pentatomidae are also known pests, such as the green stink bug (*Nezara viridula*) and the powdery stink bug (*Atelocera raptoria*) (Bruwer 2004). The aforementioned species are not necessarily closely related but share similar preferences for host plants. This convergence makes it convenient and in some cases necessary to substitute the habits and preferences of the better-studied species (*A. raptoria* and *N. viridula*) with the tendencies of *C. nervosa*. This is sometimes necessary due to the lack of available literature that refers directly to *C. nervosa*.

Coenomorpha nervosa has not distinguished itself as a key pest and any development of control measures occurs with methods for *A. raptoria*, *N. viridula*, and other hemipteran species (Bruwer 2004). This indicates that *C. nervosa* is considered very similar to *A. raptoria* and *N. viridula* from a pest control viewpoint and as such can be treated as having similar biologies (Bruwer 2004).

1.4.2 Biology of the grey-brown stink bug

1.4.2.1 Eggs

Coenomorpha nervosa eggs are laid in clusters, and are very similar in appearance and in the number of eggs laid to that of the brown marmorated stink bug, *Halyomorpha halys* (Bernon *et al.* 2004). The egg clusters usually number around 25 eggs, of which each is about 1 mm in diameter (Bernon *et al.* 2004). The individual eggs are light green in colour, with a barrel-like appearance, and are securely attached to the substrate on which they are located. The clusters are laid underneath bark or within the nook of a branch on a tree. All the eggs of a cluster are laid at the same time, and as such the nymphs emerge with a high degree of synchronicity (Bernon *et al*. 2004). The incubation period of the eggs depends on factors associated with the time of the year, such as the temperature of the immediate environment. Drake (1920) observed that the eggs of *N. viridula* hatch within a week of being laid in the summer, and up to three weeks at the turn of seasons. Drake (1920) also noted that female *N. viridula* have oviposition preferences for certain plants, preferentially selecting weeds such as Mexican clover (*Richardia brasiliensis*) and blackberry (*Rubus* sp.).

1.4.2.2 Nymphs

Pentatomidae nymphs emerge from the egg by opening a disc-shaped cap that is present at the top of the egg body. Egress from the egg takes about five minutes. As with all pentatomid nymphs, they lack developed wings (Hoebeke & Carter 2003). The first-instar nymphs congregate around the egg cluster until their first moult. After the first moult, the second-instar *C. nervosa* nymphs acquire a darker colouration and disperse from the egg cluster. Subsequent moults from the third to the fifth instar lead to a regimented increase in size and a further shift in colouration as the nymphs begin to resemble the adult *C. nervosa*.

1.4.2.3 Adults

Adult *C. nervosa* have a characteristic mottled appearance that spreads over the head, pronotum, and hemilytra. The black-coloured posterior wings are visible at the posterior end of the abdomen. The insect is dorso-ventrally symmetrical. Each antenna has two distinctive orange bands that are present on the basal angle of each the last two antennules. There is also an orange band present on the median of the tibia on each leg. The head is elongated 1.21 times as long as it is broad and the veins in the wings are brown. Studies indicate that the adults become sexually mature two weeks after their final moult (Hoebeke & Carter 2003).

1.4.3 Lifecycle of the grey-brown stink bug

Pentatomids in temperate climates tend to have only one generation per year (Hoebeke & Carter 2003). In more tropical climates, with high temperatures all year round, adult pentatomids do not need to enter diapause to survive the winter and, as such, up to five generations can occur in a year (Hoebeke & Carter 2003). Adults that were in diapause during winter emerge in spring and start to mate about two weeks after their emergence (Hoebeke & Carter 2003). The females start to oviposit soon after mating and lay new egg clusters on a weekly basis. They are capable of producing in excess of 400 eggs over the course of their lifecycle. Egg clusters are oviposited throughout the season, thus it is possible to observe nymphal stages during most of the breeding season (Hoebeke & Carter 2003).

The first nymphs hatch within a week of oviposition and then typically feed alone after dispersal from the egg clusters; however, Bernon *et al*. (2004) observed that nymphs will aggregate in sheltered areas. The pentatomid *Halyomorpha halys* has five nymphal instars and, depending on temperature, each of the stages will last about a week (Bernon *et al*. 2004). Adult pentatomids are very active feeders that will migrate between plants in order to feed. Both the adults and nymphs initially rely on camouflage in order to avoid predation. If approached, *C. nervosa* will cease to move and emit a foul-smelling defensive pheromone. When a potential predator keeps encroaching and is not rebuffed by the smell, both adult and nymphal *C. nervosa* will attempt to flee by running away or dropping from the plant. Adults will also attempt to fly away if pursued (Bernon *et al*. 2004).

1.4.4 Hosts of the grey-brown stink bug

Pentatomids are known to use a sequence of crops as their populations increase throughout a season; the variety of hosts that pentatomids exploit can therefore be very diverse (Mizell *et al.* 2008). Known to be extremely polyphagous, stink bugs have developed a preference for feeding on specific parts of plants, primarily the seeds during the milk stage (during the filling of the kernel) of the seeds, while other succulent areas on individual plants are exploited for a limited amount of time during a season (Jones & Sullivan 1982). Stink bugs are major pests of commercial crops; this has the unintended consequence of reducing the inter-species competition on cotton, and stink bugs have recently become major pests of cotton (Mizell *et al.* 2008). *Halyomorpha halys* is known to attack shade trees, fruit trees, vegetables, and leguminous crops (Hoebeke 2002). In South Africa, *C. nervosa* is part of a pest complex on avocado, and is regarded as a serious pest (Bruwer 2004).

1.4.4.1 Cultivated hosts

The following list is a collection of some of the known hosts of stink bugs on cultivated plants (Mizell *et al.* 2008; Bruwer 2004; Hoebeke 2002). Due to the polyphagous habits of stink bugs, the list of cultivated hosts can be very extensive (Jones & Sullivan 1982). The following are given as an example of the breadth of possible hosts:

- Apple (*Malus domestica*)
- Avocado (*Persea americana*)
- Bean (*Phaseolus* spp.)
- Cherry (*Prunus avium*)
- Citrus (*Citrus* spp.)
- Cotton (*Gossypium hirsutum*)
- Fig (*Ficus* spp.)
- Japanese apricot (*Prunus mume*)
- Japanese persimmon (*Diospyros kaki*)
- Mulberry (*Morus* spp.)
- Okra (*Abelmoschus esculentus*)
- Peach (*Amygdalus persica*)
- Pear (*Pyrus pyrifolia*)
- Peas (*Pisum sativum*)
- Pecan (*Carya illinoiensis*)
- Soybean (*Glycine max*)

1.4.4.2 Natural hosts

Below is a list of known hosts of stink bugs on wild plants (Hoebeke 2002; Bergman *et al.* 2013). Due to the polyphagous habits of stink bugs, the list of wild and ornamental hosts can be very extensive (Jones & Sullivan 1982). The following is an example of possible hosts:

- Autumn olive (*Elaeagnus umbellata*)
- Black cherry (*Prunus serotina*)
- Black nightshade (*Solanum nigrum*)
- Black walnut (*Juglans nigra*)
- Celosia (*Celosia argentea*)

- Ceylon spinach (*Basella rubra*)
- Common hackberry (*Celtis occidentalis*)
- Hibiscus (*Hibiscus rosa-sinensis*)
- Invasive witch hazel (*Hamamelis japonica*)
- Leafy wild parsley (*Musineon divaricatum*)
- Multiflora rose (*Rosa mulitflora*)
- Princess tree (*Paulownia tomentosa*)
- Witch hazel (*Hamamelis virginiana*)

1.4.4.3 Damage symptoms

Stink bug damage on avocado can be very extensive and it changes during the growth period of the flowers and fruit (Bruwer 2004). During the early stages of flower development, feeding by *C. nervosa* will cause flowers to fall from the tree. Feeding on the immature fruit during this stage will also result in the drop of avocados (Bruwer 2004). Feeding on fruit that have been developing for one to six weeks will result in scarring on the surface of the fruit and damage to the underlying tissue; feeding during this stage may also result in fruit that are malformed (Bruwer 2004).

Pecan nuts are attacked by pentatomids from early-season fruit development until after the endosperm hardens. While the pecan nuts are still in the water stage, damage results in a condition referred to as “black pit” or “black heart”. Indications of pentatomid damage at this stage are symptoms such as fluid seeping from inside the fruit and accumulating on the exterior of the nut. The area of the husk surrounding a puncture site turns black within an hour and the vascular system between the shuck and shell darkens within two days. These symptoms spread to the rest of the nut and the nut proceeds to drop from the tree within five days. Husks damaged by stink bugs which do not drop from the trees; adhere tightly to the shell. This is in contrast to healthy husks, which can easily be removed from the nut; similarly, husks due to normal summer nut abortions separate easily (Yates *et al.* 1991).

After the water stage, hemipteran feeding can be ascertained only by cracking the nuts and examining the kernels (Polles 1977). Feeding sites on kernels are characteristically black spots that become sunken and pithy and have a bitter taste (Yates *et al.* 1991). These injuries are commonly referred to as “kernel spot”. Feeding that occurs after the endosperm hardens can only be observed by taking sections of the husk and using electron micrographic techniques (Yates *et al.* 1991).

1.4.4.4 Economic significance

The pecan industry suffers severe losses due to the feeding action of stink bugs (Deuce & Suber 1986). The losses recorded during the 1985 season in Georgia, USA, was estimated at $3.5 million. This was calculated for the state of Georgia alone and did not take into consideration the impact on the industry in other parts of the USA (Deuce & Suber 1986). Stink bug damage in 1989 attributed $13 million worth of damage in the soybean industry in Georgia (Douce & McPherson 1991). Tree fruit in particular are vulnerable to *H. halys*, with some growers losing entire crops of stone fruit in the Mid-Atlantic region of the USA (Leskey *et al.* 2012). Apple growers in the Mid-Atlantic region reported losses in excess of $37 million in 2010 throughout the region (United States Apple Association 2010).

1.4.5 Geographical distribution of the grey-brown stink bug

Coenomorpha nervosa is endemic to Southern Africa and has not been found outside of this geographical area. South Africa is listed as a potential quarantine risk for accidental phytosanitary breaches for Australian exports of avocado (Plant Health Australia 2011). Many pentatomid pests have an essentially cosmopolitan distribution and even *H. halys*, which is not distributed worldwide, is spreading due to increasing trade and lax phytosanitary measures (Hoebeke & Carter 2003). It was found in 2001 that *H. halys* was already established on the eastern seaboard of the USA (Hoebeke & Carter 2003). The southern green stink bug (*Nezara viridula*) is believed to have originated in Ethiopia; however, the expansion of monocultures and intercontinental trade has expanded its range to include the tropical and subtropical regions of Europe, Asia, and the Americas (Panizzi 2008).

1.4.6 Control measures for grey-brown stink bug

1.4.6.1 Cultural control

Due to the active nature, hemimetabolous lifecycle, and reproductive preferences of *C. nervosa* and stink bugs in general, there are no widespread cultural practices designed to curtail stink bug populations. During the progression of this study, the researcher noticed that stink bugs prefer to diapause in artificial structures, such as irrigation tubes that are usually left behind in orchards. The removal of such artificial structures will deny stink bugs a more substantial habitat and lead to a higher mortality rate due to climactic exposure and increased contact with predators during the winter months.

1.4.6.2 Attractant chemicals

A trap developed by W.L. Tedders for monitoring and capturing *Euschistus* spp. captured many adult male and female stink bugs as well as nymphs that were in close proximity of the trap (Mizell & Tedders 1995). The trap needs to be used in conjunction with an aggregation pheromone in order to produce effective results (Aldrich *et al.* 1991). Mizell and Tedders (1995) found that the trap did not efficiently monitor the other true bug genera in a study area; although presence-absence data were garnered from the visual attraction of the yellow-coloured trap. Virtually no other biologically based strategies and tactics of practical use are available for the suppression of stink bugs in small-farm, organic, or homeowner production (Mizell & Tedders 1995). Since the introduction of this trap, proprietary traps have entered the market for the domestic control of stink bugs in the USA. The RESCUE!® trap (Making Pest Control Smarter, USA) was introduced as a possible means to control stink bugs in a lawn-and-garden-based environment. No trap has been developed specifically for use against *C. nervosa*.

1.4.6.3 Trap cropping

Tillman (2006) reported that in the southern USA, sorghum acts as an effective trap crop for *N. viridula* when cultivated with cotton. White mustard (*Sinapis alba*) and peas (*Pisum sativum*) were evaluated and recommended as trap crops for *N. viridula* to reduce damage to maize (Rae *et al.* 2002). Shuster (2004) found that a trap crop of squash cultivated adjacent to tomatoes reduced the occurrence of whitefly-vectored tomato yellow leaf curl. The early spring planting of triticale (× *Triticosecale*), crimson clover (*Trifolium incarnatum*), and buckwheat (*Fagopyrum esculentum*) as trap crops within the vicinity of cash crops minimise damage to early-season cash crops (Mizell *et al.* 2008). For the summer to fall period, sunflower (*Helianthus* spp.), buckwheat (*F. esculentum*), and sorghum (*Sorghum* spp.) are recommended trap crops due to the tendency of these crops to draw stink bugs away from cash crops (Mizell *et al.* 2008). Okra (*Abelmoschus esculentus*) and field peas (*Pisum sativum*) were found to augment the ability of other summer trap crops to draw away stink bugs, and they also increased the population of beneficial insects in the trap crop. This was found to increase the number of predators acting in and around the cash crop (Mizell *et al.* 2008).

1.4.6.4 Sterile insect technique

The principle of an SIT is that the number of sterile males released must be great enough to prevent reproduction (Knight & Gurr 2007). The higher the initial ratio of sterilised individuals introduced to the population, the more rapidly the fertility of the population as a whole will decline (Pedigo 1992). There is no known SIT programme underway for *C. nervosa*. Exposing fourth-instar *N. viridula* nymphs to 10 Gγ of ionising radiation rendered them partially sterile (Dyby & Sailer 1999). Irradiated female *N. viridula* laid mostly non-viable eggs with a significantly lower level of fecundity. The surviving *N. viridula* progeny of the partially sterilised females were, however, capable of reproductive recovery (Dyby & Sailer 1999). The cost of rearing sufficient numbers of *N. viridula* to adulthood is prohibitive and the idea of an SIT programme is unlikely to garner much traction until less expensive methods of rearing are developed (Knight & Gurr 2007).

1.4.6.5 Biological control

No biological control methods are currently practised specifically for *C. nervosa*. Haddad and Louw (2006) suggested that *Trissolcus basalis* (Hymenoptera: Scelionidae) might be an effective biological control component of an IPM programme for *Atelocera raptoria*. Many methods of biological control for *N. viridula* have been developed (Knight & Gurr 2007). The egg parasitoids *Trissolcus basalis* and *Trichopoda giacomellii* are known to parasitise the eggs of *N. viridula* (Waterhouse 1998). Subsequently, as a means of control, *T. basalis* was released in the western states of Australia. It is not possible to accurately assess the impact of *T. basalis* on the *N. viridula* population (Knight & Gurr 2007). In New South Wales, *N. viridula* is still regarded as a major pest of soybeans (Knight & Gurr 2007). As such, the effectiveness of biological control on *N. viridula*, especially in Australia, cannot be considered completely effective (Knight & Gurr 2007). Gurr and Wratten (1999) considered the conservation of natural predators an important factor in biological control. The adoption of within-field monocultures, combined with an increased reliance on pesticide usage, has the effect of lowering the overall predator balance within an agroecosystem (Knight & Gurr 2007). The use of broad-spectrum pesticides for the control of *N. viridula* on soybeans had the effect of lowering the overall population of predators and parasitoids within a field, and, in the process, this increased the reliance on pesticides (Knight & Gurr 2007). Also, the practice of removing surrounding vegetation not only limited available habitats for parasitoids, but also limited their food sources (Knight & Gurr 2007). It is thus preferable to incorporate trap-cropping models with biological control to ensure sufficient habitat and food for predator and parasitoid species. Trap cropping also has the theoretical benefits of making a cash crop more difficult for *N. viridula* to locate and to attract predator and parasitoid populations (Knight & Gurr 2007).

1.4.6.6 Chemical control

Stink bugs are naturally resistant to many pesticides; therefore efficacious insecticide products for the management of stink bug pests are rare (Mizell *et al.* 2008). Payne and Wells (1984) found that the incidence of kernel spot – dark lesions on the kernel of pecan nuts that were not treated – was 1.8 %, whereas trees that did not receive treatment had a kernel spot incidence of 5.8 %. Chess® (Pymetrozine: Syngenta SA (Pty) Ltd, South Africa) is a systemic insecticide registered for the control of *C. nervosa* and similar pests on avocado. The application of Bulldock (Beta-Cyfluthrin: Bayer CropScience; South Africa) registered for use on *C. nervosa* was found to reduce the incidence of *C. nervosa* in avocado orchards from a high of 29% to a low of <1 % after a single treatment (Bruwer 2004). The application of Thioflo (Eendosulfan: Bayer, South Africa) was found incapable of reducing *C. nervosa* numbers to acceptable levels with one treatment; instead, three treatments are necessary in order to ensure damage lower than 2% (Bruwer 2004). An organic product, Organo Z (Azadirachtin: Impact Zone Chemicals, South Africa), which functions by means of plant extracts, was capable of reducing *C. nervosa* damage to below 1% with two applications (Bruwer 2004).

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CHAPTER 2

SHUTTLING OF FALSE CODLING MOTHS BETWEEN CITRUS AND PECAN ORCHARDS

2.1 Introduction

False codling moths (FCM) (*Thaumatotibia leucotreta*) are known as an equatorial pest of cotton in Africa (Newton 1998) and citrus in Southern Africa, particularly in the southern areas (Catling & Aschenborn 1974). Twenty-one cultivated plants and 14 indigenous plants were identified as hosts for FCM in South Africa (Schwartz 1981). This polyphagous habit of FCM makes the accurate discernment of host specificity problematic and, as such, FCM can be expected to function as a pest of opportunity that will infest cultivated or wild host plants as opportunity or necessity requires. The economic implications of FCM infestation can be considerable. The local market for citrus started to shrink during the period 2013–2015, whilst exports have remained constant (Citrus Growers Association 2016). Revenues for citrus exports have increased significantly during the same period (Citrus Growers Association 2016). False codling moths have not been reported to represent an economic threat to the pecan industry by means of direct damage to crops; however, due to the phytosanitary status of the moth, export markets could be placed in jeopardy if shipments are found to contain FCM. Strict phytosanitary measures are therefore in place to prevent transference of the moth; this in turn requires tighter control measures by producers (DAFF 2010). Knowledge of potential shuttling behaviour between adjacent orchards can therefore be of great importance to future export competitiveness.

The high degree of similarity encountered between the larvae of FCM and carob moth (CM) (*Ectomyelois ceratoniae*) makes it difficult to differentiate the immature stages of these moths from each other (Rentel *et al.* 2011). There is thus a concern that these two lepidopteran species can be mistaken for the other. This confusion can lead to phytosanitary complications for products that have their primary markets overseas. The close proximity of citrus and pecan cultivation that is encountered in the Vaalharts region in the Northern Cape and North West provinces of South Africa leads to a concern that shuttling of the two lepidopteran species might cause increased infestation in potential export crops. The original protocol for the study indicated that snap surveys conducted showed both FCM and CM present in the Vaalharts region and that both species were actively infesting pecan nuts (Moore 2010).

