ASSESSMENT OF PHEROMONE SPECIFICITY IN *THAUMATOTIBIA LEUCOTRETA* (MEYRICK) POPULATIONS WITH FOCUS ON PEST MONITORING AND THE REGIONAL ROLLOUT OF THE STERILE INSECT TECHNIQUE IN CITRUS

Submitted for the fulfilment of the requirements for the degree of

MASTERS OF ENTOMOLOGY

at

Rhodes University

by

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January 2018

DECLARATION

The following thesis has not been submitted to a university other than Rhodes University, Grahamstown, South Africa. The work presented here is that of the author.

Signed: …… Date: ……

ACKNOWLEDGEMENTS

- My supervisor, Martin Hill and co-supervisor Sean Moore for their guidance, lots of patience and constructive criticism.
- Unathi Heshula who helped with the initial setup of the Y-tube olfactometer trial.
- Mathew Goddard, Pippa Muskett, Claire Love and Sam Johnson for their help during the field trials.
- NRF-THRIP Programme of the Department of Science and Technology, Rhodes University and Citrus Research International for the funding of the project.
- Mellissa Peyper for her help collecting FCM pupae and maintaining the FCM colonies.
- Jeanne Van der Merwe who helped me source the equipment that I required.
- Xsit who provided me with all the sterile moths that were essential in conducting the field trials.

ABSTRACT

False codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) is considered the most important indigenous pest of citrus in southern Africa. It is recognized by several markets as a phytosanitary organism and the efficient control of this pest is now more important than ever. The pheromone communication between the male and female moths has been exploited in order to control FCM through the sterile insect technique (SIT). The sterilized males used for all SIT programmes across South Africa come from a colony that originates from wild material collected from the Citrusdal area of the Western Cape Province. The aim of this study was to determine if any differences in attractiveness of females to males exist between different geographical populations of FCM and if so what impact this would have on the male's ability to locate females from other populations via the volatile sex pheromone released by the female. Laboratory trials with Y-tube olfactometers and flight tunnels tested the attraction of male moths to virgin females, but did not yield any consistent results. Field experiments were conducted with sterile male Citrusdal moths released and recaptured in yellow delta traps in two separate trials. For one trial, the traps were baited with live virgin females from five different geographical populations including Addo, Nelspruit, Marble Hall, Citrusdal and the Old colony, which is a mixture of several populations. For the other trial traps were baited with various synthetic pheromone blends including three regional blends which included South Africa, Ivory Coast and Malawi and three commercial blends including Pherolure, Isomate and Checkmate. For the virgin female trial the Citrusdal males showed a significant preference for females from their own population. There was also a significant difference in the recaptures from the different synthetic pheromones. The South African blend was the most attractive of all the regional and commercial blends. A cross-mating trial was also conducted under laboratory conditions in petri dishes with five different FCM populations including Citrusdal, Addo, Marble Hall, Nelspruit and Old (mixed origin). Females produced more eggs when mated with males from the same population for the Addo, Marble Hall, Nelspruit and Old (mixed origin) populations. The only case in which this was statistically significant was for the Marble Hall population. All the crosses produced viable eggs and the

origin of the male or female did not influence egg hatch. The results from this study may lead to improvements in both the control and monitoring of FCM populations. The control methods include mating disruption, attract-and-kill and SIT. Tailoring these methods for a specific growing area with a pheromone blend originating from the area or releasing sterile moths from a colony that originates from the area may optimize the available monitoring and control options.

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LIST OF ABBREVIATIONS

ALPPs - areas of low pest prevalence

ANOVA - Analysis of variance

CGA - Citrus Growers Association

CM - codling moth

cm - centimetres

CRI - Citrus Research International

CrleGV - *Cryptophlebia leucotreta* granulovirus

dH_2O - distilled water

EAG - electroantennograms

e.g. - example

EPN - entomopathogenic nematode

EPPO - European and Mediterranean Plant Protection Organization

et al. - et alia (and others)

F1 - first filial generation

F2 - second filial generation

FCM - false codling moth

g - grams

Gy - gray

i.e. - id est (namely)

IPM - integrated pest management

kg - kilogram

L - litre

Ltd - limited

mg - milligram

ml - millilitre

mm - millimetre

SA-DAFF - South African Department of Agriculture, Forestry, and Fisheries

SE - standard error

SIT - sterile insect technique

XSIT - X Sterile Insect Technique Pty. (Ltd)

& - and

% - percentage

°C - degrees Celsius

± - standard error

° - degree

Chapter 1: General introduction

1.1 THE PEST (FALSE CODLING MOTH, *Thaumatotibia leucotreta*)

1.1.1 Taxonomy and distribution

False codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Torticidae), is considered to be the most important pest of citrus in South Africa (Moore *et al.* 2008). This species was first noted as being a pest of citrus in South Africa during the early 1900s when larvae were recorded within infested citrus fruit in KwaZulu-Natal Province of South Africa (Fuller 1901). The species was first referred to as the Natal codling moth and identified as *Carpocapsa* sp. (Fuller 1901). During the early 1900s the species was also referred to as the orange codling moth. Meyrick later re-described the species as *Argyroploce leucotreta* (Eucosmidae, Olethreutinae) (Meyrick 1912). It was Meyrick who gave the name false codling moth and this common name has remained throughout the years. The species classification did not remain for long before Clarke (1958) classified the moth under the genus *Cryptophlebia*. This species was later moved to the family Tortricidae from the family Olethreutidae during the 1980s (Annecke & Moran 1982). The classification was most recently changed to *Thaumatotibia leucotreta* (Meyrick) (Komai 1999). There are two other members of the same or previous genus that are economically important to tropical and subtropical fruit industries of South Africa (Timm *et al.* 2008), the macadamia nut borer, *Thaumatotibia batrachopa* (Meyrick), and the litchi moth, *Cryptophlebia peltastica* (Meyrick). False codling moth is indigenous to South Africa and its native range includes much of sub-Saharan Africa (Figure 1.1) (Venette *et al.* 2003). This moth has also been reported to inhabit the islands of both the Indian and Atlantic oceans that surround sub-Saharan Africa (Hill 1975). False codling moth has also established in Israel where it is a pest of macadamias, cotton, castor bean and most recently it was intercepted in citrus exports (Wysoki 1986; EPPO 2016; Europhyt 2017).

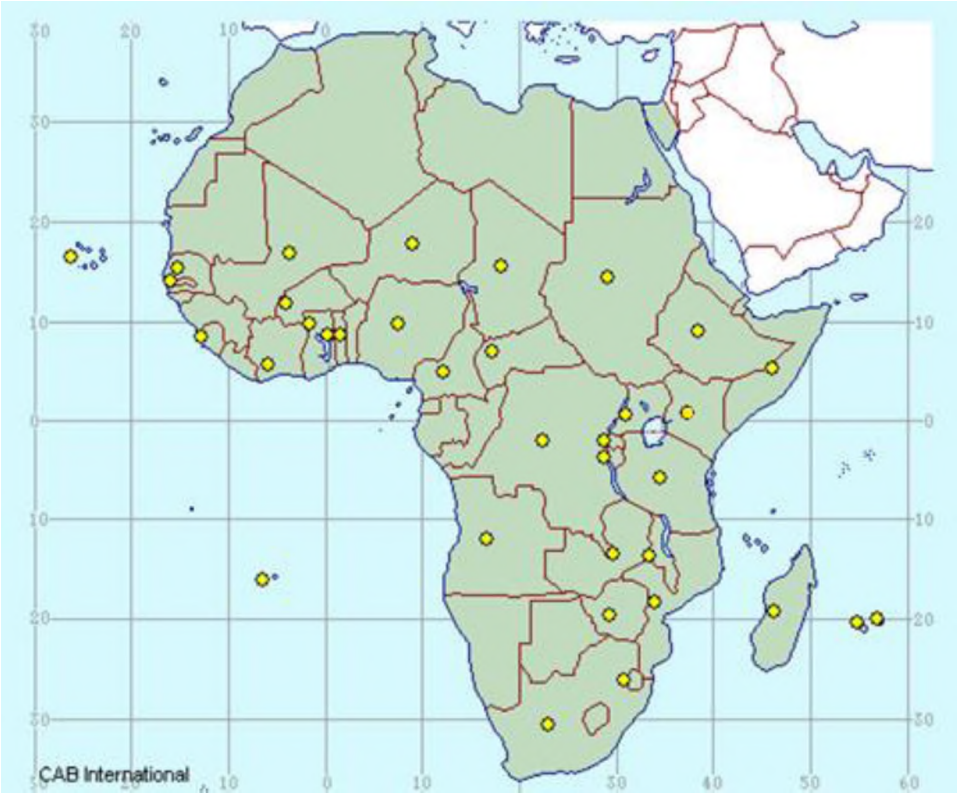

Fig. 1.1 The reported geographic distribution of FCM across Africa and its neighbouring islands highlighted by the yellow dots (http://www.cdfa.ca.gov.html)

1.1.2 Host range

The host range of FCM is extensive and comprises both wild and commercially cultivated hosts (Table 1.1) (Newton 1998). The large number of hosts that FCM can exploit and in which they can complete their life cycle is concerning for the pest status of the insect, as even if FCM is successfully controlled in citrus orchards, natural areas in the vicinity could harbour a FCM population (Kirkman & Moore 2007). It is however important to note that some of these hosts are unverified and lack original references. The European and Mediterranean Plant Protection Organization (EPPO) conducted a thorough review of the host list (EPPO 2013).