One of the more efficacious methods of control that remains an important foundation for FCM management is the cultural practice of removing fallen fruit from orchards (Moore & Hattingh 2012). The removal of visibly damaged fruit from trees should also be considered in order to deny as many potential oviposition sites as possible for lepidopteran pests (Hashemi-Fesharaki *et al.* 2011). However, even with stringent orchard sanitation, some FCM larvae will have the opportunity to escape from the dropped fruit and pupate in the soil. Schwartz (1981) indicated that weekly orchard sanitation in navel orange orchards brought fruit loss down from 7.6% in non-sanitised orchards to 5.9% and 6.3% in sanitised orchards.

With all these risks and limitations under consideration, the following had to be addressed:

- The lepidopteran borers which infect citrus fruit and pecan nuts in the Vaalharts region had to be identified.
- How the borer species interact to form a borer complex between the citrus fruit and pecan nuts needed to be investigated. This was to be accomplished by ascertaining if any shuttling occurs between adjacent orchards.
- Seasonal fluctuations that cause variation in the FCM population also had to be investigated.

2.2 Materials and methods

The study area is located in the Vaalharts region of the Northern Cape province in South Africa, at the approximate geographical coordinates of S27°71'700", E24°78'020". The seven study sites, located on five individual farms, are scattered around this point. Collection of citrus fruit and pecan nuts started in November 2011 and concluded in July 2014. This allowed three growing seasons to be monitored for each of the crops under investigation.

Two separate types of collections were performed during the course of the study. Figure 2.10 to Figure 2.14 show experimental sites where 50 pecan nuts (25 from the ground and 25 from the trees) as well as 25 citrus fruit (12/13 from the ground and 12/13 from the trees) were collected at each transect. In each successive month, one extra fruit was collected from either the ground or the trees in order to obtain the required amount of material and prevent bias from occurring between the two collection sites. With two transects located at each of the study sites

(10 m from the edge and 50 m from the edge), a total of 100 pecan nuts and 50 citrus fruit were collected per month per site. At various stages during the study, it was not possible to collect the required material; either due to orchard sanitation having been conducted by the farmer or because no material was available for collection.

At the two control sites (Figures 2.8 and 2.9), double the amount of material was collected during each sampling. At the pecan nut control site, 100 pecan nuts (50 from the ground and 50 from the trees) were collected within a specific area close to the middle of the control orchard. At the citrus control site, 50 citrus fruit (25 from the ground and 25 from the trees) were collected within a specific area closer to the edge of the orchard. Similar to the experimental sites, it was not possible to collect the required material at various stages during the study for the reasons previously stated. The collected study material was transported to the University of the Free State (UFS) where the samples were incubated within a temperature-controlled room. The pecan nuts were cracked (Figure 2.1) and placed in emergence boxes (Figure 2.2) that incubated all the pecan nuts from a specific transect for a period of two months at 25 °C with a natural light cycle. Collected citrus fruit (Figure 2.3) were dissected in order to locate candidate larvae (Figure 2.4) and said larvae were removed from the fruit to be incubated on a pre-prepared growth medium (Figure 2.5) that was supplied by Citrus Research International (CRI). The medium proved sufficient in rearing larval FCM to adulthood if the vials were monitored for fungal encroachment. In most cases, larval incursion occurred at the navel end of the fruit body (Figure 2.4). The larvae collected from citrus fruit were incubated under the same conditions as the pecan nuts. After a two-month period, any eclosed moths were removed and identified. Dr M. Krüger of the Ditsong National Museum of Natural History performed confirmation of the specimens.

Figure 2.1: Lepidopteran larvae and pupae within a cracked pecan nut.

Figure 2.2: An emergence box wherein pecan nuts were placed and moths were allowed to eclose.

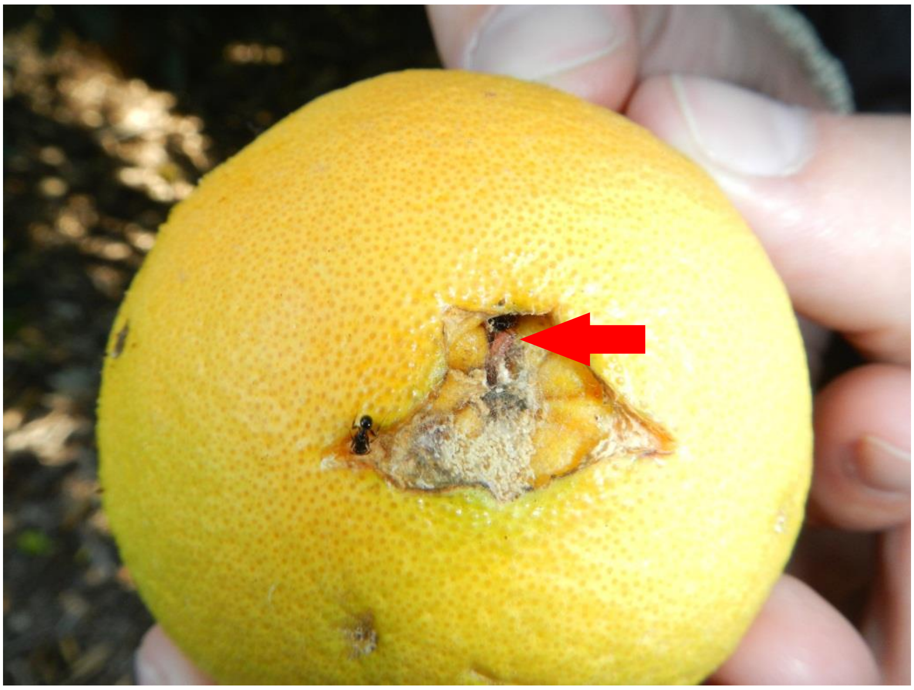

Figure 2.3: Citrus fruit with a lepidopteran borer larva visible on the surface of the fruit.

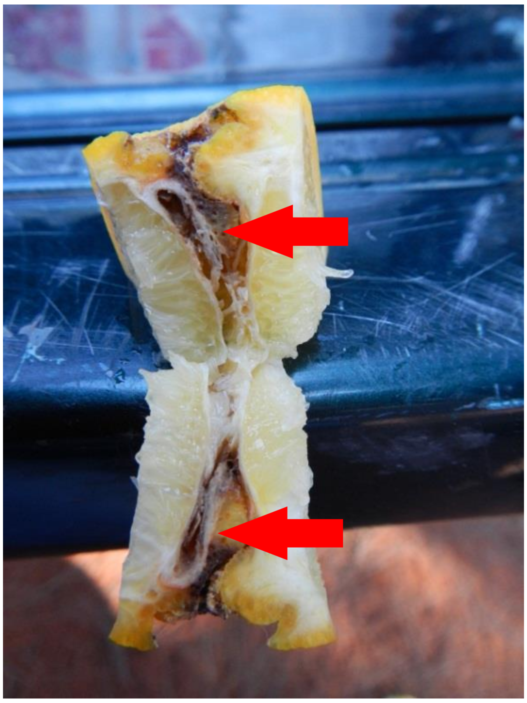

Figure 2.4: Feeding canals and faecal matter were evident throughout the fruit.

Figure 2.5: Glass vials filled with the prepared growth medium wherein larvae collected from citrus fruit were allowed to mature, pupate, and eclose. Moths that emerged were found within the cotton wool.

An extension to the original study was incorporated with the deployment of Lorelei® pheromone lure traps in January 2013. Yellow delta traps from both Chempac and Insect Science™ were deployed (see Figure 2.6). The Lorelei® pheromone ampules were provided by CRI and replaced every six months as required. Sticky pads from both Chempac and Insect Science™ were used as available (see Figure 2.7). Sticky liners were replaced approximately every two months. As a rule, the FCM pheromone trap was placed as equidistant as possible between the 10 m and 50 m transects in order to maximise the capture area over the two transects. The later addition of CM pheromone traps necessitated their placement between 20 m and 40 m from the FCM pheromone traps in order to minimise instances of cross-trapping. A description of each of the study sites follows.

Figure 2.6: Yellow delta trap suspended in a pecan tree (*Carya illinoinensis*); traps were generally placed where they were both easy to spot at a distance and easy to reach without the use of a ladder.

Figure 2.7: A sticky liner placed within the yellow delta traps.

The sites for the lepidopteran borer study were selected based on the proximity of the two test orchards to each other and the willingness of the affected farmer to participate in the study.

In all situations, sites were selected where the distances between pecan and citrus orchards were minimal.

The orchard selected for the citrus control (Figure 2.8) was surrounded by Beefwood (*Casuarina* sp.) windbreaks with citrus orchards on all other sides. The closest pecan orchard was 750 m away from the control site. The two roughly parallel lines indicate the region where the sample material was collected for the control. The area selected for collecting material was purposely small and toward the edge of this orchard to mitigate the effect of Lorelei® pheromone lures placed closer to the centre of the orchard. A high standard of orchard sanitation was maintained throughout the season on this farm, including integrated pest management (IPM) measures to combat both FCM and CM.

Note that all aerial photographs were retrieved from Google Maps. In all photographs, FCM and CM captions indicate the approximate location of the pheromone traps.

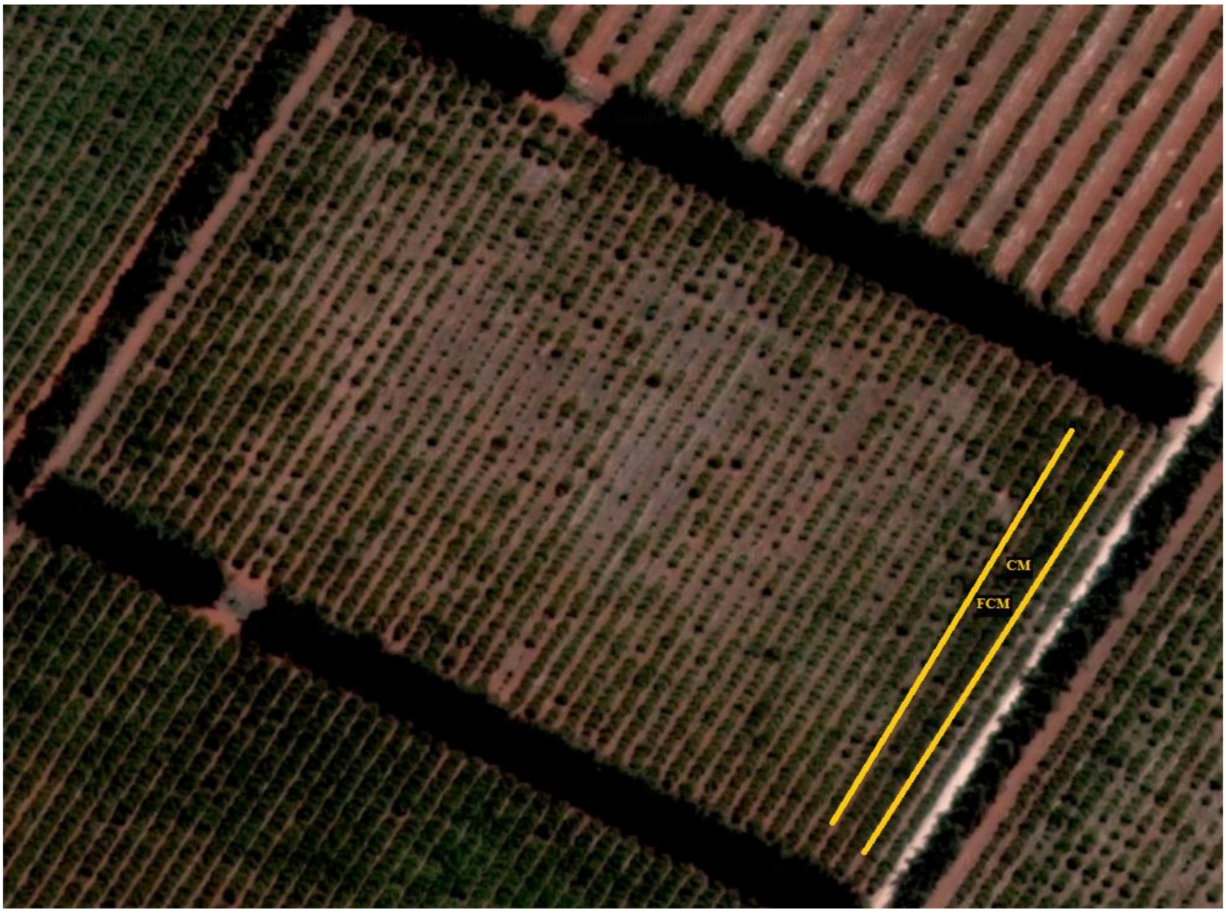

Figure 2.8: Citrus control study site (located at S27°99'785", E24°75'174").

The orchard used for the pecan control site (Figure 2.9) had no Beefwood windbreaks and was surrounded by more pecan orchards and cereal (and later cotton) irrigation. The closest citrus orchard was approximately 1 500 m from the control site. Orchard sanitation occurred twice per season after both harvests. No IPM measures were employed within the orchard. Similar to the citrus control site, sampling occurred between the two indicated green lines with the pheromone traps located within this area.

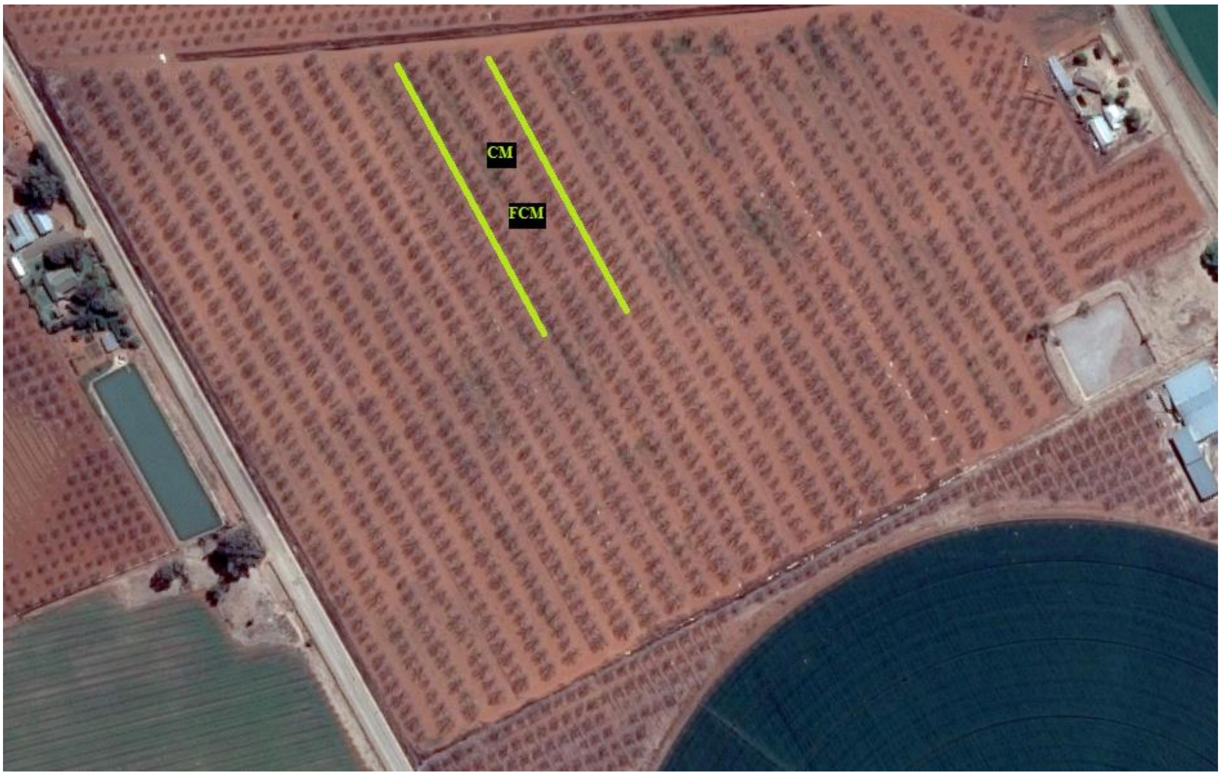

Figure 2.9: Pecan control study site (located at S27°64'218", E24°74'293").

The citrus and pecan orchards at the Gillyfrost study site (Figure 2.10) were separated by a Beefwood windbreak. The distance between the orchards was less than 30 m. The pecan orchard was bordered by a cereal/cotton field. The pecan trees in this orchard were widely spaced, in contrast to the tightly spaced and much more intensively cultivated citrus orchard. Orchard sanitation for the citrus occurred throughout the season. Orchard sanitation for the pecan nuts was less intensive, only being noted once every season.

Figure 2.10: Gillyfrost study site (located at S27°64'432", E24°77'325").

The pecan and citrus orchards at the Ouplaas study site (Figure 2.11) were separated by a single-lane dirt road. No windbreak was present around either of the orchards. Orchard sanitation was thorough but only noted once per season for both orchards.

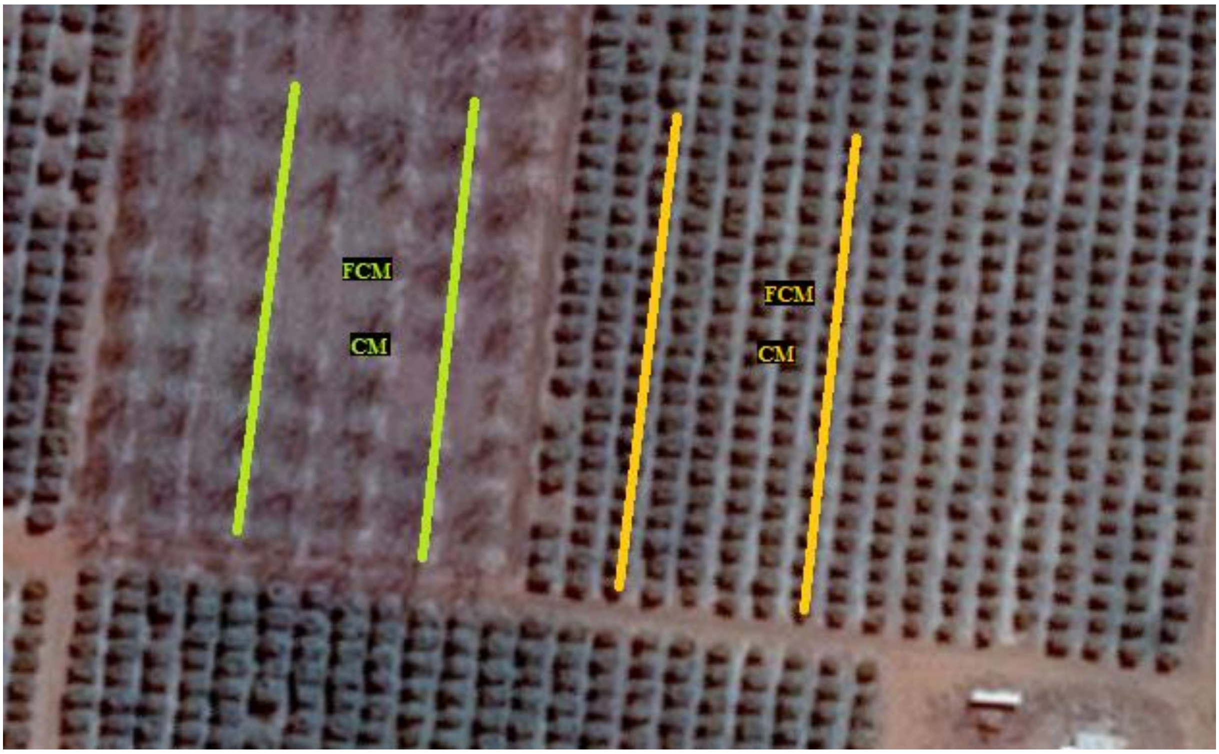

Figure 2.11: Ouplaas study site (located at S27°66'702", E24°74'226").

The orchards at the Floors Farm study site (Figure 2.12) were separated by a Beefwood windbreak which was destroyed on the owners' initiative between and during the samplings of October 2013 and December 2013. The low density of the trees within the pecan nut orchard necessitated a longer transect in order to obtain the required materials without biasing the collection of pecan nuts from too few trees. Orchard sanitation was observed to occur twice per season for both orchards.

Figure 2.12: Floors Farm study site (located at S27°65'078", E24°75'950").

A Beefwood windbreak and a single-lane dirt road separated the orchards at Saamfarm A (Figure 2.13). During the harvest season, tarpaulin sails were erected in order to further prevent chilling and fruit loss. Orchard sanitation in the pecan orchards was observed once per season. Orchard sanitation in the citrus orchards occurred throughout the season. During the growing period in late 2011, successive hailstorms caused significant nut drop in the pecan orchard. At the farmer’s request, nuts were not collected from the trees in the pecan orchard for the 2012 season; however, nut collection from the ground proceeded as normal.

Figure 2.13: Saamfarm A study site (located at S28°00'216", E24°75'846").

A Beefwood windbreak and a double-lane dirt road, resulting in a distance of approximately 30 m between the two orchards, separated the orchards of Saamfarm C (Figure 2.14). Orchard sanitation within the citrus orchard occurred throughout the season. Orchard sanitation within the pecan orchard was never observed. In addition, significant foliage was allowed to grow between the trees in the pecan orchard, which obstructed nut gathering and allowed local wildlife to consume a large proportion of the dropped nuts.

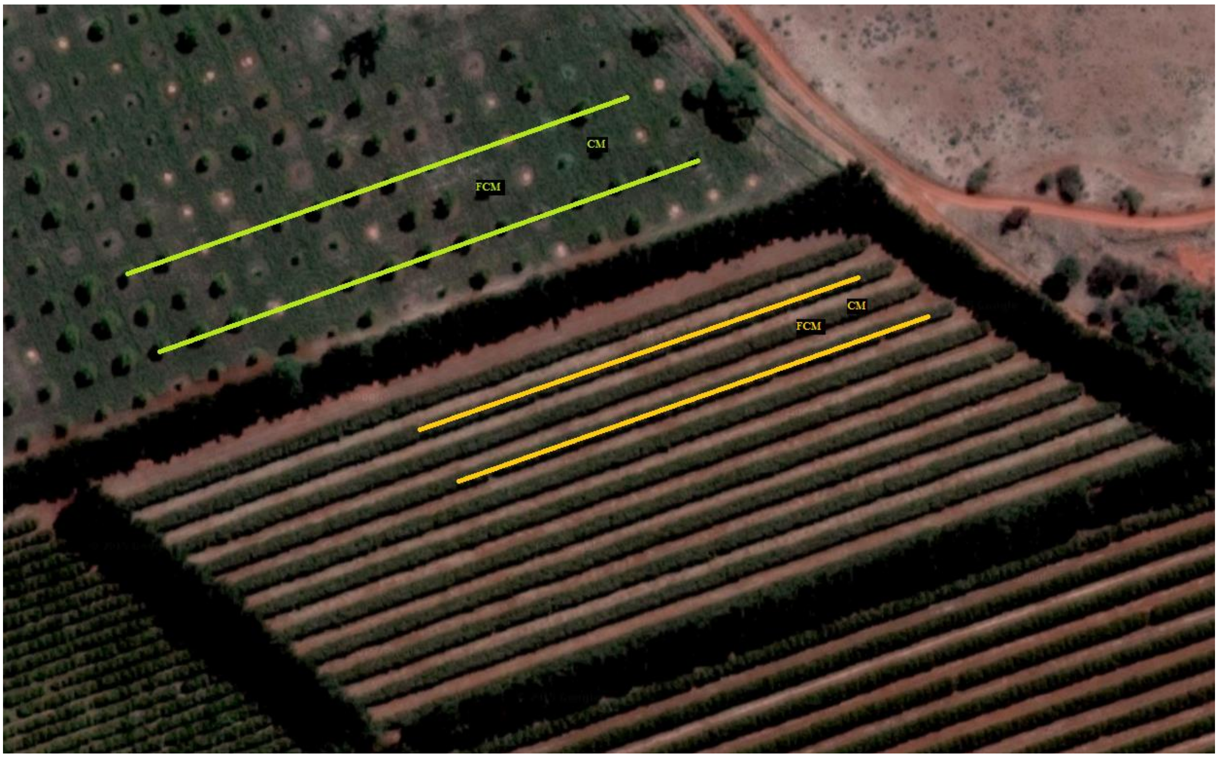

Figure 2.14: Saamfarm C study site (located at S27°99'640", E24°73'903").

The statistical analysis was conducted using the 2009 version of Statistical Analysis Software (SAS). To ascertain whether FCM populations overlapped between pecan and citrus orchards, Spearman correlation coefficients were calculated between the counts of CM (in both the pecan and citrus orchard) and the counts of FCM (in both the pecan and citrus orchards). Spearman correlation coefficients were calculated in preference to conventional Pearson correlation coefficients because the Spearman correlation coefficient is more robust to outliers. This correlation was calculated for the pheromone data.

In order to assess potential shuttling between orchards, Spearman correlation coefficients were calculated between the moth counts for the pecan and citrus orchards. A positive correlation between populations might indicate that there is a link between the FCM and CM populations in the two orchards. Furthermore, a Poisson regression (using the GENMOD Procedure) was conducted on the pheromone data for FCM. The counts of FCM in the pecan nut orchard (dependent variable) were regressed against the counts of FCM in the citrus orchard, as well as the total number of citrus fruit and pecan nuts collected in the citrus and pecan nut orchards respectively (independent variables). A positive regression slope for the independent variable "Citrus" (namely the count of FCM in the citrus orchard) would indicate that the count in the citrus orchard is a reliable method of predicting the count in the pecan nut orchard. In the same Poisson regression model, if a negative slope for the independent variable "Total Citrus" (namely total count of citrus fruit collected) is found, lower counts of FCM in the pecan nut orchard are to be expected for high counts of citrus fruit. This in turn would suggest that high numbers of citrus fruit attract moths from the adjacent pecan nut orchard.

2.3 Results

Adult FCM were collected at all study sites; the samples of the first moths were sent to Dr M. Krüger, and confirmed as *Thaumatotibia leucotreta*.

The total number of FCM recovered from the collected material was 51 individuals from 2 942 citrus fruits. In addition, the total number of FCM recovered from collected pecan nuts was 47 individuals from 10 325 pecan nuts. This provides an overall infestation percentage for the study area of 0.01% for citrus fruit and 0.004% for the pecan nuts.

2.3.1 Combined captures

The following graphs indicate the combined pheromone trap capture and collected material results for the different study sites. In all graphs, the pheromone trap captures of FCM are shown as bar graphs, while eclosion results (FCM obtained from collected material) are shown as line graphs. All values are on a monthly scale for the duration of the study.

Figure 2.15 shows the combined pheromone trap captures and collected material results for the Gillyfrost study site. Pheromone trap captures indicated a year-round presence of adult FCM at this study site, with only sporadic infestation of citrus fruit and pecan nuts detected.

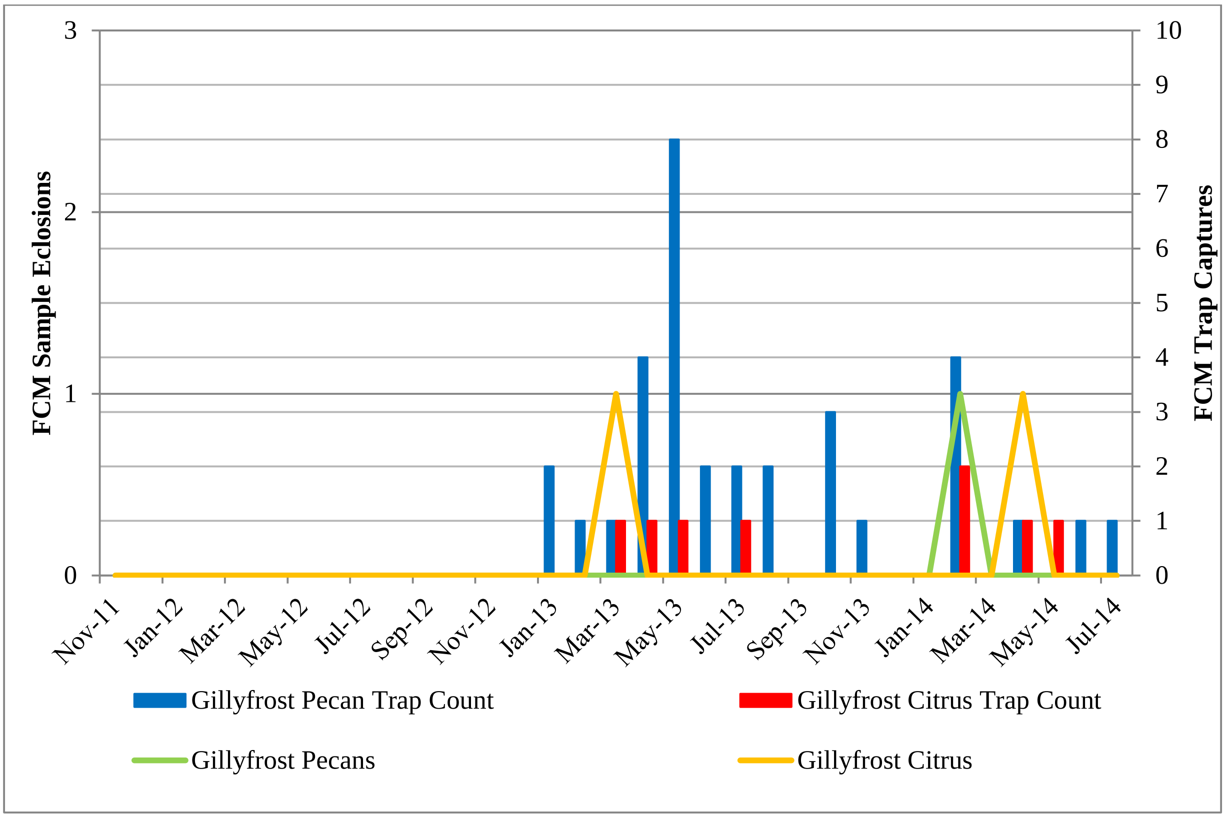

Figure 2.15: Results from the Gillyfrost study site.

Figure 2.16 indicates the combined pheromone trap captures and collected material results for the Floors Farm study site. Pheromone trap captures indicated a year-round presence of adult FCM at this study site, with substantial infestation of the citrus fruit and pecan nuts.

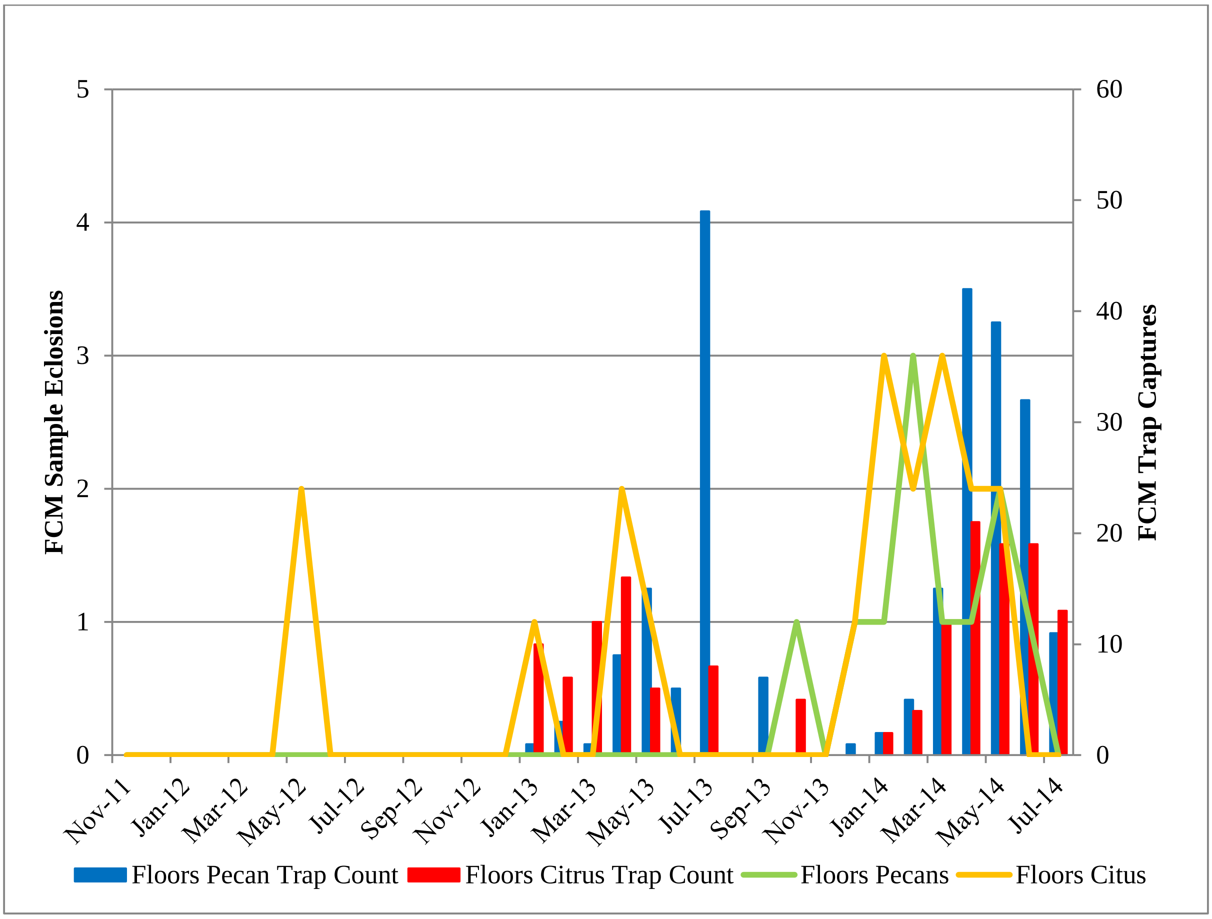

Figure 2.16: Results from the Floors Farm study site.

Figure 2.17 shows the combined pheromone trap captures and collected material results for the Ouplaas study site. Pheromone trap captures indicated a year-round presence of adult FCM at this study site, with substantial infestation of both citrus fruit and pecan nuts.

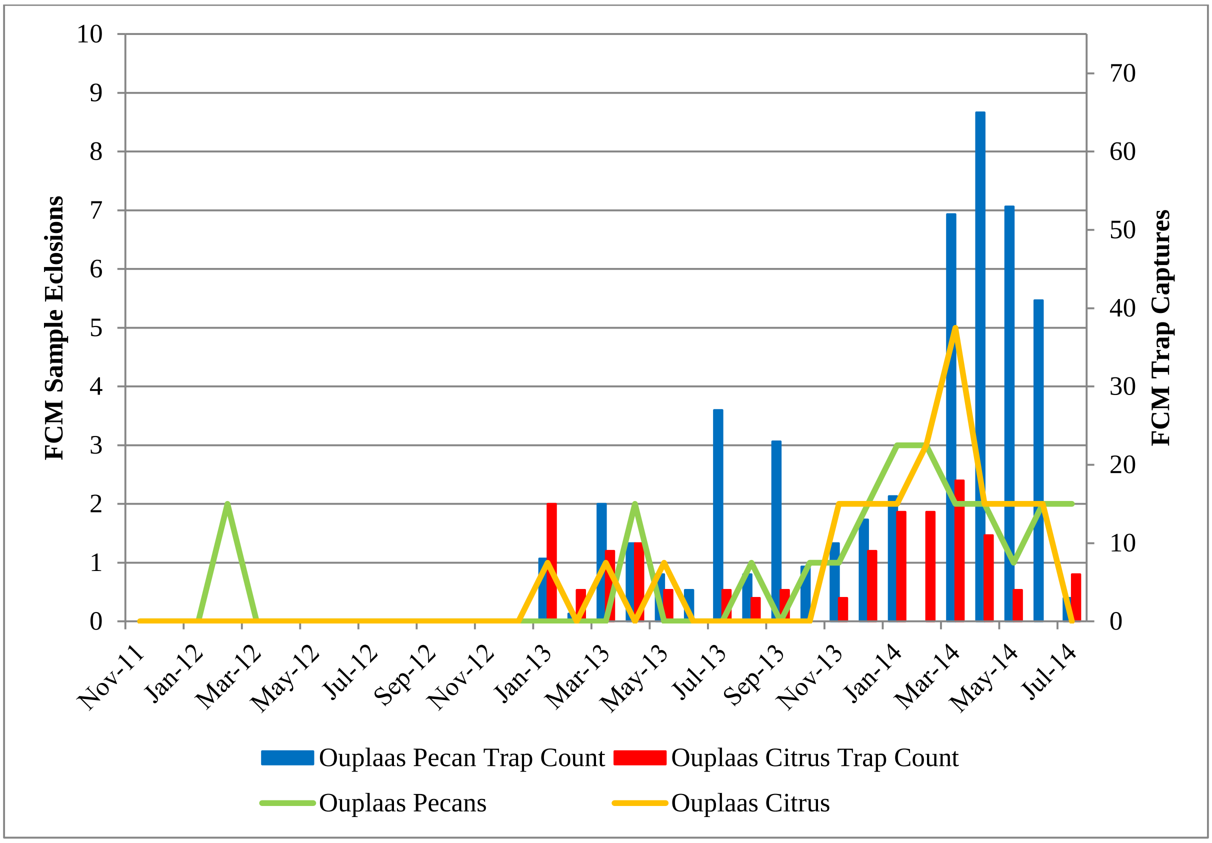

Figure 2.17: Results from the Ouplaas study site.

Figure 2.18 presents the combined pheromone trap captures and collected material results for the Saamfarm A study site. Both pheromone trap captures and FCM reared from collected material indicated that FCM only had a marginal presence at this site.