Table 1.1 The plants that have been linked to damage that has been caused by false codling moth (FCM) (Kirkman & Moore 2007; Stibick 2006; Venette *et al.* 2003).

Scientific Name	**Common Name**
Abelmoschus esculentus	Okra
Abutilon hybridum	Indian mallow
Abutilon spp.	Flowering maple, Mallow
Ananas comosus	Pineapple
Annona cherimola	Cherimoya
Annona glabra	Pond apple
Annona muricata	Soursop
Annona reticulata	Custard apple
Annona squamosa	Sugar apple
Annona spp.	Sweetsop
Averrhoa carambola	Carambola
Azanza garckeana	Snot apple
Bauhinia galpini	Pride of De Kaap
Butryospermum parkii	Butterseed
Caesalpinia pulcherrima	Peacock flower
Calotropis procera	Sodom apple
Camellia sinensis	Tea
Capparis tomentosa	Woolly caper-bush
Capsicum spp.	Pepper
Cassia petersiana	Monkey pod
Catha edulis	Khat
Ceiba pentandra	Kapok
Chrysophyllum cainito	Star apple
Citrus aurantiifolia	Lime
Citrus limon	Lemon
Citrus paradisi	Grapefruit
Citrus paradisi x Citrus reticulata	Tangelo
Citrus reticulata	Mandarin orange
Citrus reticulata x Citrus sinensis	Temple orange
Citrus sinensis	Sweet orange
Citrus spp.	Orange
Coffea arabica	Coffee
Coffea spp.	Coffee
Cola nitida	Cola
Combretum apiculatum	Red bushwillow, Rooibos
Combretum zeyheri	Large fruited bushwillow
Cyphomandra betacea	Tree tomato
Diospyros mespiliformis	Jakkalsbessie
Diospyros spp.	Persimmon
Englerophytum magalismontanum	Stemfruit
Eriobotrya japonica	Loquat
Eugenia uniflora	Surinam cherry
Ficus capensis	Wild fig
Flacourtia indica	Governor's plum
Garcinia mangostana	Mangosteen

Gossypium hirsutum	Cotton
Gossypium spp.	Cotton
Harpephyllym cattrum	Kafir plum
Hibiscus spp.	Hibiscus
Juglans regia	English walnut
Juglans spp.	Walnut
Litchi chinensis	Litchi
Lycopersicon esculentum	Tomato
Macadamia ternifolia	Macadamia nut
Macadamia spp.	Macadamia
Mangifera indica	Mango
Mumisops zeyheri	Red milkweed
Musa paradisiaca var. sapientum	Banana
Olea europaea	Olive
Pennisetum purpureum	Elephant grass
Persea americana	Avocado
Phaseolus lunatus	Lima bean
Physalis ixocarpa	Husk tomato
Physalis spp.	Ground cherry
Podocarpus falcatus	Outeniqua yellowwood
Prunus armeniaca	Apricot
Prunus domestica	Prune
Prunus persica	Peach
Prunus spp.	Cherry (all), Plum
Pseudolachnostylis maprounaefolia	Kudu-berry
Psidium guajava	Common guava
Punica granatum	Pomegranate
Quercus spp.	Oak
Ricinus communis	Castor bean
Royena pallens	Bluobos
Schotia afra	Boerboon
Schotia speciosa	Hottentot kafir bean tree
Sclerocarya birrea	Marula
Sclerocarya caffra	Marula, Kafir marvolanut
Sechium edule	Chayote
Sida spp.	Sida
Solanum melongena	Eggplant
Sorghum vulgare	Sorghum
Sorghum spp.	Sorghum
Syzygium cordatum	Water-bessie
Syzygium jambos	Rose apple
Synsepalum dulciticum	Miraculous berry
Theobroma cacao	Cacao
Triumfetta spp.	Burrbark
Vangueria infausta	Wild medlar
Vigna unguiculata	Cowpea

Vigna spp.	Cowpea
Vitis vinifera	Grape
Vitis spp.	Grape
Xeroderris stuhlmannii	Wing bean
Ximenia caffra	Large sour plum
Yucca alofolia	Spanish bayonet
Yucca gloriosa	Spanish dagger
Yucca spp.	Yucca
Zea mays	Corn
Ziziphus jujube	Jujube
Ziziphus mucronata	Buffalo thorn

Table 1.2 The hosts that can support the completion of the false codling moth (FCM) life cycle (EPPO 2013). Lemons are listed as a minor host, however Moore *et al.* (2015a) have shown that lemons are not a suitable host for FCM.

Major
Capsicum annuum
Caspicum chinense
Citrus paradisi
Citrus sinensis
Gossypium hirsutum
Minor
Citrus
Citrus limon
Gossypium
Macadamia ternifolia
Persea americana
Prunus persica
Ricinus communis
Zea mays
Unclassified
Abelmoschus esculentus
Abutilon x hybridum
Ananas comosus
Annona muricata
Averrhoa carambola
Camellia japonica
Camellia sinensis

Ceiba pentandra
Coffea arabica
Diospyros kaki
Eriobotrya japonica
Juglans regia
Litchi chinensis
Mangifera indica
Musa x paradisiaca
Olea europaea
Phaseolus
Psidium guajava
Punica granatum
Quercus
Sorghum
Theobroma cacao
Vigna unguiculata
Vitis

1.1.3 Biology and life history on citrus

False codling moth females lay their eggs singly on the surface of the target citrus fruit. Up to 65 eggs can be laid on a single fruit, but this high number of eggs on a single fruit is rare (Stofberg 1954). The cream coloured eggs are small, approximately 0.8 mm in length and 0.6 mm in width (Daiber 1979a). The time the eggs take to hatch varies between the cooler winter and warmer summer months. It takes approximately 9 to 12 days during winter and a shorter 6 to 8 days in summer ((Daiber 1979b; Newton 1998). As soon as the larvae hatch they start to burrow into the fruit and feed on the pulp (Newton 1998). Despite several eggs sometimes being laid on a fruit, usually only one larva is present within each fruit. This may be partly due to the cannibalistic nature of the larvae, but also due to the high mortality rate of the neonate larvae. The completion of larval development also varies between winter and summer. It takes approximately 35 to 67 days during winter and a much shorter 25 to 35 days during summer (Daiber 1979b; Newton 1998). During the development of the larva it goes through five instars. The mature larva of approximately 15 mm and larger has a distinct dark pink colour with a

black head. It is during the last instar that the larva emerges from the fruit; at this stage the fruit may still be on the tree, but most likely it has dropped to the ground. As soon as the larva emerges from the fruit it starts to spin a cocoon and leaf litter and soil is incorporated into the cocoon. The pupa is dark brown once it has hardened. The duration of the pupal stage in the field varies between 29 to 40 days in the winter and 12 to 24 days in the summer (Stofberg 1954). The adult moths are cryptic in nature. The total development of FCM takes 2.5 to 4 months during winter and 1.5 to 2 months during summer with a total of 5 to 6 generations each year (Daiber 1980; Newton 1998).

1.1.4 Impact of false codling moth on the citrus industry

South Africa is a major exporter of citrus fruit and as of 2014 was the second largest exporter globally (CGA 2015). Like most fruit citrus is adversely affected by a number of insect pests. In South Africa there are numerous important arthropod and molluscan pests of citrus (Moore *et al.* 2008; Moore & Hattingh 2012; Grout & Moore 2015). False codling moth is one of these pests and is considered to be the most important pest of citrus in South Africa (Grout & Moore 2015). The impact of this pest can be accredited to the nature in which this pest utilizes citrus fruit and its endemism to sub-Saharan Africa. After infestation by an FCM larva, the entrance wound that the larva makes is not always visible to the naked eye (Moore 2002). If the fruit is harvested close to this event, then there is a possibility of this wound not being noted during the fruit processing and packing procedure. The infested fruit can then cause problems once it is packaged and shipped overseas: the fruit may rot causing other fruit in its immediate vicinity to also spoil; consumers may find infested fruit; or the consignment of fruit may be rejected by the importing country for phytosanitary reasons as the moth is not present in the export market localities (Moore 2002). Currently some citrus export markets have very strict phytosanitary requirements in place for FCM. The Peoples Republic of China, United States of America, Japan, South Korea and most recently, the European Union, regulate it as a quarantine pest and mandatory post-harvest cold treatments are in place for citrus from South Africa (SA-DAFF 2014). The expectation for high quality fruit and strict phytosanitary

conditions, implemented for some export markets, force farmers to prioritize the control of FCM (Moore & Kirkman 2009). False codling moth numbers need to be kept to a minimum and this means optimizing all the available control methods.