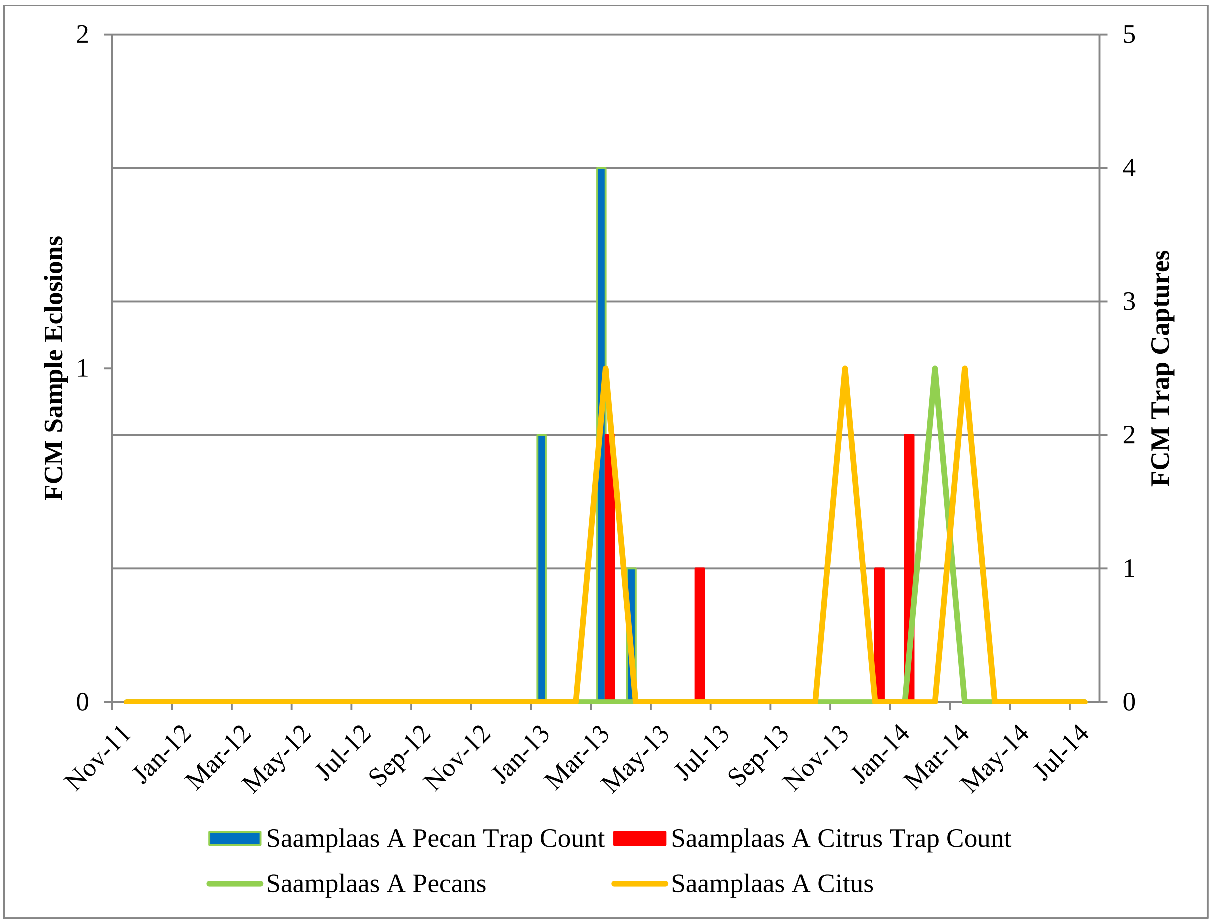

Figure 2.18: Results from the Saamfarm A study site.

Figure 2.19 shows the combined pheromone trap captures and collected material results for the Saamfarm C study site. Similar to the Saamfarm A study site (Figure 2.18), FCM only had a marginal presence at this site.

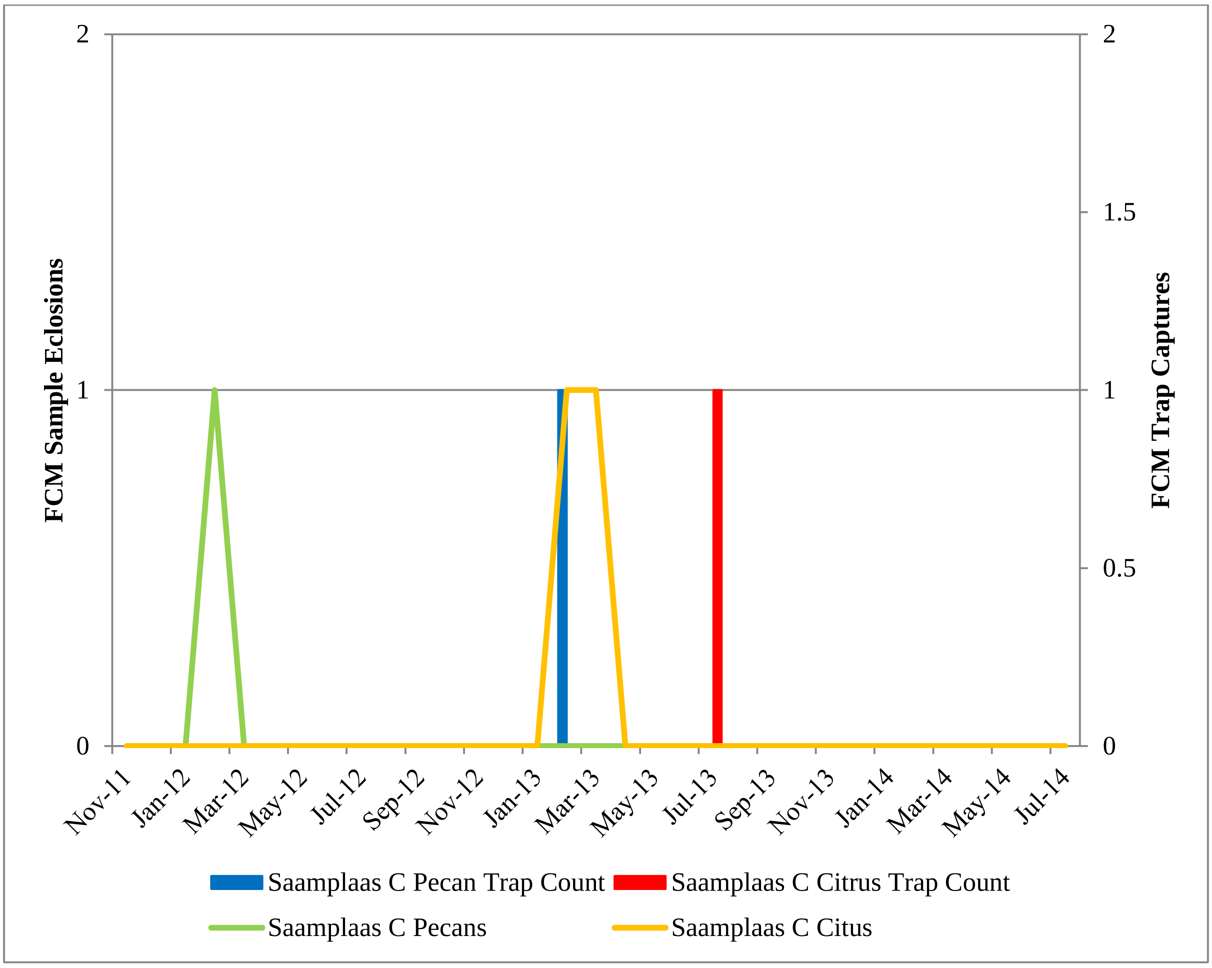

Figure 2.19: Results from the Saamfarm C study site.

Figure 2.20 presents the combined pheromone trap capture and collected material results for both the control study sites. The FCM infestation was more severe at the pecan control site. This is attributable to the lack of any IPM measures at the pecan control site.

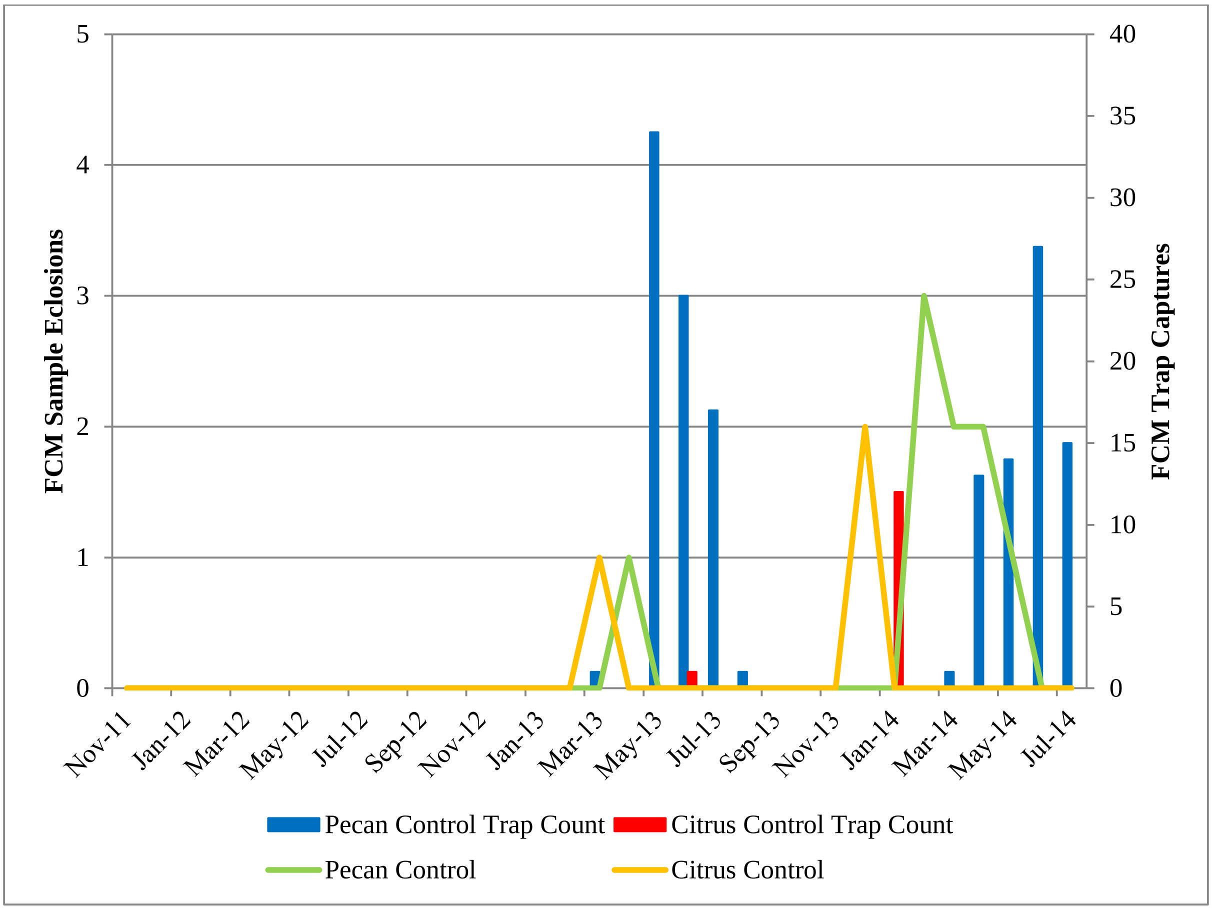

Figure 2.20: Results from the control study sites.

2.3.2 Seasonal fluctuation

Figure 2.21 shows the combined pheromone trap captures, which indicates that FCM are active throughout the year and remain active during the winter months, with male flight activity decreasing during months when potential hosts for oviposition are in short supply.

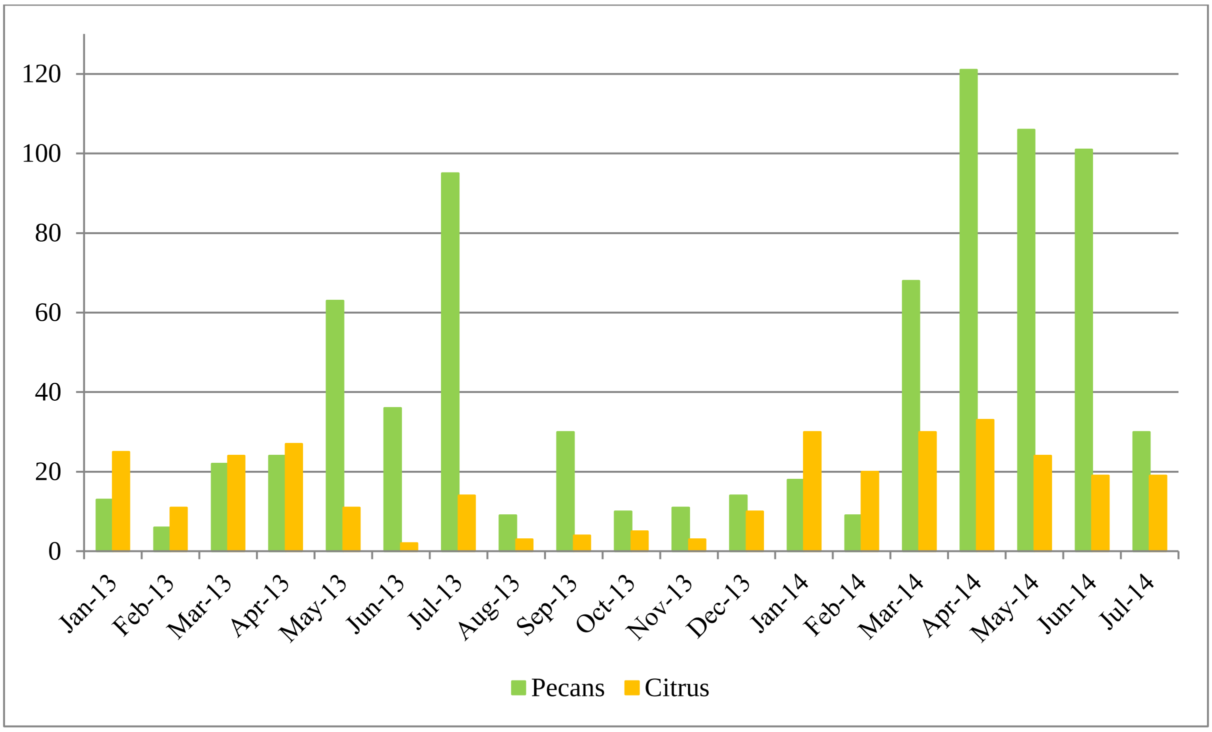

Figure 2.21: Overview of FCM pheromone trap captures over the course of the study from all the sites, indicating year-round adult activity.

The correlation results that follow only include sites that were found to have a significant indication of overlap between adjacent orchards.

2.3.3 Statistical analysis

The significant ($p < 0.05$) correlation between the pheromone trap captures of FCM in both orchards and CM in pecan orchards indicates that there was possible overlap of these two populations, as can be seen in Table 2.1. The positive significant positive correlation (in bold) indicates that the two populations overlapped.

Table 2.1: Spearman correlation coefficients from pheromone trap captures for the Floors Farm study site.

N = 15	FCM Pecan	FCM Citrus
CM Pecan	0.59051 0.0205	0.71905 **0.0025**
CM Citrus	0.11459 0.6843	0.22022 0.4303

First value indicates Spearman correlation coefficient; second number indicates the *p*-value.

The significant ($p < 0.05$) correlation between the pheromone trap captures of FCM in citrus orchards and CM in pecan orchards indicates that there was possible overlap of these two populations, as can be seen in Table 2.2. The positive significant positive correlation (in bold) indicates that the two populations overlapped.

Table 2.2: Spearman correlation coefficients from pheromone trap captures for the Ouplaas study site.

N = 15	FCM Pecan	FCM Citrus
CM Pecan	0.27260 0.3256	0.57568 **0.0247**
CM Citrus	-0.24580 0.3772	0.42032 0.1188

First value indicates Spearman correlation coefficient; second number indicates the *p*-value.

The significant ($p < 0.05$) positive correlation between moth populations reared from collected citrus fruit and pecan nuts of the Floors Farm study site indicates that there was possible shuttling of FCM between the adjacent orchards (see Table 2.3). The analysis was conducted for FCM only, between adjacent orchards. This indicates that there is a statistical relationship between the FCM populations in adjacent orchards. The significant result is indicated in bold.

Table 2.3: Spearman correlation coefficients of FCM reared from collected citrus fruit and pecan nuts for the Floors Farm study site.

N = 33	Pecan	Citrus
Pecan	1.000	0.60796 **0.0002**
Citrus	0.60796 **0.0002**	1.000

First value indicates Spearman correlation coefficient; second number indicates the *p*-value.

The significant ($p < 0.05$) positive correlation between moth populations collected from pheromone traps in the pecan and citrus orchards of the Floors Farm study site further reinforces that there was possible shuttling of FCM between the adjacent orchards (see Table 2.4). The analysis was conducted for FCM only, between adjacent orchards. This indicates that there is a statistical relationship between the FCM populations in adjacent orchards. The significant result is indicated in bold.

Table 2.4: Spearman correlation coefficients from the pheromone trap data for the Floors Farm study site.

N = 19	**Pecan**	**Citrus**
Pecan	1.000	0.64074 **0.0031**
Citrus	0.64074 **0.0031**	1.000

First value indicates Spearman correlation coefficient; second number indicates the *p*-value.

The significant ($p < 0.05$) positive correlation between FCM populations in the pecan and citrus orchards of the Ouplaas study site indicates that there was possible shuttling of FCM between the adjacent orchards (see Table 2.5). The analysis was conducted for FCM only, between adjacent orchards. This indicates that there is a statistical relationship between the FCM populations in adjacent orchards. The significant result is indicated in bold.

Table 2.5: Spearman correlation coefficients for FCM reared from collected citrus fruits and pecan nuts from the Ouplaas study site.

N = 33	**Pecan**	**Citrus**
Pecan	1.000	0.60426 **0.0002**
Citrus	0.60426 **0.0002**	1.000

First value indicates Spearman correlation coefficient; second number indicates the *p*-value.

The Poisson regression summarised in Table 2.6 suggests that “Citrus”, acting as the independent variable, has a significant ($p < 0.05$) predictive value for FCM counts in the adjacent pecan orchard. The significant ($p < 0.05$) negative regression slope for “Total Citrus” (namely total count of citrus fruits collected) suggests that the more citrus collected, the lower the count of FCM in the pecan orchard. The pecan orchard captures are the dependent variable in this regression.

Table 2.6: Poisson regression of the FCM pheromone captures for the Floors Farm study site.

Poisson Regression for Floors Farm Study Site (Citrus as Independent)							
Parameter	**DF**	**Estimate**	**Standard Error**	**Wald 95% Confidence Limits**		**Wald Chi-Square**	**Pr > ChiSq**
Intercept	1	-5.1653	4.9609	-14.8884	4.5575	1.08	0.2978
Citrus	1	0.1491	0.0324	0.0852	0.2129	20.95	< 0.0001
Total Citrus	1	-0.042	0.0141	-0.0696	-0.0144	8.9	0.0029
Total Pecans	1	0.0751	0.05	-0.023	0.1731	2.25	0.1335
Scale	0	2.4736	0	2.4736	2.4736		

The Poisson regression summarised in Table 2.7 suggests that that the “Pecans” (namely the count of FCM in the pecan orchard), acting as the independent variable, has a significant ($p < 0.05$) predictive value for FCM counts in the adjacent citrus orchard. The citrus orchard captures are the dependent variable in this regression.

Table 2.7: Poisson regression of the FCM pheromone captures for the Floors Farm study site.

Poisson Regression for Floors Farm Study Site (Pecan as Independent)							
Parameter	**DF**	**Estimate**	**Standard Error**	**Wald 95% Confidence Limits**		**Wald Chi-Square**	**Pr > ChiSq**
Intercept	1	0.6349	1.7318	-2.7593	4.0291	0.13	0.7139
Pecan	1	0.0298	0.0088	0.0125	0.0472	11.4	0.0007
Total Citrus	1	0.0352	0.0124	0.0108	0.0596	8.02	0.0046
Total Pecans	1	-0.0027	0.0202	-0.0422	0.0368	0.02	0.8941
Scale	0	1.819	0	0.819	0.819		

2.4 Discussion

2.4.1 Species overlap

The positive correlations summarized in Table 2.1 and Table 2.2 indicate that there is a significant ($p < 0.05$) chance for the overlap of FCM and CM populations in adjacent orchards. As the test orchards were adjacent to each other, the likelihood that the populations will overlap is almost certain. The polyphagous habits of FCM, as reported by the European and Mediterranean Plant Protection Organization (EPPO) (2013), along with the eclosion results, suggest that FCM can exploit both citrus fruit and pecan nuts for reproduction. This outcome indicating the polyphagous nature of the moths was also reported in Catling and Aschenborn's (1974) study on FCM and Gothilf's (1964) study on CM. Both species were reported as citrus pests. False codling moths were found to infest macadamia nuts (EPPO 2013; Timm 2005; La Croix & Thindwa 1986). This tendency for overlap is a concern for potential complications during phytosanitary inspections, as the larvae of FCM and CM are known to be difficult to distinguish (Rentel *et al.* 2011; Wysoki 1984). These similar host preferences, along with the Spearman correlations, indicate that if both FCM and CM are present in an area, overlap of the populations can be expected.