1.1.5 The methods available for FCM management

1.1.5.1 Inspection and monitoring

Inspection and monitoring are an integral part of any management programme being implemented to effectively control a pest (Schwartz 1972; Hofmeyr 2003; Moore *et al.* 2008). Knowing the levels of FCM infestation can be crucial to helping the farmer to decide whether and when to implement a control measure. The inspection of fruit for the presence of eggs has been used. This technique is not very effective due to the small size of the eggs and the presence of eggs on a fruit does not guarantee that the fruit will become infested (Hofmeyr 2003). A more common method of inspection is the collection of weekly fruit fall data and the subsequent dissection of some fruit to determine the degree of larval infestation. This method has its drawbacks because it does not help the farmer to act before the damage has been done (Hofmeyr 2003). The most effective and popular approach is the monitoring of FCM population numbers with the aid of pheromone traps (Moore *et al.* 2008). At this stage it is only possible to trap the male moths with the aid of synthetic pheromone lures used as an attractant. Future studies may find a way to attract and trap female moths. The most popular trap used in South African citrus orchards is the Delta trap. These traps are baited with an synthetic pheromone lure and the base of the trap is lined with paper coated with a sticky adhesive that traps the moths (Moore *et al.* 2008). The traps are checked weekly and the number of trapped moths per week gives the farmer an indication of when to implement preventative control measures. The historic threshold before control is implemented is a weekly FCM trap catch count of 10 male moths per week per trap (Hofmeyr 2003; Moore *et al.* 2008). This threshold should be ignored as it is only an indication of when pre-harvest fruit losses will become substantial and it does not consider the likelihood of post-harvest fruit being infested with FCM. With the phytosanitary status of FCM it is recommended that fruit fall and

trap catch data be used to help with the timing of corrective measures (Moore *et al.* 2008; Moore 2016; Grout & Moore 2015). A peak in flight activity of the male moths, which is recorded by trap catches, is followed by a peak in egg hatch one to two weeks afterwards (Moore *et al.* 2008).

1.1.5.2 Cultural control

Basic orchard sanitation is a common method of FCM control on the majority of citrus farms (Moore & Kirkman 2009). Orchard sanitation was the main method of FCM control approximately 30 years ago (Moore & Kirkman 2009), when very few other options were available. The efficacy of the method was shown in field trials approximately 65 years ago (Stofberg 1954). The method remains unchanged and consists of the weekly removal of all fallen fruit from the orchards. The collected fruit is then ground to a pulp in a fruit mulcher to destroy any of the larvae that might be in the fruit. Orchard sanitation is considered to be the foundation of FCM control in citrus (Moore 2016). If an efficient weekly orchard sanitation programme is followed, fruit infestation can be reduced by 75 % (Moore 2016).

1.1.5.3 Chemical control

The first chemical products for the control of FCM were only registered during the late 1980s (Homeyr & Pringle 1998). Prior to this, trials with chemicals such as DDT and parathion were conducted and showed considerable promise, however no products were registered. Synthetic pyrethroids and their use for the control of FCM was investigated during the early 1980s (Hofmeyr 1983a; Hofmeyr 1983b). Fruit loss was shown to decrease by up to 90 % after an application two to three months prior to harvest (Hofmeyr 1983a; Hofmeyr 1983b). Effective control was most likely achieved through the loss of viability of eggs that were present on the fruit. Fenopropathrin and cypermethrin are two synthetic pyrethroids still in use for the control of FCM (Croplife 2016; Moore & Hattingh 2016). There are six pyrethroid products registered for the control of FCM on citrus, five containing cypermethrin and one containing fenopropathrin (Table 1.3). These products have a direct impact on the viability of the eggs.

The main way in which these chemicals impact on FCM is via the inhibition of the female's egg laying ability, the chemical also has a negative impact on the eggs decreasing their probability of hatching (Hofmeyr 1983a; Hofmeyr 1983b). However, as with the chitin synthesis inhibitors discussed below, these chemical products also have a negative impact on natural enemies (Moore *et al.* 2004). A study by Moore *et al.* (2004) showed that orchards treated with these chemicals may even experience elevated levels of FCM infestation compared to an untreated control orchard, as a result of their effect on egg parasitoids. Non-target effects are thus the biggest downfall of synthetic pyrethroids. Hofmeyr (1983b) reported repercussions of the citrus pest red mite *Panonychus citri* (McGregor) (Acari: Tetranychidae), after the use synthetic pyrethroids.

Table 1.3 Products registered for the chemical control of FCM on citrus in South Africa (Croplife 2016).

Product name	Active ingredient	Company name
Coragen	chorantraniliprole	Du Pont de Nemours
Ag-Cypermethrin 200 EC	cypermethrin	Ag-Chem Africa (Pty) Ltd
Avi-Sipermetrin	cypermethrin	Avima (Pty) Ltd
Chanrai Cypermethrin 200EC	cypermethrin	Chanrai SA (Pty) Ltd
Cypermethrin 200 EC	cypermethrin	Agro-Serve t/a Efekto
Novacord	cypermethrin	Global Agrosciences
Meothrin	fenpropathrin	Philagro South Africa (Pty) Ltd
Runner 240 EC	methoxyfenozide	Dow Agroscience SA
Rimon 10 EC	novaluron	Makhteshim-Agan SA (Pty) Ltd
Delegate 250 WG	spinetoram	Dow Agroscience SA
Nomolt	teflubenzuron	BASF South Africa (Pty) Ltd
Alsystin 480 SC	triflumuron	Bayer Crop
Triflumuron 480	triflumuron	RT Chemicals

Teflubenzuron and triflumuron are insect growth regulators that inhibit chitin synthesis in many insects, including FCM (Hofmeyr 1984). These benzoylated ureas have been popular

for the control of FCM (Newton 1987; Moore & Hattingh 2016). Newton (1987) showed that a single application of these chemicals can reduce fruit loss by as much as 86 %. Three products are currently registered in South Africa; Alsystin 480 SC (Bayer Crop), Triflumuron 480 (RT Chemicals) and Nomolt (BASF South Africa (Pty) Ltd) (Croplife 2016; Moore & Hattingh 2016). These products specifically target embryonic development, preventing egg hatch. The timing of application is essential to ensure that the product is applied before the eggs are laid (Newton 1987). If the eggs are laid on the surface of the citrus fruit coated with the chemical product it will take effect. Weather conditions also influence the efficacy of these products. Rainfall soon after or during application of the chemical as a spray onto the trees will remove the chemical residue from the fruit. Export markets periodically change their views and restrictions on the use of chemicals and chemical residues on fruit; this has led to a decrease in the usefulness and popularity of these products. The biggest downfall of the chitin synthesis inhibitors is their negative impact on natural enemies, specifically concinellid beetles (Moore *et al.* 2004). This has led to fewer farmers utilizing these products because they prefer to have an IPM approach implemented on their farms. The popularity of these products and their regular use has resulted in FCM developing resistance to these products (Homeyr & Pringle 1998).

The two recently registered chemicals spinetoram (Delegate 250 WG) and methoxyfenozide (Runner 240 EC), both currently produced by Dow Agroscience SA, have grown in popularity. Moore (2015b) showed that these products are also very effective in controlling FCM and are better suited for an integrated pest management (IPM) approach, due to their less severe negative impact on beneficial insects.

1.1.5.4 Biological control

False codling moth has a wide range of natural enemies consisting of predators, parasitoids and pathogens (Grout & Moore 2015). Ullyett (1939) initiated the research effort to investigate the natural enemies of FCM in South Africa. A total of 25 natural enemies of FCM have been recorded and 12 of these have been identified in citrus orchards in South Africa (Ullyett 1939; Schwartz 1977; Catling & Aschenborn, 1974). The most effective natural

enemy of FCM in South Africa is the egg parasitoid *Trichogrammatoidea cryptophlebiae* (Nagaraja) (Hymenoptera: Trichogrammatidae) (Newton & Odendaal 1990; Moore & Richards 2002; Moore & Hattingh 2012). The way in which farmers maximize the benefits of this parasitoid is to augment the natural population with additional releases of the parasitoid (Moore & Hattingh 2012). It is recommended that 100 000 parasitoids per ha should be released each season, starting in October and it has been shown that following this recommendation can lead to a reduction in fruit infestation of up to 60% (Newton & Odendaal 1990; Moore & Hattingh 2012). There are other natural enemies that are viable alternatives for release in citrus orchards and the current movement to avoid chemical control has ensured that environmentally friendly alternatives receive considerable attention from growers. For example a natural enemy that shows considerable potential is the larval parasitoid *Agathis bishopi* (Nixon) (Hymenoptera: Braconidae). It has been shown that this parasitic wasp can parasitize approximately 34 % of FCM larvae in the field (Gendall 2007; Zimba *et al.* 2015).

There are also other natural enemies that have the potential to be valuable assets to farmers. Those that are receiving considerable attention from researchers include entomopathogenic nematodes (EPN) and entomopathogenic fungi (EPF) (Malan *et al.* 2011; Manrakhan *et al.* 2014; Coombes *et al.* 2016; Malan & Moore 2016). A number of EPFs have been identified as effective control agents for FCM under laboratory conditions and include the fungal isolates *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) isolate G Ar 17 B3 and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) isolate FCM Ar 23 B3 (Coombes *et al.* 2016). Coombes *et al.* (2016) tested the efficacy of these EPF isolates in commercial citrus orchards, and showed that these isolates can reduce FCM infestation by 28% to 82%. The EPN species that have been identified as candidates for the control for FCM larvae and pupae include *Heterohabditis bacteriophora* (Poinar) (Rhabditida: Heterorhabtidae) and *Steinernema feltiae* (Filijev) (Nematoda: Steinernematidae) (Malan *et al.* 2011). Manrakhan *et al.* (2014) showed that the naturally occurring EPN species in Mpumalanga, *H. bacteriophora*, can account for a 58.6%

lower infestation of FCM compared to a block where the nematodes were suppressed by nematicide application.

Controlling pests with a virus has little to no negative impact on the environment due to the very narrow host range of viruses (Evans 2000). Baculoviridae comprises of four genera, *Alphabuaculorirus*, *Betabaculovirus, Gammabaculovirus* and *Deltabaculovirus* (Herniou *et al.* 2011). However, only the first two of these are important for biological control in agriculture and are also known as Nucleopolyhedrovirus (NPV) and Granulovirus (GV) (Murphy *et al.* 1995). The baculovirus *Cryptophlebia leucotreta* granulovirus (CrleGV) is a naturally occurring indigenous pathogen that is used as a biological control agent for FCM (Moore 2002). *Thaumatotibia leucotreta* used to be known as *Cryptophlebia leucotreta* and was changed during the 1990s (Komai 1999), thus the virus was named after FCM when it was still classified under a different genus. There are three registered products available that utilize this pathogen for the control of FCM in South Africa; Cryptogran (River Bioscience, South Africa), Cryptex (Andermatt, Switzerland) and most recently Gratham (Andermatt, Switzerland). These products are applied to citrus trees in the form of a spray that coats the citrus fruit. Once the fruit has been coated any FCM larva that hatches and burrows into the fruit will ingest virus particles causing the infection and subsequent death of the larva (Moore *et al.* 2004). The time of application is crucial to ensure the effectiveness of these products (Moore 2016). Monitoring of the FCM population numbers help farmers to maximize the effectiveness. Moore *et al.* (2004) conduced a trial in the Sundays River Valley (SRV) with Cryptogran and reported a reduction in FCM infestation in a Navel orange orchard by up to 70% for 17 weeks. Unlike chemicals these products do not harm natural enemies of FCM because the virus is host specific. Chemical control can be used to compliment this form of control as the registered chemicals do not have any impact on the virus (Moore *et al.* 2004).

1.1.5.6 Mating disruption

There are approximately 160 000 known species of moths and the predominant system for mate location is the upwind flight of a male towards a sex pheromone released by the female (Cardé & Minks 1995; Johansson & Jones 2007). Moths have evolved a communication system where the females release a pheromone specific to their species and the males then respond to this signal to locate a female (Cardé & Minks 1995). The release of a sex pheromone by a female moth leads to strong competition between males to be the first to locate the calling female (Cardé & Haynes 2004). This leads to scramble competition among male moths (Cardé & Haynes 2004; Johansson & Jones 2007). Gaston *et al.* (1967) showed in their pioneering field trials 50 years ago, that it is possible to disrupt a male moth's ability to locate virgin females. In their trials the ability of cabbage looper (*Trichoplusia ni*) males to locate virgin females was disrupted after the widespread release of synthetic sex pheromone in a small plot (Gaston *et al.* 1967). Mating disruption has become a widely used method of control for cryptic lepidopteran pests (Witzgall *et al.* 2010; Mori & Evenden 2013). The larvae of pests such a leafminers, bollworms and borers are very cryptic and they avoid contact with conventional insecticides because the host plant that they enter provides them with a barrier from direct exposure (Witzgall *et al.* 2010). Mating disruption can be achieved in different ways which fall under two categories, including competitive and non-competitive (Miller *et al.* 2006). With competitive mechanisms the males are oriented away from a calling female in favour of a dispenser that releases synthetic pheromone (Miller *et al.* 2006). The non-competitive mechanisms are quite diverse and are not independent of one another (Mori & Evenden 2013). They include sensory adaptation of the pheromone receptors on the antennae, habituation of the central nervous system to pheromone, imbalance of sensory system, and camouflage of the natural pheromone plume from calling females (Cardé & Minks 1995).

Mating disruption is also a widely used method of FCM control used by citrus farmers. The synthetic pheromones are made up of different ratios of (E)-7-dodecenyl acetate, (E)-8-dodecenyl acetate and (Z)-8-dodecenyl acetate to mimic the pheromone released by the

female (Moore & Hattingh 2012). Isomate (Bioglobal Limited, Australia) is registered as a mating disruption product for FCM (Moore & Hattingh 2012). The product comes in the form of a polyethylene tube dispenser that is hung in a citrus tree and releases synthetic pheromone. Isomate has been shown to reduce FCM infestation by 55% in an orchard with high FCM abundance and by 75% in an orchard with low FCM abundance from December to April (Moore & Hattingh 2012). This reduction was shown to be as high as 86% and 95% closer to harvest (Moore & Hattingh 2016). Checkmate (Suterra, USA) is also registered as a mating disruption product for FCM (Moore 2016). The product comes in the form of a pheromone containing capsulated suspension formulation which is applied as a spray every 21-28 days to the top third of the citrus trees in an orchard (Moore 2016; Moore & Hattingh 2012). Within the middle of this year two new products have been registered including Splat (River Bioscience) and X-Mate (Insect Science). The drawback of these products is that they interfere with the monitoring of FCM population numbers utilizing synthetic pheromone lures. The amount of the synthetic pheromone that is released into the orchards masks the pheromone output from traps baited with a synthetic pheromone lure. This leads to lower trap catches and produces data on FCM numbers which does not represent the actual population levels of FCM in that area.

1.1.5.7 Attract and kill

Attract-and-kill is another method of pheromone-based suppression that is a combination of an synthetic sex pheromone and an insecticide (Charmillot *et al.* 2000; Krupke *et al.* 2002). There are exceptions where the attractant is not a pheromone. For example, the attract-and-kill formulation for *Amyelois transitella* (Walker) contains an allomone as an attractant (Phelan & Baker 1987). Attract-and-kill is a widely used method used for the species-specific control of lepidopteran pests and the products used are often referred to as attracticides (Charmillot *et al.* 2000; Poullot *et al.* 2001; Krupke *et al.* 2002; Kroschel & Zegarra 2010; Campos & Phillips 2014). Instead of flooding the target area with large amounts of synthetic pheromone, the synthetic pheromone is used at a lower concentration along with a

pyrethroid or pyrethrin, in order to attract and kill the male moth (Charmillot *et al.* 2000). The problem with the attract-and-kill strategy is that it is not very effective at reducing pest numbers in areas of high pest abundance (Charmillot *et al.* 2000; Poullot *et al.* 2001; Krupke *et al.* 2002). The attracticide is in direct competition with calling females releasing pheromone (Poullot *et al.* 2001). The use of an attracticide is much more effective when used in an area where the pest abundance is lower and there is thus less competition from females (Poullot *et al.* 2001). Attract-and-kill is also used for the control of FCM. Last Call FCM (Insect Science, South Africa) is registered for the control of FCM (Moore & Hattingh 2012). The formulation consists of a synthetic pheromone, a pyrethroid and protective gel. The product is applied to the trees, the male moths are attracted to the droplets and are killed soon after they make contact with a droplet (Moore & Hattingh 2012). This method has been shown to not to be very effective against high levels of FCM, as with other attract-and-kill products developed for other lepidopterans (Poullot *et al.* 2001; Krupke *et al.* 2002).

1.1.5.8 Sterile insect technique

The sterile insect technique (SIT) dates back to early 1930s (Klassen 2005). Three researchers independently developed SIT in three very different environments, including A.S. Serebrovskii (Moscow State University), F.L. Vanderplank (Tanzania) and E.F. Knipling (United States Department of Agriculture) (Robinson 2002; Klassen 2005). Serebrovskii focused on genetic studies on *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) during the 1930s and 1940s (Robinson 2002). It was determined that inherited sterility can be achieved by releasing insects with translocation homozygotes. Vanderplank (1947) did his work on tsetse fly while working at a tsetse field research station in Tanzania. He found that sterility can be induced when two tsetse fly species are crossed. When *Glossina swynnertoni* (Austen) and *Glossina morsitans* (Westwood) (Diptera: Glossinidae) were crossed the subsequent hybrids had different genitalia and both the male and female hybrids showed partial sterility (Vanderplank 1947). Following this study the *G. swynnertoni* population was virtually eradicated from the area after releases of male *G. morsitans.* Knipling (1955) did his

work on the mating behaviour of the New World screwworm, *Cohliomyia hominovorax* (Coquerel) (Diptera: Calliphoridae). By releasing a high number of sterile screwworms, leading to a high ratio of sterile:fertile screwworms in the field, he found that the pest could be suppressed (Klassen 2005). His work on mathematical models determined the probability of eradicating the screwworm from an area (Klassen 2005). The eradication of this pest from large areas in North America during the 1950s is the first major success story where SIT was implemented as an area-wide method of control (Vargas-Teran *et al.* 2005).

The basis of SIT is to mass-rear the species which is being targeted for control, to sterilize it with ionizing radiation and release high numbers of the sterilized insects into the target area (Klassen 2005). The number of sterile insects that are released is crucial, so that the correct sterile male to wild female ratio is achieved (Klassen *et al.* 2005; Barnes *et al.* 2015). This ensures that sufficient sterility is introduced into the target areas wild population and the required ratio (sterile male:wild female) differs between species (Klassen 2005). The sterile males then mate with the females from the wild population and they then produce eggs which are infertile. Consequently the population numbers of the following generation are much lower (Barnes *et al.* 2004). In its early stages of development and implementation SIT was not always very effective and was met with a lot of resistance from sceptics (Krafsur 1998). Since the initial stages of SIT, the method has grown and numerous insect species have been controlled successfully using this method (Klassen 2005). The possible strategic outcomes of control with SIT include pest eradication, suppression, containment and prevention (Hendrichs *et al.* 2005). Although eradication may be seen as the ultimately desired outcome, the difficulty and costs involved in securing an area as pest-free are extremely high (Barnes *et al.* 2015). Consequently, the use of an SIT programme is now more often seen as a tool to help suppress the wild population of a pest in order to create 'areas of low pest prevalence' (ALPPs) as part of a 'systems approach' (Klassen 2005). The use of SIT is now often included in an area-wide integrated pest management (IPM) programme to optimise the suppression of the target pest (Moore and Hattingh 2012; Barnes *et al.* 2015).