2.4.2 Shuttling

The significant ($p < 0.05$) positive correlation indicated in Table 2.3 was obtained from the analysis of the eclosion data for the Floors Farm study site, and indicates that shuttling is possibly taking place between the pecan and citrus orchards. The positive correlation indicated in Table 2.4 was obtained by analysing the pheromone trap captures for the Floors Farm study site. The significance ($p < 0.05$) of this data further reinforces the eclosion data, as the shuttling of FCM was detected both from eclosion data and pheromone trap data. The significant ($p < 0.05$) positive correlation shown in Table 2.5 was obtained from analysis of the eclosion data for the Ouplaas study site, and indicated that possible shuttling is taking place between the pecan and citrus orchards. With FCM being able to infest both citrus fruit (Catling & Aschenborn 1974) and various nuts (EPPO 2013; Timm 2005; La Croix & Thindwa 1986), shuttling between adjacent orchards can be expected. Only the Floors Farm and Ouplaas study sites produced significant correlations that are indicative of shuttling between the adjacent orchards. The other study sites – Gillyfrost ($p = 0.8040$), Saamfarm A ($p = 0.7573$), and Saamfarm C ($p = 0.8040$) – all had statistically insignificant results.

2.4.3 Pheromone trap predictability

The direction of the majority of the shuttling is difficult to determine and is most likely linked to whichever orchard provides the most oviposition sites. The Poisson regression shown in Table 2.6 produced a significant ($p < 0.05$) positive regression for the "Citrus" parameter of the FCM pheromone captures at the Floors Farm study site. The pheromone trap captures from the pecan orchard were used as the dependent variable. The significant regression suggests that the number of FCM from the citrus pheromone trap captures will provide a prediction for the number of FCM that can be expected to be captured in the adjacent pecan orchard. Furthermore, the significant (p < 0.05) negative regression slope for “Total Citrus”, as seen in Table 2.6, indicated that the more citrus fruit are collected, the lower the count of moths in the pecan nut orchard, which in turn would suggest that high numbers of citrus fruit attract moths from the adjacent pecan nut orchard. This value provides an indication that there is a tendency for FCM to shuttle from pecan orchards to citrus orchards. The possible reasons for this were not investigated, but citrus is known to be a preferred host of FCM (Newton 1998). The preference of FCM to oviposit on citrus fruit that are very close may encourage shuttling to citrus orchards.

The Poisson regression shown in Table 2.7 produced a significant (p < 0.05) positive regression for the “Pecan” parameter of the FCM pheromone captures at the Floors Farm study site. The pheromone trap captures from the citrus orchard were used as the dependent variable. The significant regression suggests that the number of FCM from the pecan pheromone trap captures will provide a prediction of the number of FCM that should be expected to occur in the adjacent citrus orchard. The value of the regression slope for the pecan independent Poisson regression was very low, indicating a weak (yet still significant) capacity to predict the number of FCM to be captured in the adjacent orchard.

2.4.4 Seasonal fluctuation

Pheromone trap data (see Figure 2.14) indicated that the FCM adults are active all year round. The data indicated that minimal activity occurs during the coldest months of the year. The most active period for FCM was found to be from January to May, when both potential host crops are susceptible to infestation. Peak flight activity was recorded during the January to May period. This was found to be consistent with the seasonal variation described by Newton (1998). Due to the limited area wherein collections could be done, FCM pheromone traps already in place had CM pheromone traps placed within close proximity. At no point during the study was any cross-trapping between the two traps observed.

2.5 Conclusion

False codling moths were found at all of the study sites, including the control sites. This indicates that FCM are present throughout the Vaalharts region, and capable of infesting both pecan nuts and citrus fruit.

The statistical analysis of the pheromone data indicated that there was significant overlap of the FCM and CM populations across both orchards. Populations of FCM and CM are able to inhabit both pecan and citrus orchards at the same time. Overall CM infestation was very low.

The statistical analysis of the eclosion data showed that there was qualitative evidence of shuttling of FCM between adjacent citrus and pecan orchards. Significant Spearman correlations for the eclosion data were established for the Floors Farm and Ouplaas study sites. The application of Poisson regression modelling on the pheromone data indicated that the number of FCM from the citrus pheromone trap captures will predict the number of FCM captured in the adjacent pecan orchard. The same model run with dependent variables exchanged indicated that pecan pheromone trap captures will predict the number of FCM infestation in the adjacent citrus orchard. The direction of the shuttling could not be directly established, but the Poisson regression modelling of the pheromone data also indicated that the availability of citrus fruit in adjacent orchards affects the number of FCM captures in the adjacent pecan orchard. This seems to suggest that high numbers of citrus fruit attract moths from the adjacent pecan orchard. Seasonal fluctuations of the FCM population in the study sites were as expected for a temperate region. Year-round availability of at least some host plants means that there is always some FCM activity. Peak FCM flight activity was recorded during the January to May period. No cross-trapping of FCM and CM was observed, and the species specificity of the Lorelei® and PheroLure™ pheromone lures was thus found to be accurate during this study.

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CHAPTER 3

SHUTTLING OF CAROB MOTHS BETWEEN CITRUS AND PECAN ORCHARDS

3.1 Introduction

Carob moths (CM) (*Ectomyelois ceratoniae*) are a known pest of many cultivated hosts, including almonds, carob, castor oil bean, citrus, dates, figs, pomegranates, and mesquite. There is also ample evidence to support the polyphagous nature of CM (Hashemi-Fesharaki *et al.* 2011; Mehrnejad 1993; Gothilf 1964). Carob moths have been observed completing generations on pomegranate, then moving to a more suitable host plant where conditions are more amenable for oviposition, such as the grooves and tracks that occur on pistachio nuts, which CM will proceed to infest as well (Mehrnejad 1993). A preceding infestation by the citrus mealybug, *Planococcus citri*, has also been noted as being necessary for grapefruit to be infested by CM. A study by Serghiou (1983) found that where no *P. citri* infestation is present on the surface of the grapefruit, the exuded gum of the fruit is able to kill the first-instar CM larvae before significant damage to the fruit is caused. Carob moth females are also known to show preference for oviposition onto already damaged pomegranate fruit, which increases the survivability of the vulnerable first-instar larvae (Hashemi-Fesharaki *et al.* 2011). Gothilf (1964) observed that CM develops mostly in cracked carob pods; of the pods investigated at harvest, 70% to 90% of all cracked pods were found to be infested by CM. For the control of CM in almonds, Madge (2012) found that the removal of almonds that remain after harvest proved to be effective in reducing the pest population. It was reported that pomegranate orchards with lax sanitation had a higher incidence of CM than comparative orchards which were stringently sanitised (Al-Izzi *et al.* 1985). Mozaffarian *et al.* (2007) concluded that infested pomegranates might not be the only overwintering sites for CM, which suggests that the collection of pomegranates remaining after harvest may not effectively prevent the overwintering of CM.

Madge (2012) found that the large-scale deployments of a selected pheromone solely for the purpose of CM mating disruption could be a viable option. This is done by the dispersal of large quantities of sex pheromone within an area to confuse males. Peyrovi *et al.* (2011) also showed that oil extracts of *Ferula asafoetida* are an efficient CM repellent. The development of a sterile insect technique (SIT) programme for CM and other species was reported to be in the laboratory-testing phase in multiple countires (Simmons *et al.* 2009), while the development of an SIT programme specifically for CM has commenced in Tunisia (Mediouni & Dhouibi 2007).

The limited exposure lifecycle and boring behaviour of CM larvae make the widespread use of chemical insecticides for control ineffective (Kishani Farahani *et al.* 2012). There are chemicals that can act as effective control methods when applied correctly. An effective measure of chemical control over CM can be established with the application of trichlorfon (Organophosphate: Miles, Inc., USA) or fenthion (Organophosphate: Bayer CropScience, South Africa) over a period of 10 days. This combination was determined to be effective against CM (Reuther *et al.* 1989). The combined application of chlorpyriphos (Organophosphate: DowElanco, USA), mecarbam, methomyl (Carbamate: DuPont Agricultural Products, USA), and methidathion (Organophosphate: Ciba-Geigy Corporation, USA) was effective in controlling both citrus mealybug and CM (infestation complex) (Serghiou 1983).

The high degree of similarity in appearance between CM and false codling moth (FCM) (*Thaumatotibia leucotreta*) larvae makes the immature stages of these moths difficult to differentiate from each other (Rentel *et al.* 2011). If the larvae of CM are mistaken for those of FCM, there can be incorrect phytosanitary implications (DAFF 2010). Considering these risks and limitations, the following issues had to be addressed:

- The lepidopteran borers which infest citrus fruit and pecan nuts in the Vaalharts region had to be identified.
- How the borer species interact to form a borer complex between the citrus fruit and pecan nuts needed to be investigated. This was to be accomplished by ascertaining if any shuttling occurs between the two crops grown adjacently.
- Seasonal fluctuations, which cause a variation in the CM populations, also had to be investigated.

3.2 Materials and methods

The study area is located in the Vaalharts region of the Northern Cape province in South Africa, located at the approximate geographical coordinates of S27°71'700", E24°78'020".

The seven study sites, located on five individual farms, are situated around this point. Collection of citrus fruit and pecan nuts started in November 2011 and concluded in July 2014. This allowed for three growing seasons to be monitored for each of the crops under investigation.

As indicated by Figure 2.1 and Figure 2.2 in Chapter 2, the collected pecan nuts were cracked and placed inside emergence boxes. Collected citrus fruit were cut open (see Figure 2.4) and examined for larvae; any larvae found were placed on the same growth medium used for the FCM larvae (see Figure 2.5). The adult moths that eclosed from the collected citrus fruit and pecan nuts were sent to Dr M. Krüger at the Ditsong National Museum of Natural History for identification. The method by which the material for the study was collected was identical to the methods described in Section 2.2 for FCM collection.

The only difference between the investigations for CM shuttling and FCM shuttling was the pheromone trap component. CM pheromone traps were purchased from InsectScience™, specifically the PheroLure™ brand pheromone traps. Starting in April 2013, yellow delta traps from both Chempac and InsectScience™ were deployed at the study sites (see Figure 2.6). The PheroLure™ pheromone bait lures were replaced approximately every six weeks, as directed. It was required that the bait from the PheroLure™ was placed directly upon the sticky pads (see Figure 3.1). Sticky pads from both Chempac and InsectScience™ were used as available (see Figure 2.7). Sticky liners were replaced every two months, or as necessary if significant dust had accumulated. Trapping was done in order to gather more information about the population dynamics of CM, which the collection of pecan nuts and citrus fruit could not directly provide. As a rule, the CM pheromone traps were located as equidistant as possible between the 10 m and 50 m transects in order to maximise the capture area over the two transects. It was necessary to hang the CM pheromone traps between 20 m and 40 m from the FCM pheromone traps in order to minimise the possibility of cross-trapping.

Figure 3.1: Sticky liner placed within the yellow delta traps, with dust build-up already visible in this example. The PheroLure™ pheromone lure is visible in the centre of the sticky trap, affixed directly onto it.

The statistical analysis was conducted using the 2009 version of SAS. Spearman correlation coefficients were used to calculate the overlap of species between the counts of CM and the counts of FCM.

In order to assess potential shuttling between orchards, Spearman correlation coefficients were calculated between the moth counts for the pecan and citrus orchards. A positive correlation between populations might indicate that there is a link between the FCM and CM populations in the two orchards. Poisson regression was also conducted on the pheromone data for CM.

3.3 Results

Adult CM were collected from all the study sites; samples of the first recovered moths were sent to Dr M. Krüger, which were identified as *Ectomyelois ceratoniae*.

The total number of CM recovered from the collected material comprised 94 individuals from 2 942 citrus fruits. In addition, the total number of CM recovered from collected pecan nuts was 169 individuals from 10 325 pecan nuts. This provides an overall infestation percentage for the study area of 0.03% for citrus fruit and 0.01% for the pecan nuts.

3.3.1 Combined captures

The following graphs indicate the combined pheromone trap captures and collected material results for the different study sites. In all the graphs, all values are on a monthly scale for the duration of the study. Pheromone trap captures of CM are shown as bar graphs, while eclosion results (CM obtained from collected material) are shown as line graphs.

Figure 3.2 shows the results of the Gillyfrost study site. Infestation of citrus fruit and pecan nuts occurred on a year-round basis. Pheromone trap captures only occurred on one occasion.

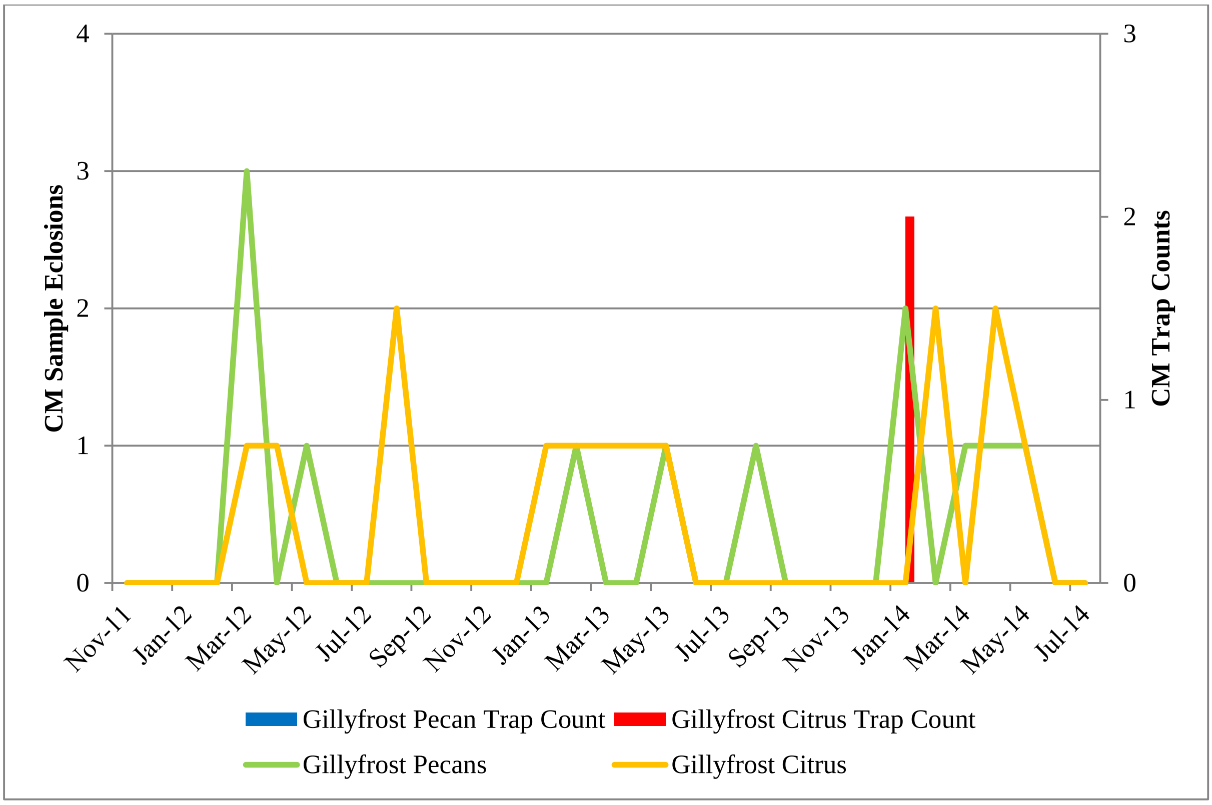

Figure 3.2: Results from the Gillyfrost study site.

Figure 3.3 indicates the combined pheromone trap captures and collected material results for the Floors Farm study site. Infestation of citrus fruit and pecan nuts followed a regular pattern, where material collected from July until November was found to have no CM infestation. Pheromone trap captures indicated that the moths were mostly active during the summer.

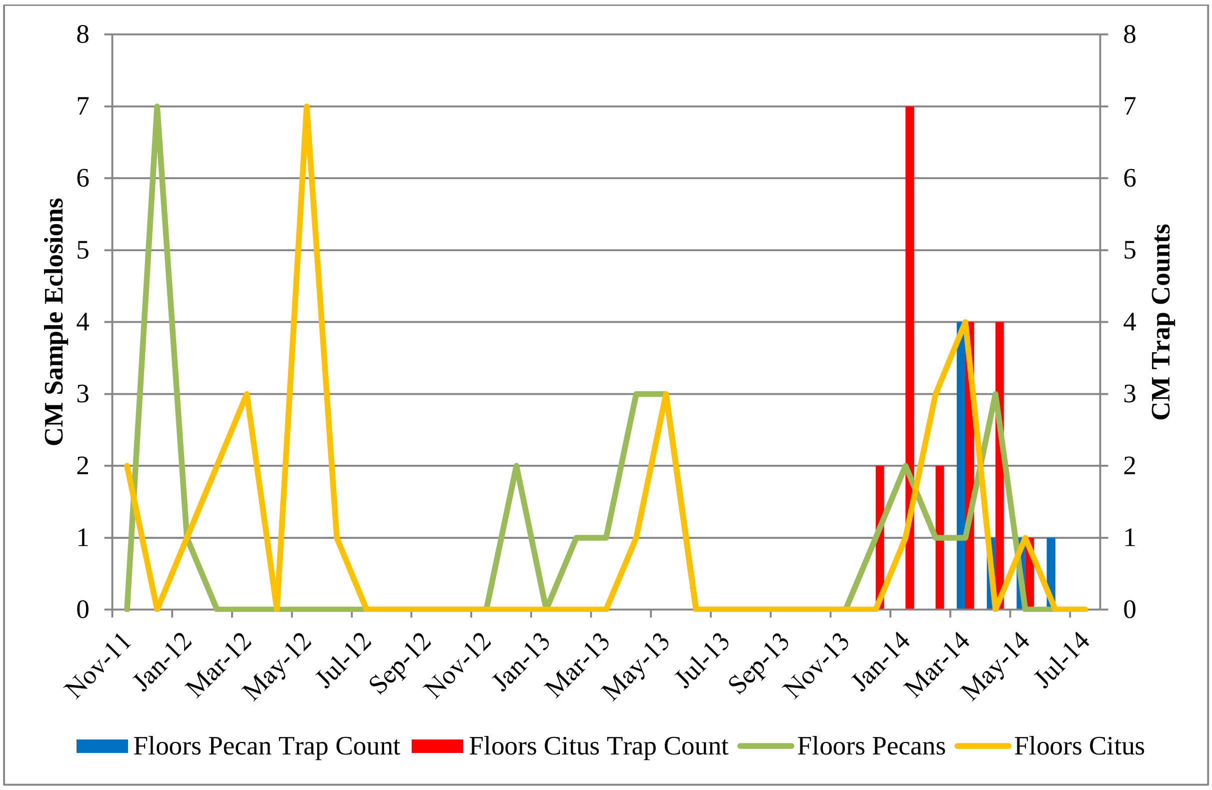

Figure 3.3: Results from the Floors Farm study site.

Figure 3.4 shows the combined pheromone trap captures and collected material results for the Ouplaas study site. Infestation of citrus fruit and pecan nuts followed a regular pattern, where material collected from July to November was found to have low CM infestation. Pheromone trap captures indicated that the moths were mostly active during the summer.

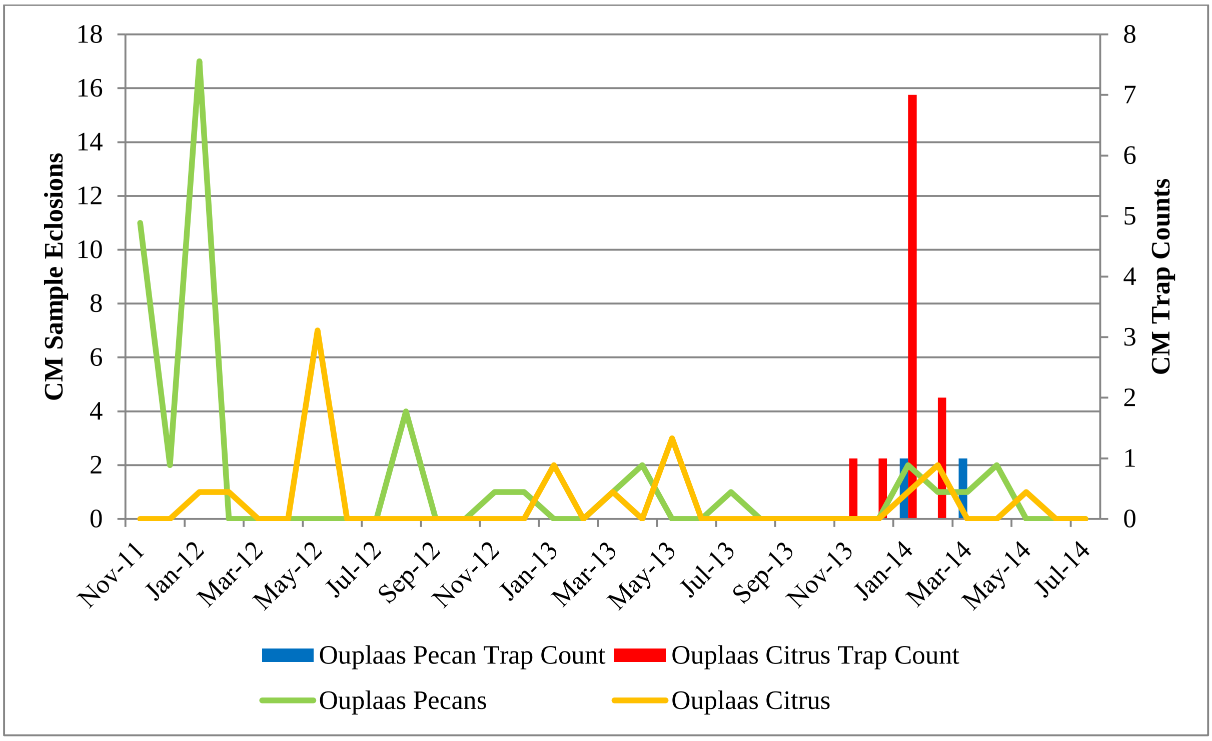

Figure 3.4: Results from the Ouplaas study site.

Figure 3.5 presents the combined pheromone trap captures and collected material results for the Saamfarm A study site. Infestation of citrus fruit and pecan nuts occurred on a year-round basis. Pheromone trap captures indicated that the moths were mostly active during the summer.

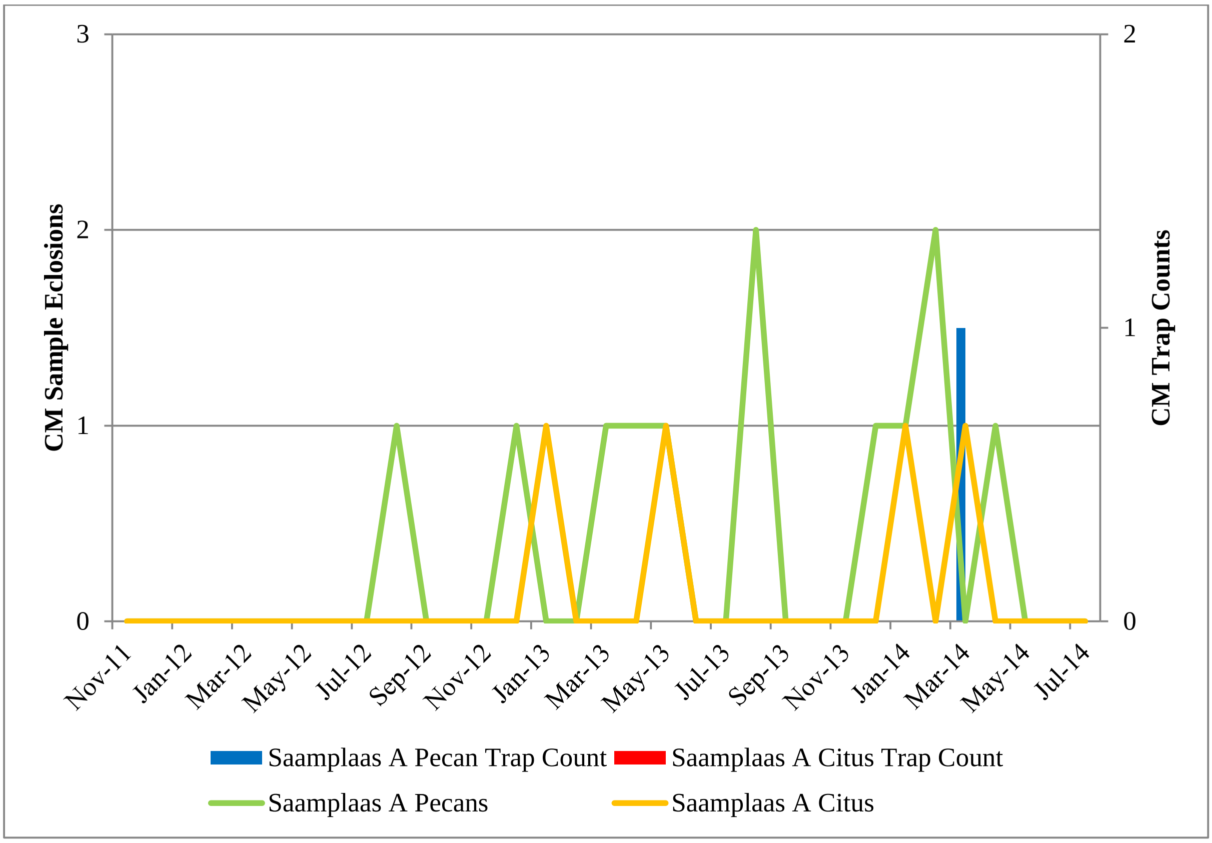

Figure 3.5: Results from the Saamfarm A study site.

Figure 3.6 shows the combined pheromone trap captures and collected material results for the Saamfarm C study site. Infestation of citrus fruit and pecan nuts followed a regular pattern, where material collected from June until November was found to have no CM infestation. Pheromone trap captures indicated that the moths were mostly active during the summer.

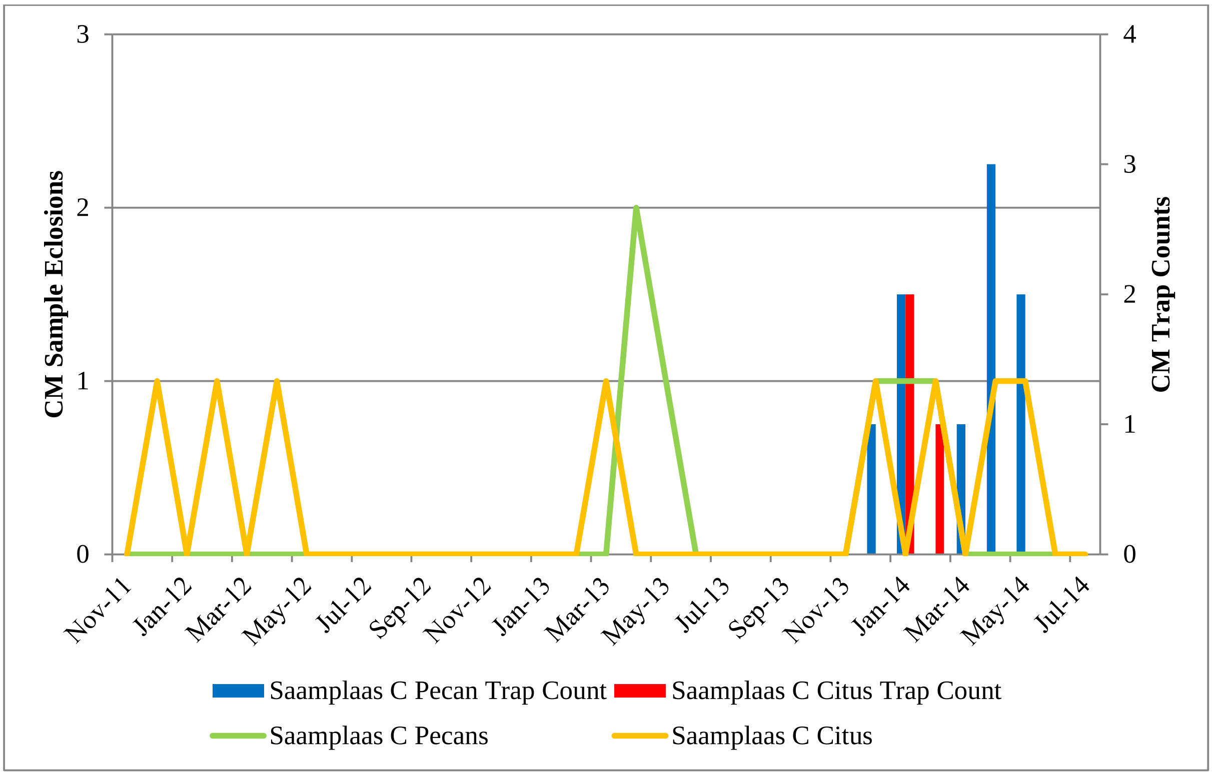

Figure 3.6: Results from the Saamfarm C study site.

Figure 3.7 indicates the combined pheromone trap captures and collected material results for the control study sites. Infestation of citrus fruit and pecan nuts followed a regular pattern, where material collected from June until November was found to have no CM infestation. Pheromone trap captures indicated that the moths were mostly active during the summer.

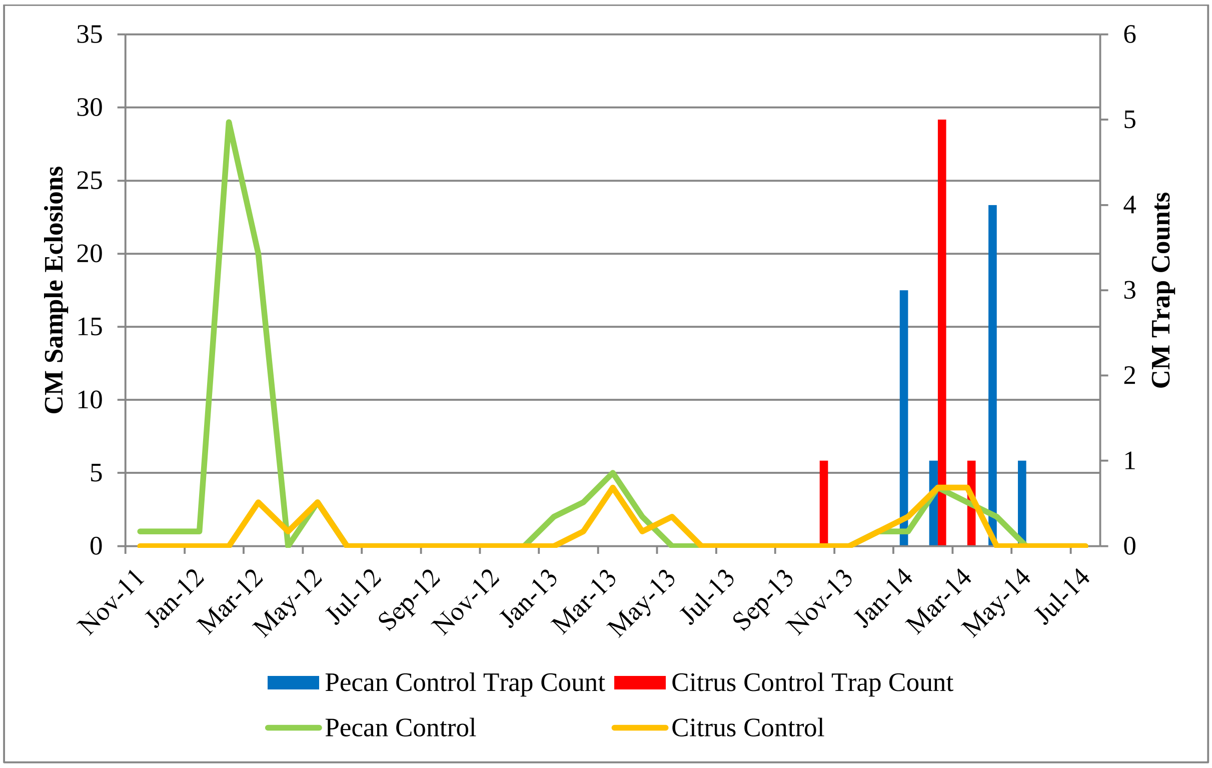

Figure 3.7: Results from both the pecan and citrus control sites.

3.3.2 Seasonal fluctuation

Figure 3.8 presents the combined pheromone trap captures, which indicate that CM was only active throughout the warmer months of the year, with decreased male flight activity during months when potential hosts for oviposition were in short supply.

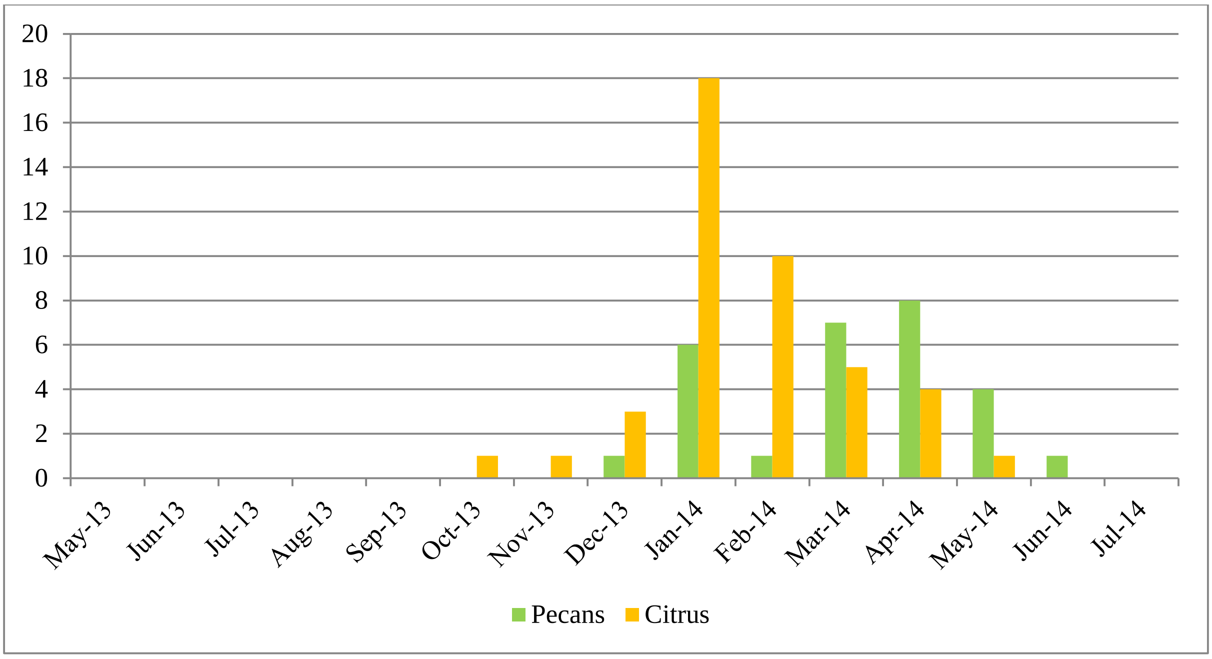

Figure 3.8: This combined graph offers an overview of CM pheromone trap captures, over the course of the study, from all the sites. This indicates a lack of activity during the winter months.

The significant ($p < 0.05$) correlation between the pheromone trap captures of CM in pecan nut orchards and FCM in both orchards (see Table 2.1 and Table 2.2) indicates that there was a possible overlap of the two moth populations at the Floors Farm and Ouplaas study sites. These were the only sites found to have a significant population correlation between the orchards.

The correlation results that follow indicate the sites that produced the best results; however, no positive significant results could be obtained.

3.3.3 Statistical analysis

The best result obtained using Spearman correlation on the eclosion data found a weak and non-significant ($p > 0.05$) positive correlation between CM populations collected from the Gillyfrost study site, as shown in Table 3.1. The analysis was conducted for CM only, between adjacent orchards. This indicates that there was no statistical relationship between the CM populations in adjacent orchards.

Table 3.1: Spearman correlation coefficients for CM reared from collected citrus fruit and pecan nuts for the Gillyfrost study site.

N = 33	**Pecan**	**Citrus**
Pecan	1.000	0.26160 0.1414
Citrus	0.26160 0.1414	1.000

First value indicates Spearman correlation coefficient; second number indicates the *p*-value.

The best result obtained using Spearman correlation on the pheromone trap data found a non-significant ($p > 0.05$) positive correlation between CM populations of the Floors Farm study site, as shown in Table 3.2. The analysis was conducted for CM only, between adjacent orchards. This indicates that there was no statistical relationship between the CM populations in adjacent orchards.

Table 3.2: Spearman correlation coefficients for CM reared from collected citrus fruit and pecan nuts for the Floors Farm study site.

N = 15	**Pecan**	**Citrus**
Pecan	1.000	0.42229 0.1169
Citrus	0.42229 0.1169	1.000

First value indicates Spearman correlation coefficient; second number indicates the *p*-value.

The best Poisson regression summarised in Table 3.4 provided a non-significant ($p > 0.05$) result. This indicates that “Pecan”, acting as the independent variable, almost has a predictive value for CM counts in the adjacent citrus orchard. The citrus orchard captures are the dependent variable in this regression.

Table 3.3: Poisson regression of the CM pheromone captures for the Ouplaas study site.

Poisson Regression for Ouplaas Study Site							
Parameter	**DF**	**Estimate**	**Standard Error**	**Wald 95% Confidence Limits**		**Wald Chi-Square**	**Pr > ChiSq**
Intercept	1	12.075	10.7894	-9.0717	33.2222	1.25	0.2361
Pecan	1	1.2609	0.743	-0.1954	2.7172	2.88	0.0897
Total Citrus	1	0.2715	0.2408	-0.2005	0.7435	1.27	0.2593
Total Pecans	1	-0.2512	0.2226	-0.6875	0.1852	1.27	0.2593
Scale	0	0.9252	0	0.9252	0.9252	-	-

3.4 Discussion

3.4.1 Species overlap

The positive correlations summarized in Tables 2.1 and 2.2 indicate that there was a significant ($p < 0.05$) chance for the overlap of the CM and FCM populations in adjacent orchards. As the test orchards were adjacent, the likelihood that the populations overlap is almost certain. Carob moths are known to be polyphagous (Harvey 2013). Pheromone data, along with the eclosion results, suggest that FCM can exploit both citrus fruit and pecan nuts for reproduction. The polyphagous nature of the moths was reported by Catling and Aschenborn (1974) for FCM, and by Gothilf (1964) for CM. Both species were reported as citrus pests. Both FCM and CM were found to infest macadamia nuts (EPPO 2013, Timm 2005, La Croix & Thindwa 1986, Wysoki 1984).