When it comes to starting an SIT programme, certain principles need to be satisfied in order to ensure its success and sustainability (Barnes 2007). All these principles can be considered to be of equal importance, because if any single one is not satisfied the programme will not be successful (Barnes 2007). These principles include cost-effective mass-rearing of the target pest, isolation of the target area, an area-wide approach, integration into an IPM programme, adequate funding, support from beneficiaries, realistic promises and realistic expectations (Barnes *et al.* 2015). Some examples of SIT programmes that have been successfully implemented worldwide include programmes for the Mediterranean fruit fly (medfly), *Ceratitis capitata (*Wiedemann*)* (Diptera: Tephritidae) in Guatemala, Mexico and South Africa, the tsetse fly *Glossina austeni* (Newstead) (Diptera: Glossinidae) in Zanzibar, the onion fly, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae) in the Netherlands, the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) in the Ryukyu Islands of Japan, the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechidae) in the Central Valley of California, USA, the codling moth (CM) *Cydia pomonella,* (Linnaeus) (Lepidoptera: Tortricidae) in British Columbia, Canada and the false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) in the Western Cape and Eastern Cape provinces of South Africa (Bloem *et al.* 2003).

Xsit (Pty) Ltd commercialized the use of SIT for FCM in South Africa in 2007 (Barnes *et al.* 2015). They utilize the method that aims to flood the environment with sterile males so that the wild males have to compete with the sterile moths for mates. The moths that are used for all their programmes are reared and sterilized at their facility in Citrusdal with a low dose of gamma radiation (150 Gy) from a ^{60}Co panoramic point source radiator (Hofmeyr *et al.* 2015). Sterile moths are released on a regular basis to ensure a high number of sterile males. The ratio they aim to reach is at least 10 sterile to 1 wild male FCM (Hofmeyr *et al.* 2015). The competitive ability of the sterile moths remains sufficient for them to be able to compete with wild moths. The first area where this control method was utilized was in the Olifants River

Valley (32°35'21"S19°00'54"E) in the Citrusdal area of the Western Cape (Bloem *et al.* 2003; Hofmeyr *et al.* 2015; Barnes *et al.* 2015).

After the implementation of routine aerial releases of the sterilized moths in the Olifants River Valley, the number of wild FCM trap catches and fruit infestation by FCM was reduced significantly (Barnes *et al.* 2015). The weekly average trap catches of male moths per trap was at 13.0 during 2006 and was reduced to 2.0 in 2012, 0.4 in 2013 and 0.1 in 2014 (Figure 1.1) (Barnes *et al.* 2015). The infestation of fruit with FCM measured from collecting and cutting fallen fruit was reduced from 2.6 % in 2010/11 to 0.1 % in 2013/2014 (Figure 1.2) (Barnes *et al.* 2015). Programmes have also been implemented in the Sundays River Valley (33°27'39"S25°33'45"E) and Gamtoos River Valley (33°44'21"S 24°46'24"E) of the Eastern Cape (Bloem *et al.* 2015). The method was introduced to the Sundays River Valley during 2011 and Xsit plans to roll out the SIT method to other citrus growing areas of South Africa.

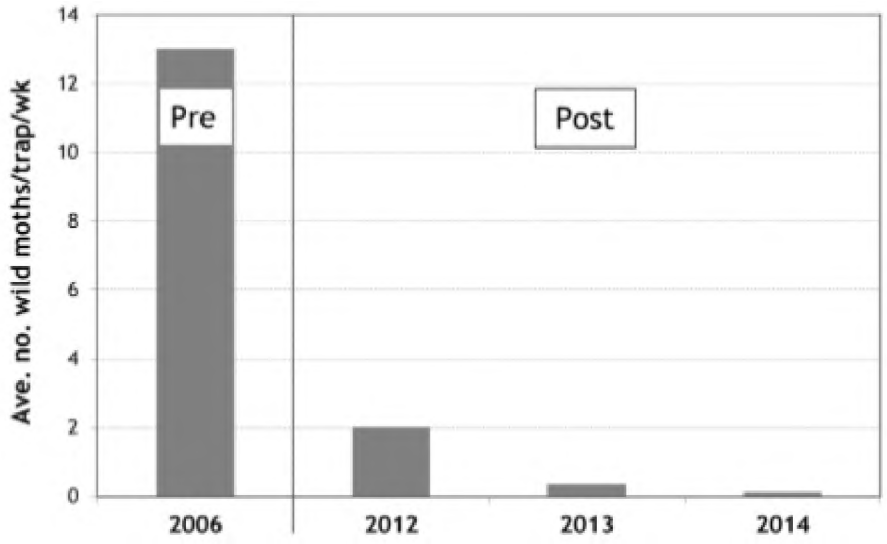

Fig. 1.2 Average number of wild false codling moth, *Thaumatotibia leucotreta*, adults caught in pheromone traps before and after the releases of sterile adults (Barnes *et al.* 2015).

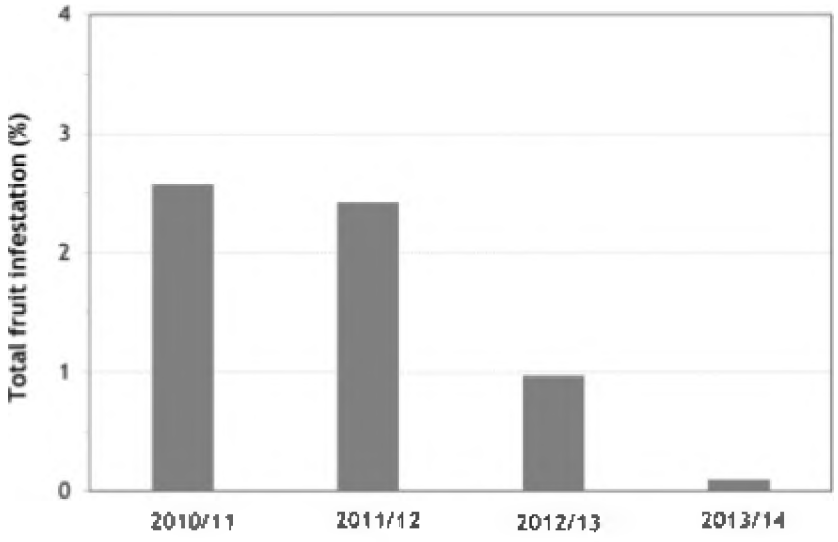

Fig. 1.3 Percentage of total fallen fruit infested by false codling moth, *Thaumatotibia leucotreta*, larvae in citrus orchards in three successive seasons after the release of sterile adults (Barnes *et al.* 2015).

1.2 INTER-POPULATION PHEROMONE VARIATION

The technology to analyse and identify the compounds that make up the sex pheromones secreted by female moths has been available for around 50 years (Bellas *et al.* 1983). This has improved significantly over the past 20 years (Klun *et al.* 1973; Lacey & Sanders 1992; McElfresh & Millar 2001; Lassance *et al.* 2010). It is possible to produce synthetic pheromones that are similar in constitution and effect to the naturally occurring pheromones (Persoons *et al.* 1974; Campos & Phillips 2014). Research regarding the sex pheromone produced by female FCM has enabled other improvements in the monitoring of this pest and to control it with methods such as mating disruption and attract-and-kill. The focus of this study was to investigate the possibility of pheromone variation between FCM populations. The literature shows numerous examples of other Lepidoptera that have interpopulation pheromone variation (Klun *et al.* 1973; Löfstedt & Van der Pers 1985; Glover *et al.* 1987; Linn *et al.* 1997; Kawazu *et al.* 2000; McElfresh & Millar 2001; Lassance *et al.* 2010). For example the European corn borer (ECB), *Ostrinia nubilalis* (Hübner), utilises two distinct sex pheromone communication systems, including a Z strain using a 97:3 mix of (Z)-

and (E)-11-tertradecenyl acetates (Z11-14:OAc and E11-14: OAc) and E strain using a 4:96 blend of (Z/E11-14: Ocs) (Klun *et al.* 1973; Glover *et al.* 1987). Kawazu *et al.* (2000) studied the sex pheromone variation of the rice leaffolder moth, *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae). This study explored the geographical variation in the female sex pheromone between populations from India and the Philippines. The sex pheromones of different populations consisted of the same compounds, however, the blend ratios of the compounds differed. This is a good example of reproductive isolation under extreme circumstances where the populations are geographically far apart. McEfresh and Millar (2001) conducted a similar study with the saturniid moth, *Hemileuca eglanterina* (Boisduval) (Lepidoptera: Saturniidae) and found two distinct pheromone types. Lassance *et al.* (2010) considered the origin of pheromone variation in the Z and E strains of *O. nubilalis* and found that allelic variations in a fatty-acyl reductase gene was the cause of the variation. This gene is a crucial part of the biosynthesis of the pheromone and the variations in the gene causes phenotypic differences in the pheromone. This was shown to be the origin of the sex pheromone signalling becoming race-specific (Lassance *et al.* 2010).

False codling moth is nocturnal and females release a sex pheromone to attract males (Angelini *et al.* 1981; Hall *et al.* 1984; Attygalle *et al.* 1986; Hofmeyr & Calitz 1991). A number of studies have set out to determine the composition of the pheromone released by the female (Persoons *et al.* 1977; Angelini *et al.* 1981; Zagatti *et al.* 1983; Hall *et al.* 1984; Attygalle *et al.* 1986). Early studies reported that the female sex pheromone comprised of the isomers trans-8-dodecenyl acetate and cis-8-dodecenyl acetate, with Persoons *et al.* (1977) reporting equal amounts of each isomer in the pheromone. Angelini *et al.* (1981) and Zagatti *et al.* (1983) found that the isomer dodecyl acetate also forms part of the pheromone and recorded a trans-8-dodecenyl acetate: cis-8-dodecenyl acetate: dodecyl acetate ratio of 69:23:8. Hall *et al.* (1984) reported a pheromone blend of 38:62 with almost the opposite amounts of the two main isomers, trans-8-dodecenyl and cis-8-dodeceyl acetate. A 1:1 mixture of trans-8-dodecenyl acetate and cis-8-dodecenyl acetate has also been used successfully as an synthetic

pheromone blend to attract male moths in Malawi (La Croix *et al.* 1985). However, Attygalle *et al.* (1986) proposed that all these reported variations in the pheromone blend are most likely due to a difference in the pheromones produced by females from different geographical areas and not due to individual variations. The consensus is that the pheromone consists of a blend of isomers of which three are most important to the female FCM sex pheromone and include 1) trans-8-dodecenyl acetate, 2) cis-8-dodecenyl acetate and 3) dodecyl acetate (Hofmeyr & Calitz 1991), and these blends differ regionally (Attygalle *et al.* 1986). In Africa, variation in these geographical pheromone blends (trans-8-dodecenyl acetate: cis-8-dodecenyl acetate: dodecyl acetate) include Ivory Coast (69:23:8), Malawi (32:52:16) and South Africa (76:10:14) (Angelini *et al.* 1981; Hall *et al.* 1984; Attygalle *et al.* 1986). With this knowledge there is a strong possibility that FCM may have some pheromone variation between populations in South Africa. If this is the case, the SIT programme against FCM in South Africa might be improved by using sterilized male moths from the area where SIT is being implemented.

1.3 PROJECT PROPOSAL

1.3.1 Justification

South Africa is a major exporter of citrus fruit and as of 2014 was the 2nd largest exporter globally (CGA 2015). Like most fruit, citrus is also adversely affected by a large number of insect pests. In South Africa there are 23 arthropod and molluscan pests of citrus that can be regarded as of major importance (Moore *et al.* 2008; Moore & Hattingh 2012). False codling moth is one of those pests and is considered to be the most important pest of citrus in South Africa (Grout & Moore 2015). The expectation by international markets for high quality fruit and strict phytosanitary conditions, forces farmers to prioritize the control of FCM (Moore & Kirkman 2009). FCM numbers in the orchard needs to be kept to a minimum and this means optimizing all of the available control methods. One of the non-chemical based methods that is used is the sterile insect technique (SIT). It was first implemented in South Africa during 2007 by Xsit Pty (Ltd) (Citrusdal, South Africa) (Hofmeyr *et al.* 2015). Xsit only used FCM from the Citrusdal area for establishment of their laboratory colony for sterilization of males. This

method has been shown to be effective in the Citrusdal area of the Western Cape, but is also being implemented in other parts of the country (Hofmeyr *et al.* 2015). Numerous studies have looked into the composition of the female sex pheromone (Persoons *et al.* 1977; Angelini *et al.* 1981; Zagatti *et al.* 1983; Hall *et al.* 1984; Attygalle *et al.* 1986), and variations in the sex pheromone have been identified across Africa (Angelini *et al.* 1981; Hall *et al.* 1984; Attygalle *et al.* 1986). Opoku-Debrah *et al.* (2014; 2016) showed distinct genetic and biological differences between geographic populations of FCM throughout South Africa. Timm *et al.* (2010) showed that the differences between populations could be linked to a lack of gene flow between these populations. The results of this genetic study showed that certain populations could be tracked to their area of origin or in some cases the exact orchard from which the samples were collected (Timm *et al.* 2010).

1.3.2 Aims

The main aims of this study were to 1) establish if regional variation in attractiveness of female FCM to male FCM (likely a result of pheromone variation) is a factor that may influence the efficacy of SIT and if so to propose a way in which we can benefit from this knowledge and 2) what impact possible pheromone variations and preferences will have on the sensitivity and accuracy of FCM monitoring with pheromone baited traps.

1.3.3 Objectives

In order to achieve the aims, a number of objectives had to be accomplished. First a laboratory trial was conducted with a Y-tube olfactometer and flight tunnel. This study attempted to reproduce the attraction of males to virgin FCM females under laboratory conditions. This would help to test the attraction of males to females from different geographical populations. The second objective was to test the attraction of male moths to different synthetic pheromone blends and virgin females from different geographical populations under field conditions. This would shed light on the impacts on SIT and FCM monitoring. The third objective was the least related to the main objective, but will shed light on possible postmating isolation that may take place when a male and female FCM mate and reproduce. This objective was investigated with a cross-mating trial.

Chapter 2: Testing the olfactory response of *Thaumatotibia leucotreta* males to females from different populations under laboratory conditions

2.1 INTRODUCTION

In simple terms, olfactometry is the measurement and control of odours and is implemented in laboratory research and industrial studies (Mateson 1955). The methods used, and applications for olfactometry have grown significantly over the past 50 years (Du *et al.* 1996). Olfactometry has become very popular in the field of entomology and considerable research has been undertaken on host location using Y-tube olfactometers (Du *et al.* 1996; Du *et al.* 1998; de Kogel *et al.* 1999; Geier & Boeckh 1999; Boff *et al.* 2001; Blackmer *et al.* 2004). This method helps scientists simulate and study natural behaviours of various organisms based on responses to a volatile compound (Read *et al.* 1970: Turlings *et al.* 1990). The system requires an insect to be presented with a choice of two different odours. The two odours are fed into two branches that join into one where the insect is located in the shape of a "Y" and hence the experiments are often referred to as Y-tube experiments (Read *et al.* 1970). Based on the insect's natural response to the odours, it moves up one of the branches representing a positive response to that particular odour (Read *et al.* 1970). This method has seen considerable use in the study of herbivore-induced plant volatiles (HIPV) (Turlings *et al.* 1990; Zhang *et al.* 2013; Menzel *et al.* 2014).

There have been numerous studies on mate location in Lepidoptera (e.g. Charlton & Cardé 1990; Hansson 1995). Generally it is the role of the male to locate a potential mate using either visual or olfactory cues or a combination of both. Diurnal butterflies are prone to make use of visual cues, while nocturnal moths rely heavily on olfactory cues consisting of volatile sex pheromones released by females to attract males (Charlton & Cardé 1990; Hansson 1995).

Female Lepidoptera may also have a "choice" in selecting males with favourable genes (Rutowski 1982). The males are expected to experience some filtering and selection pressures as a result of the difficulty of reaching adulthood and locating females (Rutowski 1982). Higher genetic quality can be expected from males that do manage to locate a female (Lloyd's "Passive filtering") and locating a female while having to compete with other males (Lloyd's "Passive selection") (Lloyd 1979).

The efficacy of the Xsit SIT programme in the Olifants River Valley is beyond doubt. The trap catches of male moths and the infestation of fruit by FCM have decreased significantly since the programmes implementation (Hofmeyr *et al.* 2015; Barnes *et al.* 2015). This success is based in the natural attractiveness of wild females in the region to the released sterile males. However, it has been shown that the FCM pheromone varies across Africa (Angelini *et al.* 1981; Hall *et al.* 1984; Attygalle *et al.* 1986). The possibility of further variations in the pheromone across South Africa needs to be investigated. In order to determine if the efficacy of SIT can possibly be increased in other areas via the use of moths for SIT from the target area. The aim of this chapter was to determine if there were differences in the attractiveness of females from different populations to males, under laboratory conditions.

2.2 METHODS AND MATERIALS

2.2.1 The Insect

There were five colonies of *T. leucotreta* maintained at Rhodes University, which were originally maintained in a laboratory at Citrus Research International (CRI) in Port Elizabeth. These colonies were established by Moore (2002) and Opoku-Debrah (2014). The first colony was started during 1996 and is a mixture of FCM larvae collected from Citrusdal (Weastern Cape), Zebediela (Limpopo) and Addo (Eastern Cape) (Moore 2002). This is a mixed colony and is referred to as the Old colony mixed origin). At the time of this study, the colony had been maintained for approximately 226 generations, estimated at a rate of one generation per month. The remaining colonies were started by Opoku-Debrah *et al.* (2014) and included

Addo (Eastern Cape), Nelspruit (Mpumulanga), Citrusdal (Western Cape) and Marble Hall (Limpopo) colonies, which were in there 92nd, 90th, 91st and 90th generations respectively, at the time of this study. During repeated sampling throughout 2015 when the laboratory trials were conducted, it was found that the average sex ratio was 1:1 (male: female) for all the colonies.

2.2.2 Setup of Y-tube olfactometer

The attraction of FCM males to FCM females was tested using a glass Y-tube olfactometer. The setup borrowed heavily from the design used by Yu *et al.* (2010). The system had two air pumps (adjustable) supplying air to two polytetrafluoroethylene tubes with a diameter of 10 mm. These tubes where connected to two flow meters (l/h) that could be used to adjust airflow. From the flow meters the tubes where connected to 500 ml conical flasks containing activated charcoal to ensure the air was sterilized. Sterilized air was then supplied to 10 mm polytetrafluoroethylene tubes leading from these flasks and connected to another set of 500 ml conical flasks filled with 250 ml of distilled water. A glass pipe fed the air into the distilled water and ensured it bubbles up to the surface so that the air was moistened. The sterilized and moistened air was then led by more 10 mm polytetrafluoroethylene tubing to two flasks that held the treatments, 1) the virgin FCM female and 2) an empty flask, which was the control. From the treatment flasks additional 10 mm polytetrafluoroethylene tubing was connected to each branch of the glass Y-tube that was housed in an observation chamber (Figure 2.1). Two glass Y-tubes used for the trials were 1) Y-tube with inner diameter of 25 mm and 2) Y-tube with inner diameter of 35 mm.