3.4.2 Shuttling

These similar host preferences, along with the Spearman correlations, indicate that if both FCM and CM are present in an area, overlap of the populations can be expected. No significant results could be obtained from the Spearman correlations performed on all CM data. Spearman correlations were performed on pecan nuts and citrus fruit collected at the study sites. No correlations between adjacent test orchards could be shown to be statistically significant. The best correlation (see Table 3.1) was obtained from the Gillyfrost test site (p = 0.1414). Spearman correlations performed on the pheromone data found no statistically significant results, with no correlations between adjacent test orchards found to be significant. The best correlation (see Figure 3.2) was obtained from the Floors Farm study site (p = 0.1169). The higher overall population of CM obtained from sampled material is the factor considered for the lack of reliable statistical evidence for shuttling, because the smaller variations of potentially shuttling CM are lost in the background data of the much larger overall population size. The eclosion data from the Gillyfrost study site represented the best results; the other study sites had the following even less significant *p*-values: Floors Farm ($p = 0.3187$), Ouplaas ($p = 0.9048$), Saamfarm A ($p = 0.4596$), and Saamfarm C ($p = 0.4269$).

3.4.3 Pheromone trap predictability

Unlike the FCM Poisson regressions from Chapter 2, CM pheromone data could not show that captures from one orchard will act as a prediction of captures in another orchard. Furthermore, no significant negative regression slope was present in any of the test orchards for CM pheromone data. Therefore, unlike the results for FCM (Chapter 2) which indicated a link between infestation of citrus fruit and pheromone captures in an adjacent pecan orchard (see Figure 2.6), no such relationship was found for the CM data.

3.4.4 Seasonal fluctuation

The lack of pheromone trap captures during the winter months of June to August (see Figure 3.2) lends further credence that CM larvae may have entered diapause within the nuts during the winter (Mehrnejad 1993). The survival of the CM larvae in the Vaalharts region can be expected as CM have been shown to be tolerant to cold (Heydari & Izadi 2014). Early oviposition and thus the emergence of adult CM can in almost all cases be attributed to the presence of pecan nuts that were not removed from the trees during previous harvests. Mehrnejad (1993) indicated that after diapause, larvae continue to feed on the nuts still present on the trees and pupate in the nuts. Madge (2012) reported that adults will exploit any available nuts opportunistically at the earliest possible prospect. Removal of any fruit still present from the previous season is encouraged (Gothilf 1969). Citrus fruit still present in the orchards at this time will serve as potential oviposition sites (Gothilf 1964). Any damaged fruit present in and around the orchard will encourage multiple oviposition events on the damaged fruit (Hashemi-Fesharaki *et al.* 2011). Due to the limited area wherein collections could be done, the CM traps had to be placed in close proximity to the established FCM traps. At no point during the study was any cross-trapping observed between the two traps.

3.5 Conclusion

Carob moths were found at all the study sites, while the control sites also had CM populations. This indicates that CM are present throughout the Vaalharts region, and capable of infesting both pecan nuts and citrus fruit.

The statistical analysis of the pheromone data indicated that there was a significant overlap of the FCM and CM populations across both orchards. Populations of FCM and CM are able to infest both pecan and citrus orchards at the same time. Overall infestation was very low, and no significant evidence of shuttling was found. The potential commercial impact of this adjacency could not be quantified.

Spearman correlations of the eclosion data showed that there was no qualitative evidence for CM shuttling between adjacent citrus and pecan orchards. The Poisson regression modelling on the pheromone data from the experimental sites showed that trap captures in one orchard could not be reliably used for predicting the CM population in the adjacent orchard. The number of citrus fruit or pecan nuts in an adjacent orchard seemed to have no effect on the oviposition preference of CM.

Seasonal fluctuations of the CM population in the study sites were as expected for a temperate region. Larval diapause was observed from June to August and peak adult flight activity from January to April. No cross-trapping of FCM and CM was observed and the Lorelei® and PheroLure™ pheromone lures were thus species specific.

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CHAPTER 4

PECAN CULTIVAR PREFERENCE AND THE EFFECT OF COVER CROPS AND NATURAL VEGETATION ON THE POPULATION OF THE GREY-BROWN STINK BUG

4.1 Introduction

Pentatomidae are known to be polyphagous (Jones & Sullivan 1982). While pecan nuts are still in the water stage, damage results in a condition referred to as “black pit” or “black heart” (Yates *et al.* 1991). Indications of pentatomid damage at this stage are symptoms such as fluid seeping from inside the fruit and accumulating on the exterior of the nut (Yates *et al.* 1991). The area of the husk surrounding a puncture site turns black within an hour and the vascular system between the shuck and shell darkens within two days (Yates *et al.* 1991). The husks of nuts which do not drop from the trees as a result of stink bug damage, adhere tightly to the shell (Yates *et al.* 1991). This is in contrast to healthy husks, which can easily be removed from the nut; this is noticeably different from the husks that separate easily due to normal summer nut abortions (Yates *et al.* 1991). After the water stage, hemipteran feeding can be ascertained only by cracking the nuts and examining the kernels (Polles 1977). Feeding sites on kernels are characteristically black spots that become sunken and pithy and have a bitter taste (Yates *et al.* 1991). Similarly, stink bug damage on avocado can be very extensive, and it alters over the growth period of the flowers and fruit (Bruwer 2004). During the early stages of flower development, feeding by the grey-brown stink bug, *Coenomorpha nervosa*, will cause flowers to fall from the tree (Bruwer 2004). Pentatomids primarily feed on seeds during the milk stage of development (Jones & Sullivan 1982).

Due to the active nature, hemimetabolous lifecycle, and reproductive preferences of *C. nervosa* and stink bugs in general, there are no widespread cultural practices designed to curtail stink bug populations. Measures that have been suggested include the planting of early-season crops in order to minimise damage from stink bugs, as most will still be in diapause; however, these practices are not widely employed. A trap developed by W.L. Tedders for monitoring and capturing *Euschistus* spp. produced many captures of male and female stink bugs as well as nymphs which were in close proximity to the trap (Mizell & Tedders 1995). The trap needs to be used in conjunction with an aggregation pheromone in order to produce effective results (Aldrich *et al.* 1991). Since the introduction of this trap, proprietary traps have come on the market for the domestic control of stink bugs in the USA.

Tillman (2006) reported that sorghum acts as an effective trap crop for *Nezara viridula* when cultivated with cotton in the southern United States. White mustard (*Sinapis alba*) and peas (*Pisum sativum*), were evaluated and recommended as trap crops for *N. viridula* to reduce damage to maize (Rae *et al.* 2002). Mizell *et al.* (2008) reported that the early-spring plantings of triticale, crimson clover, buckwheat, and hairy vetch as trap crops within the vicinity of the crop will have the effect of minimising damage to early-season growth. They also found that okra and field peas augment the ability of other summer trap crops to draw away stink bugs, and also increased the population of beneficial insects in the trap crop (Mizell *et al.* 2008). Dyby and Sailer (1999) found that exposing fourth-instar *N. viridula* nymphs to 10 Gγ of ionising radiation rendered them partially sterile. Many examples of biological control for *N. viridula* have been developed (Knight & Gurr 2007). The egg parasitoids *Trissolcus basalis* and *Trichopoda giacomellii* are known to parasitise the eggs of *N. viridula* (Waterhouse 1998). The use of broad-spectrum pesticides for the control of *N. viridula* on soybeans has the effect of lowering the overall population of predators and parasitoids within a field, and in the process this increases the reliance on pesticides to a greater degree (Knight & Gurr 2007). The adoption of within-field monocultures combined with an increased reliance on pesticide usage has the effect of lowering the overall predator balance within an agroecosystem (Knight & Gurr 2007).

The practice of removing surrounding vegetation not only limits available habitats for parasitoids, it also limits their food sources (Knight & Gurr 2007). Stink bugs are known to be naturally tolerant to many pesticides; therefore efficacious insecticide products for the management of stink bug pests are rare (Mizell *et al.* 2008). Payne and Wells (1984) found that the incidence of kernel spot on pecan nuts that were sprayed with pesticide was 1.8%, whereas trees that did not receive pesticide treatment had a kernel spot incidence of 5.8%. Chess® (Pymetrozine: Syngenta SA (Pty) Ltd, South Africa) is a systemic insecticide registered for the control of *C. nervosa* and similar pests on avocado. No studies regarding its efficacy could be found. The application of Bulldock (Beta-Cyfluthrin: Bayer CropScience, South Africa), a pesticide registered for use on avocado, was found to reduce the *C. nervosa* population from a high of 29% to a low of <1% after a single treatment (Bruwer 2004).

The pecan industry suffers severe losses due to the feeding action of stink bugs (Deuce & Suber 1986). Losses recorded during the 1985 season in the state of Georgia, USA, were estimated at $3.5 million. This was calculated for the state alone, and did not take into consideration the impacts on the industry in other areas of the USA (Deuce & Suber 1986). Also in Georgia, stink bug damage could be attributed to $13 million worth of damage and control costs for the soybean industry (Douce & McPherson 1991). Due to the polyphagous habits of stink bugs, the range of potential cultivated hosts can be very extensive (Jones & Sullivan 1982).

The following points had to be addressed in order to ascertain the potential threat that stink bugs pose to the pecan industry in the Vaalharts region:

- Determine which stink bug species is the most economically important.
- Determine stink bug cultivar preference.
- Determine the importance of ground cover vegetation and densities within and around pecan orchards, with regard to the stink bug population.

4.2 Materials and methods

The study area was located in the Vaalharts region of South Africa, in the Northern Cape province, with the approximate centre of the entire study area located at the geographical coordinates of S27°71'700", E24°78'020". Both the scouting for stink bugs in the pecan trees and the sweeping of various locations in and around orchards started in January 2013 and were conducted on a monthly basis until January 2014.

The sites for the cultivar preference study were selected based on the availability of sufficient Wichita cultivar pecan nut trees in orchards which had representation of at least one other cultivar. Sites with diverse cover plants were preferred to sites which had only bare soil beneath the pecan nut trees. Cultivated fields adjacent to the pecan nut orchards were also preferred to orchards which did not have this feature. The willingness of the farmer to assist in the study was also a requirement.

During the month preceding the study (December 2012), specimens of the stink bugs from each of the test sites were collected and sent to the Agricultural Research Council (ARC) for identification by M. Stiller. The specimens, as seen in Figure 4.1, were collected directly from pecan nut trees. Figure 4.2 represents the general layout of a pecan nut orchard in the Vaalharts region, while Figure 4.3 is an example of a potential host crop being intensively cultivated in close proximity to some of the pecan orchards.

Figure 4.1: *Coenomorpha nervosa* on a pecan nut. Photo by J. Saaiman.

Figure 4.2: Example of a site where stink bug populations were determined and sweeps were done.

Figure 4.3: Example of a field of adjacent alfalfa. The pecan orchard is to the left of the field.

At the Oberholtzer study site (Figure 4.4), Wichita (green line) and Choctaw (red line) cultivars were scouted for the level of stink bug infestation. In every transect, 10 trees were scouted for five minutes each in order to count the number of stink bugs present on the trunks and branches. The sweeps conducted at this study site intersected the two scouting transects, running lengthwise along the orchard edge, in two separate transects of 100 sweeps each. Note that all aerial photographs were retrieved from Google Maps.

Figure 4.4: Layout of transects at the Oberholtzer study site (located at S27°66'332", E24°78'903").

At the Engelbrecht study site (Figure 4.5), Wichita (green line) and Choctaw (red line) cultivars were scouted for the level of stink bug infestation. In every transect, 10 trees were scouted for five minutes each in order to count the number of stink bugs present on the trunks and branches. The sweeps conducted at this study site were on the northeastern edges in two separate transects of 100 sweeps each.

Figure 4.5: Layout of transects at the Engelbrecht study site (located at S27°64'137", E24°73'702").

At the De Villiers study site (Figure 4.6), Wichita (green line) and Barton (red line) cultivars were scouted for the level of stink bug infestation. In every transect, 10 trees were scouted for five minutes each in order to count the number of stink bugs present on the trunks and branches. The sweeps conducted at this study site were on the northern edge in two separate transects of 100 sweeps each.

Figure 4.6: Layout of transects at the De Villiers study site (located at S27°71'754", E24°79'006").

At the Duvenhage study site (Figure 4.7), Wichita (green line) and Navaho (red line) cultivars were scouted for the level of stink bug infestation. In every transect, 10 trees were scouted for five minutes each in order to count the number of stink bugs present on the trunks and branches. The sweeps at this study site were conducted in the alfalfa field south of the orchard in two separate transects of 100 sweeps each.

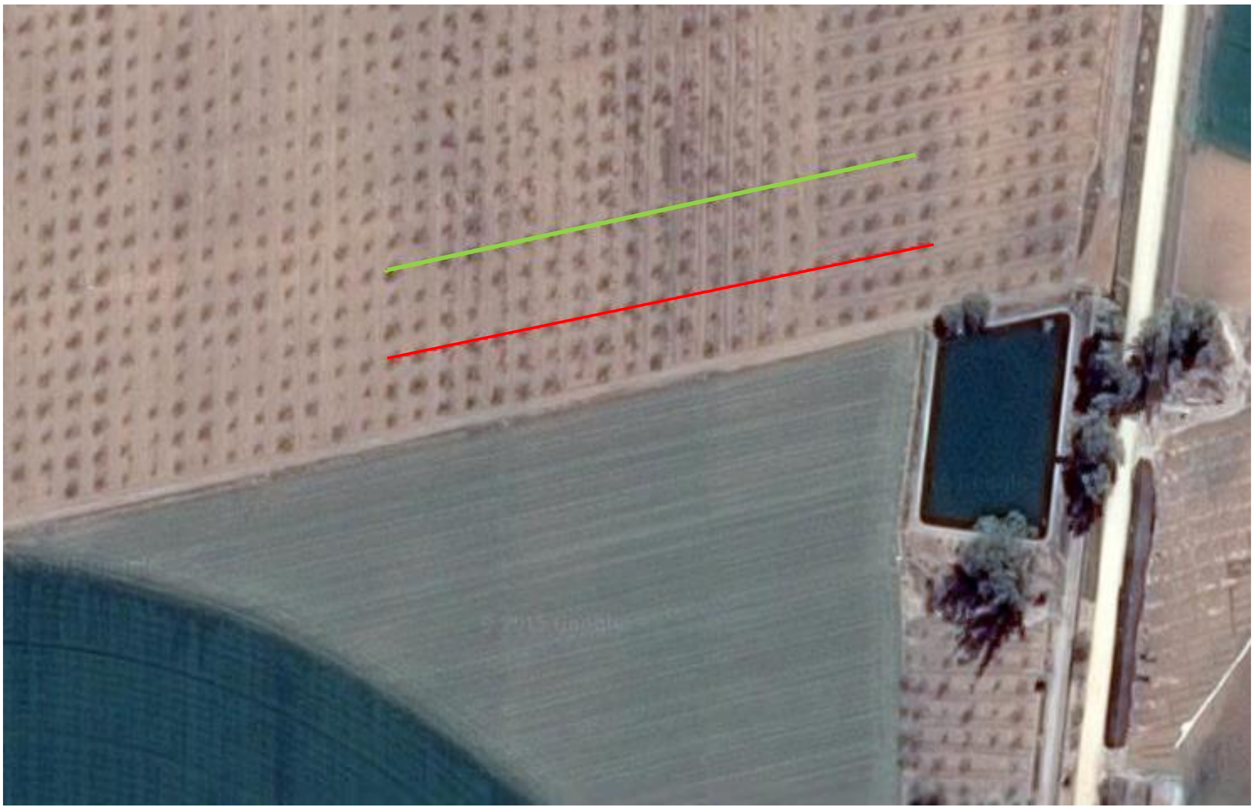

Figure 4.7: Layout of transects at the Duvenhage study site (located at S27°73'244", E24°77'508").

At the Human study site (see Figure 4.8), Wichita (green line) and Choctaw (red line) cultivars were scouted for the level of stink bug infestation. In every transect, 10 trees were scouted for five minutes each in order to count the number of stink bugs present on the trunks and branches. The sweeps at this study site were conducted in the alfalfa field to the east in two separate transects of 100 sweeps each.

Figure 4.8: Layout of transects at the Human study site (located at S27°84'901", E24°81'423").

At the Erasmus study site (see Figure 4.9), Wichita (green line) and Choctaw (red line) cultivars were scouted for the level of stink bug infestation. In every transect, 10 trees were scouted for five minutes each in order to count the number of stink bugs present on the trunks and branches. The sweeps at this study site were conducted on the eastern edge in two separate transects of 100 sweeps each.

Figure 4.9: Layout of transects at the Erasmus study site (located at S27°85'744", S24°82'529").

The statistical analysis was conducted by Prof. Robert Schall of the UFS, using the 2009 version of SAS. For each farm, the total monthly count of stinkbugs among both Wichita and competing cultivars was calculated. Conditioning on this total count, the count of stink bugs in Wichita trees followed a Binomial (p,t) distribution, where t was the total count of stink bugs, and p was the probability that a stink bug was collected among Wichita trees. Consequently, 1-p is the probability that a stinkbug was collected among the control trees. A preference for Wichita trees was shown by indicating that the probability for one of the control cultivars was ($p > 0.5$).

4.3 Results

Stink bugs collected from the study sites were identified by M. Stiller as *Coenomorpha nervosa*. During scouting, *C. nervosa* was the only species of stink bug found, apart from one instance where a single *Atelocera raptoria* was found on the pecan nut trees.

4.3.1 Cultivar preference of *C. nervosa*

The probability that stink bugs prefer Wichita to the control was labelled "Estimate"; the p-value associated with testing the null-hypothesis (H_0: $p = 0.5$) was labelled "Pr > ChisSq". A probability larger than 0.5 suggests that stink bugs prefer Wichita to the control trees; if the lower limit of the 95% confidence interval for the probability is larger than 0.5, then the preference is shown to be statistically significant; equivalently, the p-value in this is less than 0.05. The results summarised in Table 4.1 indicate that *C. nervosa* preferred pecan trees of the Wichita cultivar at all of the study sites. Total number of stink bugs scouted were 1230.

Table 4.1: Summary of the results for *C. nervosa* cultivar preference at all study sites.

Cultivar preference of *C. nervosa*				
Farm	**Control Tree**	**Probability *C. nervosa* prefers Wichita**		**Pr > ChisSq**
		Estimate	**95% Confidence Interval**	
Oberholtzer	Choctaw	0.6162	0.5165 - 0.7070	0.0228
Engelbrecht	Choctaw	0.7168	0.6174 - 0.7988	<0.0001
De Villiers	Barton	0.6484	0.5775 - 0.7132	<0.0001
Duvenhage	Navaho	0.5939	0.5136 - 0.6695	0.0221
Human	Choctaw	0.7297	0.6507 - 0.7965	<0.0001
Erasmus	Choctaw	0.838	0.6894 - 0.9234	0.0001

4.3.2 Possible alternative refuges for *C. nervosa*

Sweeps were conducted on a monthly basis on a variety of plants. The study failed to produce any *C. nervosa* specimens in vegetation growing either within the orchard, adjacent crops, or the uncultivated edges.