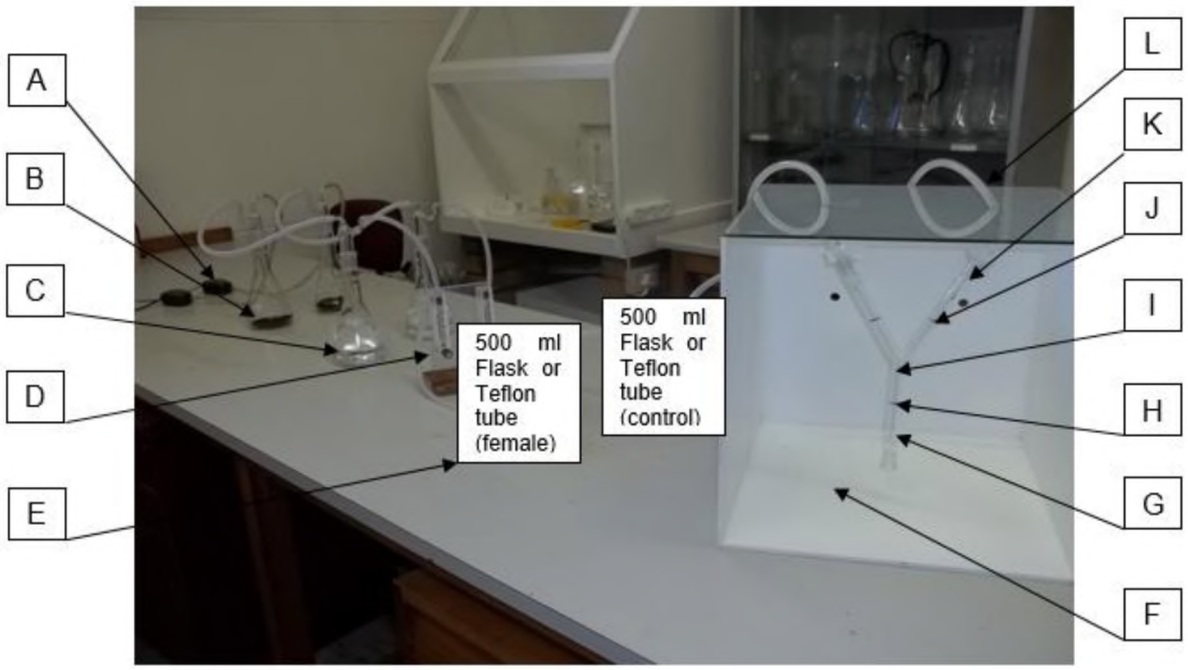

Fig. 2.1 Olfactometer setup. A= air pump; B= activated charcoal filter; C= distilled water; D= flow meter; E= Container housing virgin female; F= Perspex cage housing the Y-tube; G= base arm of Y-tube; H= starting line; I= Y-tube olfactometer; J= choice line; K= upper arm of Y-tube; L= air flow tubes.

2.2.3 Olfactometer trial

The first step was to optimise the experimental design of the Y-tube olfactometer. There have been no previous successful attempts to recreate the attraction of FCM males to FCM females using a Y-tube olfactometer. Smit (2004 and 2005) and Moore *et al.* (2013, unpublished data) have attempted to conduct laboratory studies with FCM using different olfactometer designs. The optimization focused on the 1) airflow, 2) angle of the Y-tube, 3) time of evening the experiments were carried out and 4) containment of the virgin FCM female. Each replicate of the trial consisted of five to seven separate replicates. The number of females that eclosed at the same time determined the number of replicates that could be achieved. The male was replaced after each replicate and the female after every three replicates. The equipment was cleaned after every six replicates. The equipment was cleaned with a detergent, dried with paper towels, cleaned with ethanol, rinsed in distilled water and finally dried in an oven set at 60°C for 24 hours. The experiments were done using FCM virgin

females of between 1 - 2 days old to ensure maximum pheromone output (Daiber 1980; Delisle & Royer 1994). These initial experiments were done using males and females from the same colony to first check that the protocol worked; the Addo colony was used.

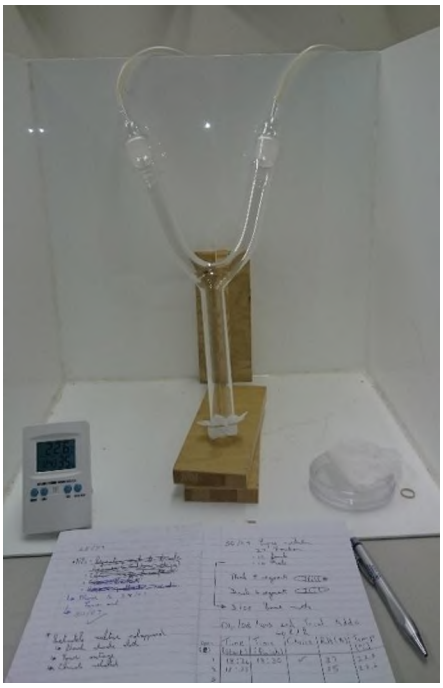

Fig. 2.2 Experimental setup of the glass Y-tube in an observation chamber.

The target was a significant positive response from the males towards the calling virgin female. Initially the airflow was set at 40 L/h, the Y-tube was angled horizontally and the experiment started one and a half hours after sunset. The laboratory was illuminated by two fluorescent tubes that were mounted to the ceiling and produced a combined light intensity of 5 lux. These initial replicates were not very successful. After changing the angle of the Y-tube, the male moths seemed to respond to the calling females. The setup that yielded the first results was as follows: 1) 40 L/h air flow, 2) Y-tube angled at 45°, 3) experiments conducted one and half hours after sunset and 4) virgin females housed in 500 ml conical flask. This was replicated three times with each replicate consisting of six separate replicates. Additional

replicates were also conducted with the virgin female housed in a section of polytetrafluoroethylene tubing with a diameter of 20 mm. The ends of a 10 cm long section of the 20 mm diameter polytetrafluoroethylene tubing was pushed over the ends of the 10 mm diameter tubing supplying the airflow towards and away from the female. This setup was replicated three times, with each replicate consisting of six separate replicates. The airflow was also changed to 50 L/h for a separate replicate and replicated six times

2.2.4 Flight tunnel trial

Due to the difficulties experienced with the olfactometer, it was decided to also run a laboratory trial with a flight tunnel. The design of the flight tunnel borrowed heavily from Segura *et al.* (2012). The flight tunnel had a length of 50 cm, height of 15 cm and width of 15 cm (Figure 2.3). The flight tunnel was made from poly(methyl methacrylate) (PMMA) with white sides and bottom and a clear top. The airflow entered the flight tunnel from one side and exited on the other. The setup of the source of airflow was as described for the olfactometer trial, but with the Y-tube substituted with the flight tunnel.

An initial replicate was conducted with virgin male and female FCM. The virgin female was placed in a perforated plastic bottle of which the lid was attached to the top of the flight tunnel. The male was placed in the middle of the flight tunnel, which represented the start of the replicate. Each replicate was run for a maximum of 15 min, at which time a new male was used, even if the male had not responded; each female was used three times. During the initial replicates the response of males to females from different colonies were tested. However due to inconsistent results, no pairings other than Addo males to Addo females were tested. This was done in order to optimize the setup of the flight tunnel. The males did not respond reliably within 15 min and it was decided to increase the cut off time to 30 min. Airflow was also increased from 40 L/h to 45 L/h, which caused the males to be more active. Results were still inconsistent, even after numerous replicates. It was therefore not possible to reliably gauge a positive response from the males to the pheromone of the female. A follow up replicate

substituted the virgin female with a commercial FCM lure (Chempac, South Africa) and was also replicated six times.

Fig. 2.3 The flight tunnel used for the trial.

2.2.5 Statistical analysis

A Kruskal-Wallis test was used to determine whether the results from the olfactometer trials were statistically significant. A probability level (*P*) of < 0.05 was used in all cases.

2.3 RESULTS

2.3.1 Olfactometer trials

During the first successful replicate, 80.00 % of the males responded positively to the virgin female, 20.00 % to the control and none of the males made no choice (Table 2.1). However in the subsequent replicate, 42.86 % of the males responded positively to the virgin female, 42.86 % to the control and 14.29 % of the males made no choice, while in replicate three male choice for the virgin female was 33.33 %, 50.00 % for the control and 16.67 % of the males made no choice (Table 2.1).

Table 2.1 The male choice (%) for the virgin female, control and no choice when the Y-tube was set at 45° with 40 L/h airflow and virgin female was housed in 500 ml flask.

Replicate	Male choice (%)		
	Virgin female	Control	No choice
1 (5 replicates)	80.00	20.00	0.00
2 (7 replicates)	42.86	42.86	14.29
3 (6 replicates)	33.33	50.00	16.67

For replicate four male choice for the virgin female was 33.33 % and 66.67 % for the control (Table 2.2). Replicate five was the same design as replicate four and male choice for the virgin female was 42.86 %, 42.86 % for the control and 14.29 % of the males made no choice (Table 2.2). In replicate six, with the same design as both replicate four and five, male choice for the virgin female was 33.33 %, 16.67 % for the control and 50.00 % of the males made no choice (Table 2.2).