The following is the complete list of plant species that were found within all transects during the course of all the sweeps. None of these plants had any *C. nervosa* at any time during the study.

- *Urocloa mosambicensis* (African Liverseed Grass)
- *Sporobolus fimbriatus* (Sacaton Grass)
- *Delosperma herbeum* (Klipvygie)
- *Conyza bonariensis* (Flax-leaf Fleabane)
- *Pollichia campestris* (Barley Sugar Bush)
- *Lippia scaberrima* (Beukessboss)
- *Gamochaeta pennsylvanica* (Cudweed)
- *Gomphrena celosiodes* (Gomphrena Weed)
- *Schkuhria pinnata* (Dwarf Mexican Marigold)
- *Felicia muricata* (Taaibloublommetjie)
- *Gazania krebsiana* (Rooi Gazania)
- *Hermannia depressa* (Rooi-opslag)
- *Verbena bonariensis* (Purpletop Vervain)
- *Physalis viscose* (Starhair Ground Cherry)
- *Solanum nigrum* (European Black Nightshade)
- *Eragrostis lehmanniana* (Lehmann Love Grass)
- *Taraxium officinale* (Dandelion)
- *Elionurus muticus* (Elsgras)
- *Hibiscus trionum* (Flower-of-an-Hour)
- *Tagetis minuta* (Southern Cone Marigold)
- *Medicago sativa* (Alfalfa)
- *Acacia erioloba* (Camel Thorn)
- *Datura stramonium* (Jimsonweed)
- *Arista canescens*

4.4 Discussion

4.4.1 Cultivar preference

The statistical analysis indicated that *C. nervosa* had a significant ($p > 0.05$) preference to inhabit pecan trees of the Wichita cultivar. The trees within the orchards were all roughly the same age and size. Transects were planned in such a way as to mitigate the edge effect. All trees were thoroughly and equally investigated, with the same amount of time spent scouting for *C. nervosa* at all locations. The primary factor for the population disparities between transects seems to be attributable to the cultivars found within those transects. *Coenomorpha nervosa* indicated a significant preference for the Wichita cultivar to Barton, Choctaw, and Navaho cultivars in the Vaalharts region.

4.4.2 Alternate hosts for *C. nervosa*

In contrast to what was noted by Mizell *et al.* (2008), that other pentatomids use a sequence of crops as their population increases throughout a season, *C. nervosa* does not seem to exploit readily available host plants within a convenient range. Even after defoliation of the pecan trees occurred in the autumn, no *C. nervosa* could be found in potential host plants that were not dead or defoliated during winter. Significant numbers of other insects, primarily lepidopteran larvae, were found during the sweeps, which indicated that there was no strict regimen of control practised on other potential habitat sites. Adjacent alfalfa fields in particular had large numbers of other insects exploiting the crop, but no *C. nervosa* in any stage of development were found in any of the potential habitats during the course of the study.

All life stages of *C. nervosa* were found to be present during the course of the study. For confirmation purposes, one set of egg packets was collected and incubated under the same conditions as the FCM and CM collections of the previous chapters. The first-instar nymphs that emerged were noticeably those of *C. nervosa*. This conclusion was reached when similar nymphs were seen congregating with adult *C. nervosa* on the pecan trees. This indicated that *C. nervosa* seem to stay within the pecan nut orchard and specifically on the pecan trees for their entire lifecycle. This also conforms to the lifecycle that pentatomids inhabiting a temperate climate demonstrate, with adults that diapause during winter to become active in spring and starting to mate two weeks after becoming active (Hoebeke & Carter 2003).

Very few *C. nervosa* were encountered on the pecan trees during the winter months. The disparity was very noticeable due to the lack of foliage, which made the detection of *C. nervosa* easier. Only adult specimens were encountered during the winter months. Egg packets were found during the winter but overall proved very difficult to find. The adult diapause of *C. nervosa* was consistent with what was reported of other Pentatomidae species (Saulich & Musolin 2012). Nymphs were only encountered from September onwards.

4.5 Conclusion

M. Stiller of the ARC identified the species of pentatomid that was most predominant in all the orchards as *C. nervosa*. The primary species of pentatomid that was encountered within the pecan orchards, and thus by exclusion the most economically significant species, was *C. nervosa.*

The population distribution of *C. nervosa* across all the study sites indicated a significant preference for the Wichita cultivar. This preference was exhibited over other pecan nut trees of similar age and health within the same orchard.

The lack of any *C. nervosa* found from sweeping vegetation underneath the pecan trees and adjacent crops led to the conclusion that the availability of potentially suitable host plants in the vicinity of the pecan nut orchards will not lead to *C. nervosa* exploitation. The year-round presence of *C. nervosa* within the pecan nut trees was also a strong indication that there is a preference for *C. nervosa* to stay within the trees to await more favourable conditions, and as such start oviposition as soon as possible.

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CHAPTER 5

GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

False codling moths (FCM) are a known pest of citrus in southern Africa, particularly in the southern areas (Catling & Aschenborn 1974). In South Africa, 21 cultivated plants and 14 indigenous plants have been identified as hosts for FCM (Schwartz 1981). False codling moths have not been reported to represent an economic threat to the pecan industry by means of direct damage to crops; however, due to the phytosanitary status of FCM, shipments due for export to certain markets could be at risk. Strict phytosanitary measures are therefore in place to prevent transference of the moth; this in turn requires tighter controls by producers (DAFF 2010). The high degree of similarity encountered between the larvae of FCM and CM makes the immature stages of these moths difficult to differentiate from each other (Rentel *et al.* 2011). Cultural control of removing fallen and unharvested fruit from the orchards is still one of the more efficacious methods for controlling FCM (Moore & Hattingh 2012). The removal of visibly damaged and infested fruit from trees should also be considered in order to decrease potential oviposition sites for lepidopteran pests (Hashemi-Fesharaki *et al.* 2011).

Carob moths (CM) are known to be polyphagous (Hashemi-Fesharaki *et al.* 2011; Mehrnejad 1993; Gothilf 1964). Hashemi-Fesharaki *et al.* (2011) reported that CM females show preference for oviposition onto already damaged pomegranate fruit, which increases the survivability of the vulnerable first-instar larvae. Gothilf (1964) reported that CM develop mostly in cracked carob pods. Of the pods investigated at harvest, 70% to 90% of all cracked pods were found to be infested by CM. In pomegranate orchards with lax sanitation, a higher incidence of CM was encountered in contrast to orchards that were stringently sanitised (Al-Izzi *et al.* 1985). Large-scale deployments of a selected pheromone solely for the purpose of CM mating disruption could be a viable option for the control of CM (Madge 2012). The limited exposure lifecycle and boring of larvae into fruit render the widespread use of chemical insecticides for control ineffective (Kishani Farahani *et al.* 2012).

Pentatomidae are known to be polyphagous (Jones & Sullivan 1982). While the pecan nuts are still in the water stage, damage results in a condition referred to as “black pit” or “black heart”

(Yates *et al*. 1991). After the water stage, hemipteran feeding can be ascertained only by cracking the nuts and examining the kernels (Polles 1977). Stink bug damage on avocado can be very extensive (Bruwer 2004). Pentatomids have been found to primarily feed on seeds during the milk stage of development (Jones & Sullivan 1982). Due to the active nature, hemimetabolous lifecycle, and reproductive preferences of *Coenomorpha nervosa* and stink bugs in general, there are no widespread cultural practices designed to curtail stink bug populations. Tillman (2006) reported that sorghum acts as an effective trap crop for *Nezara viridula* when cultivated with cotton in the southern USA. Many examples of biological control for *N. viridula* have been developed (Knight & Gurr 2007). The egg parasitoids *Trissolcus basalis* and *Trichopoda giacomellii* have proven effective for the control of *N. viridula* (Waterhouse 1998). The use of broad-spectrum pesticides for the control of *N. viridula* on soybeans has the effect of lowering the overall population of predators and parasitoids within a field, and in the process this feeds back by increasing the reliance on pesticides to a greater degree (Knight & Gurr 2007). Losses attributed to stink bug feeding have been estimated at $3.5 million for the 1985 season in the state of Georgia, USA. This was calculated for the state alone, and did not take into consideration impacts on the industry in other areas of the USA (Deuce & Suber 1986). Due to the polyphagous nature of stink bugs, the range of potential cultivated hosts can be very extensive (Jones & Sullivan 1982).

5.2 Discussion and recommendations

5.2.1 Infestation

False codling moths (*Thaumatotibia leucotreta*) and CM (*Ectomyelois ceratoniae*) were both found to be present at all the study sites in the Vaalharts region. The endemic distribution of FCM in sub-Saharan Africa (Newton 1998) and the cosmopolitan distribution of CM (Gothilf 1969) makes curtailing of further distribution in Southern Africa a non-concern.

During the course of the study, the total infestation in the citrus orchards was found to be 0.01% and 0.03% for FCM and CM respectively. In the pecan orchards, infestation was found to be 0.004% and 0.01% for FCM and CM respectively. The infestation in either crop did not indicate a significant economic impact by either of the lepidopteran species. The pest-control measures observed during the study, including orchard sanitation and the large-scale deployment of pheromone traps (particularly at the Saamfarm sites), indicated that the probability of either lepidopteran borer reaching pest status in the Vaalharts region is unlikely.

As mentioned in Chapters 1, 2, and 3, FCM and CM are easily confused when in larval form, which poses a risk of potential phytosanitary infringement if a shipment is destined for markets that have prohibitions on the presence of FCM in the product. This is specifically a concern in the event of fresh fruit and in-shell exports to nations concerned with the introduction of FCM, which includes China, EU countries, Japan, Korea, Thailand, and the USA (DAFF 2016). Considering the low level of infestation encountered during the study, and measures already in place (see Chapter 1) for preventing the transfer of phytosanitary pests, no extra precautions can be recommended, apart from extra diligence on the part of inspectors to prevent confusion between FCM and CM larvae.

5.2.2 Shuttling

The Spearman correlation indicated that there was qualitative evidence of FCM shuttling between citrus and pecan orchards. The population of FCM in one orchard could be positively correlated with the population in an adjacent orchard. This is consistent with Stotter's (2009) findings, namely that guavas and acorns were infested within a 1.5 km radius of the citrus orchards under investigation. The shuttling of FCM between adjacent orchards was consistent with Reed's (1974) findings, namely that FCM readily infest adjacent cotton of both the same and different maturities. Conversely, the Spearman correlation of the eclosion data indicated that there was no qualitative evidence for CM shuttling between adjacent citrus and pecan orchards. This is in contrast to Mehrnejad's (1993) findings, namely that CM will readily infest available hosts in adjacent orchards.

The primary reason for the lack of statistical evidence to establish shuttling was a lack of sufficient eclosing CM adults from any of the test sites. The small number of CM collected from the sites made it impossible for the Spearman correlation modelling to establish a statistically significant evidence of shuttling.

The method of sampling that restricted the collection of material and placement of pheromone traps to areas closer to the edges of the test orchards limited the ability to collect sufficient material in some cases. Although citrus fruit and pecan nuts were collected at random as far as possible, the repeated monthly collection in the same transects possibly served to deplete FCM and CM populations. This collection method also served to deplete the stocks of available material (citrus fruit and pecan nuts) within the established transects, especially later in the season, after harvesting by the owners. The decision of non-interference with the everyday management of the orchards (harvesting, sanitation, pheromone trapping, irrigation, micronutrient supplementation, and chemical or manure fertilisation) was made in order to prevent the development of a situation that would not be present in an orchard under optimal management conditions.

A slightly modified form of the Taiwan protocol is recommended for future studies. A greater number of the trees under investigation should be concentrated towards the area adjacent to other orchards in the study. Pheromone traps should also be placed before the collection of material commences in order to ensure that a significant population of FCM and CM is present in the orchards. The number of fruit was sufficient, but the number of moths was insufficient to establish shuttling of CM. In order to ensure more reliable evidence of shuttling, efforts should be directed towards the collection of infested citrus fruit and pecan nuts in order to increase the number of moths available for statistical analysis. In doing so, the amount of material that would be depleted from the orchard on every sampling occasion could be reduced in order to ensure that material will be available for future collection.

To further establish potential shuttling and to remove the effect of anthropogenic management practices, “wild” or abandoned stands of citrus and pecan trees adjacent to each other should be sought out if available, in the same area as the managed orchards. Material should also be collected and pheromone traps placed in these stands of unmanaged trees in order to receive a more baseline indication as to the shuttling habits of FCM and CM.

5.2.3 Species overlap

The Spearman correlation of the pheromone data indicated that there was a significant overlap of FCM and CM populations between adjacent orchards. During the study, both species of moths were found to have overlapping infestations in the citrus and pecan orchards. This polyphagous ability further increases the potential for confusion between the similar larvae of FCM and CM during phytosanitary inspections (Rentel *et al*. 2011). As noted in Chapters 1 and 2, this overlap can potentially cause delays during phytosanitary inspections.

5.2.4 Alternate host availability

The Poisson regression modelling indicated that the availability of fruit in the citrus orchard affected the number of FCM captured in the adjacent pecan orchard. This result indicated a tendency of FCM to shuttle from pecan orchards to citrus orchards to take advantage of oviposition opportunities. This result should be taken into consideration in the future layout of orchards and the type of orchards within the vicinity in order to curtail the extra influx of phytosanitary pests to a predominantly export crop. Carob moth feeding preferences do not seem to act as pressure for exploiting alternate hosts and thus adjacent orchards containing potential hosts were not found to attract significant numbers of CM.

The low level of infestation indicated that there was little, if any, competition between FCM and CM for oviposition opportunities. This study did not find larvae from FCM and CM infesting the same fruit. Thus the possibility for inter-species competition between FCM and CM still exists; however, in situations such as those encountered during the course of the study where infestation was low, this is unlikely.

5.2.5 Pheromone trap predictability

The Poisson regression modelling indicated that the number of FCM captured in citrus pheromone traps can be used to predict the FCM population captured in the adjacent pecan orchard. Similarly, pecan orchard pheromone trap captures can be used to predict FCM numbers in the adjacent citrus orchard. On the other hand, the same modelling applied to CM pheromone captures did not provide any predictability for populations in adjacent orchards. This result is valuable in estimating the FCM population in situations where citrus orchards are located adjacent to pecan orchards. This method of pheromone trapping is recommended to predict FCM populations in adjacent orchards, both to save on money and labour.

5.2.6 Pheromone specificity

The species-specific pheromone lures used during the study – Lorelei® for FCM and PheroLure™ for CM – were found to be 100% accurate as no cross-trapping occurred. Either of the pheromone products can be used with the confidence that species-specific trapping will occur on every occasion, even when the traps are placed within the same orchard.

5.2.7 Seasonal fluctuation

As indicated in Chapter 2, pheromone trap data showed year-round activity of FCM, with peak flight activity during the January to May period, which was consistent with Newton’s (1998) findings. The essential year-round availability of oviposition sites in the form of fallen citrus fruit and pecan nuts within the orchards, even when no fruit or nuts were present on the trees, indicated that FCM activity in the Vaalharts area was neither limited by opportunities for reproduction nor physiological limitations.

As noted in Chapter 3, pheromone trap data for CM indicated possible larval diapause during the colder months of the year (June to August), with peak flight activity from January to April. This was in contrast to the presence of FCM during the winter months. Carob moths were recovered from citrus fruit and pecan nuts, both from trees and from the ground, which indicated that the year-round availability of oviposition sites will not promote year-round activity of CM. The survival of CM within the Vaalharts area is thus consistent with and attributable to Heydari and Izadi's (2014) findings that the larvae of CM are tolerant to cold. The physiological limitation of CM to be active during the colder months of the year is the most probable answer for the lack of CM captures during the winter.

This confirmation of seasonal variation in the Vaalharts region is valuable for providing a potential guideline for when control measures will be most effective for the control of FCM and CM. Sanitation should be done constantly in order to reduce the FCM population. The control of CM will be most effective when sanitation is carried out during the winter months, on areas where the dropped citrus fruit and pecan nuts are collected, as well as the fruit and nuts that are still on the trees.

5.2.8 Cultivar preference of *C. nervosa*

Coenomorpha nervosa indicated a clear preference for pecan trees of the Wichita cultivar, and this preference was exhibited over other pecan nut tree cultivars within the same orchard, which appeared to be of a similar age and health.

In the Vaalharts region, it is recommended that this cultivar preference should be taken into consideration when new pecan nut orchards are planted, as this preference for pecan trees of the Wichita cultivar will disproportionately affect farmers who choose to plant large numbers of this cultivar.

Future studies might consider investigating orchards where pecan nut trees of the Wichita cultivar are in the minority, to ascertain if the cultivar can be used as a makeshift "trap crop". This investigation should attempt to ascertain if pecan nut trees of other cultivars planted within the proximity of the Wichita cultivar suffer lower economic damage due to the presence of the cultivar, which may attract many *C. nervosa* to the orchard. This will also establish if the preference for the Wichita cultivar is not due to the abundance of the cultivar in the Vaalharts region, which perhaps stimulates a localised adoption of the cultivar by *C. nervosa* due to its ready availability.

5.2.9 Alternative host preference of *C. nervosa*

The lack of exploitation of other palatable and readily available food sources in the Vaalharts region by *C. nervosa* is anomalous. Other pentatomids are known to exploit various crops during their lifecycle, as well as with the progression of the season (Mizell *et al.* 2008). *Coenomorpha nervosa* was only encountered on pecan nut trees, particularly on the Wichita cultivar. As mentioned in Chapter 4, various other host species were available for exploitation in and immediately around the orchards but none were ever found to harbour *C. nervosa*. This indicated a strong preference for pecan nut trees of the Wichita cultivar, and that any trap crops would have poor efficacy. As stated in the previous section, it is thus recommended that the density and overall number of Wichita pecan nut trees to be planted in an orchard should be considered thoroughly beforehand. Furthermore, it is recommended that no trap crops are planted for the control of *C. nervosa* as no preference for any other plants over pecan nut trees could be established. Thus, the introduction of other plants into orchards will do little, if anything, to control *C. nervosa* populations, and might potentially introduce potential refugia for other pest species.

5.3 General conclusion

This study investigated the interaction of FCM and CM on citrus fruit and pecan nuts in the Vaalharts region of South Africa. The two main aims were to determine if lepidopteran borer pests shuttled between adjacent citrus and pecan orchards, and to establish the cultivar preference of *C. nervosa* in pecan orchards in the Vaalharts region. The two moth populations were found to have a statistically significant overlap in adjacent citrus fruit and pecan nut orchards. Both FCM and CM were found within the citrus and pecan orchards. It was found that FCM shuttled between adjacent citrus and pecan orchards; however, the predominant direction of the shuttling could not be established. It was postulated that the abundance of citrus fruit oviposition sites drew FCM from adjacent pecan orchards, increasing infestation in the citrus orchards. No significant evidence of CM shuttling could be established. Adult FCM were found to be active throughout the winter. CM adults were almost entirely absent during the winter months, but viable larvae were still present in the collected pecan nuts and citrus fruit, and adults were found to eclose from these larvae. *Coenomorpha nervosa* was found to be the most prevalent hemipteran pest on pecan trees in the Vaalharts region. Both adults and nymphs indicated a significant preference for pecan trees of the Wichita cultivar to other cultivars in the orchards (Choctaw, Barton, and Navaho). During the course of the study, *C. nervosa* could not be found to exploit other viable food sources close to pecan nut orchards, even potential host plants that were not dead or defoliated during the winter.

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