Table 2.2 The male choice (%) for the virgin female, control and no choice when the Y-tube was set at 45° with 40 L/h airflow and virgin female was housed in polytetrafluoroethylene casing.

Replicate	Male choice (%)		
	Virgin female	Control	No choice
4 (6 replicates)	33.33	66.67	0.00
5 (7 replicates)	42.86	42.86	14.29
6 (6 replicates)	33.33	50.00	16.67

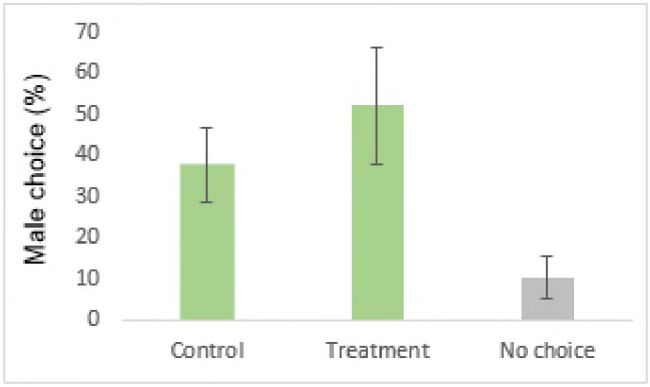

Fig. 2.4 The mean (± SE) results (male choice) for replicates 1-3. The Y-tube was set at 45° with 40 L/h airflow and virgin female was housed in a 500 ml flask.

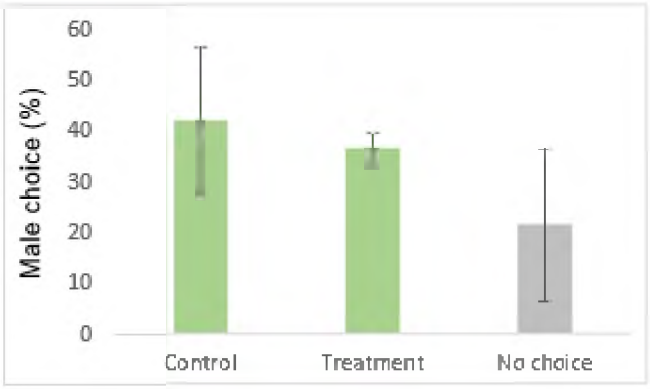

Fig. 2.5 The mean (± SE) results (male choice) for replicates 4-6. The Y-tube was set at 45° with 40 L/h airflow and virgin female was housed in polytetrafluoroethylene casing.

For replicate seven it was decided to increase the airflow slightly to potentially get better pheromone transmission from the calling virgin female to the male moth in the Y-tube. Male choice for the virgin female was 16.67 %, 33.33 % for the control and 50.00 % of the males made no choice (Fig. 2.6). After this trial it was decided to do the subsequent trial in complete darkness. The results were evaluated with the aid of a red light. For the first replicate all of the males remained stationary and made no choice. Two more attempts at the experiment were made, with still no movement recorded from the males in response to the pheromone emitted from the calling virgin female.

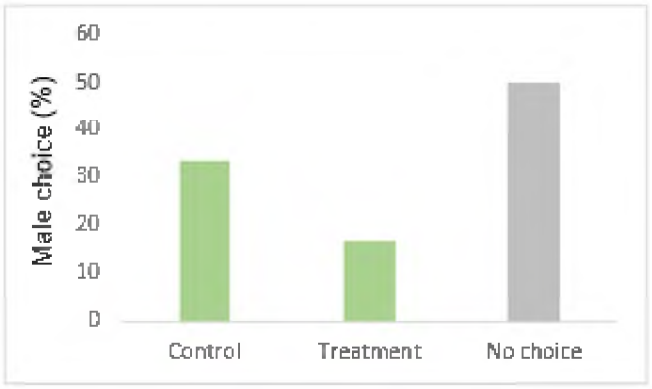

Fig. 2.6 Results (male choice) for replicate 7. The Y-tube was set at 45° 50 L/h airflow virgin female in polytetrafluoroethylene casing.

2.3.2 Flight tunnel trial

Despite changing the setup of the flight tunnel the results remained inconsistent after numerous replicates. It was not possible to gauge a positive response from the males reacting to the pheromone of the calling virgin female. Some males would fly in circles, remain motionless or take flight once and land. There was no pattern to these responses and it was decided to replicate the trial using a commercial FCM lure (Chempac, South Africa). The males responded in the same variable manner that they responded to the virgin females.

2.4 DISCUSSION

The initial step in the laboratory trials was to optimize the setup of the olfactometer. The first replicate of the Y-tube olfactometer trial was promising, but in subsequent replicates, there were no consistent results. For the first three replicates of the Y-tube olfactometer trial, the virgin females were housed in 500 ml flasks. This was followed by another four replicates where the virgin females were housed in a polytetrafluoroethylene casing. This casing promoted better airflow from the calling virgin female to the male in the Y-tube. The polytetrafluoroethylene casing seemed to be ideal to hold the females in place while promoting efficient airflow and simplifying the process of replacing virgin females between replicates. Even after all these changes, the results for the replicates using this method for housing the

virgin female still remained erratic and highly variable, even though the virgin females displayed calling behaviour during these replicates. The typical calling behaviour of waiting with the wings pointing upwards was observed (Zagatti and Castel 1987).

As FCM is a nocturnally active lepidopteran, it was decided to conduct subsequent replicates in complete darkness before continuing to change the airflow, angle of the Y-tube and other possible details of the Y-tube olfactometer setup. This replicate was conducted using red light, a method often used to observe the natural behaviour of nocturnal lepidopterans (Raina *et al.* 1986; Itagaki & Conner 1988). During these replicates, the male moths did not respond at all and further Y-tube olfactometer replicates were then halted in order to investigate a new approach.

A final attempt was then made to get FCM males to respond reliably to virgin FCM females in the laboratory with the use of a flight tunnel. Unfortunately this also did not yield any consistency from the males responding to the virgin females. Despite investigating both methods in the laboratory, consistent and repeatable results could not be achieved. Mass rearing of insects has been shown to have numerous negative impacts on the characteristics of the laboratory reared insects when compared to their wild counterparts (Boller 1972; Chambers 1977; Sørensen *et al.* 2012). The possible implications on mating competitiveness and field flight ability are most important to consider for this study (Sørensen *et al.* 2012). The use of laboratory reared insects for field and laboratory experiments may mislead researchers when considering these insects to be biologically similar to their wild counterparts (Stotter & Terblanche 2009). Unfortunately quality is often overlooked when laboratory mass rearing insects and the main focus is most often output volume (Stotter & Terblanche 2009). Smit (2005) had success with a 16 tube olfactometer design for the screening of numerous possible attractants to both male and female FCM. Further investigation into the efficacy of the attractants was however done in the field (Smit 2004; 2005), see Chapter 3. Moore *et al.* (2013, unpublished data) investigated the use of numerous olfactometer designs including variations of a Y-tube olfactometer to test the possibility of attracting male moths under

laboratory conditions but failed. The results from the Y-tube experiments should be interpreted with care, due to the inconsistencies in the results.

Chapter 3: Field testing the olfactory response of adult male *Thaumatotibia leucotreta* to different synthetic pheromone blends and to virgin females from different populations

3.1 INTRODUCTION

There has been extensive research conducted on the pheromones of lepidopterans (Nesbitt *et al.* 1979; Teal *et al.* 1981; Krupke *et al.* 2002; Bosa *et al.* 2006; Heath *et al.* 2006). This surge in research during the 1970's was initiated to a large extent by the agricultural industry trying to control the numerous lepidopteran pests (Nesbitt *et al.* 1979; Teal *et al.* 1981). Through the control and monitoring of lepidopterans, an extensive knowledge on the pheromones of these pests has been developed which has benefitted agricultural industries around the globe (Teal *et al.* 1981; Trimble *et al.* 2001; Gregg *et al.* 2016). The use of mating disruption as a method of control has become a widely used, environmentally friendly control method that can be incorporated into integrated pest management (IPM) programmes (Trimble *et al.* 2001; Moore & Hattingh 2012). The use of synthetic pheromone lures has also become crucial for monitoring populations in order to provide farmers and researchers with information regarding the population size of a particular pest (Hofmeyr *et al.* 2015).

Chapter one provided examples of lepidopteran species that show inter-population pheromone variation (Klun *et al.* 1973; Löfstedt & Van der Pers 1985; Glover *et al.* 1987; Linn *et al.* 1997; Kawazu *et al.* 2000; McElfresh & Millar 2001; Lassance *et al.* 2010). It is therefore crucial to develop a product that will work optimally not only for a particular species but also for a particular population of the target species. Angelini *et al.* (1981), Hall *et al.* (1984) and Attygalle *et al.* (1986) have shown that there are variations in the pheromone of FCM across Africa. Possible variations in olfactory systems of different FCM populations across South Africa may highlight the importance of such research.

When developing a mating disruption product or synthetic pheromone lure the first step is to determine the composition of the pheromone released by the calling virgin female. The final and most important test of these products is to assess their efficacy in the field. Studies testing the potential of mating disruption products or synthetic pheromone blends in the field have been conducted for numerous lepidopteran pests (Nesbitt *et al.* 1979; Teal *et al.* 1981; Krupke *et al.* 2002; Bosa *et al.* 2006; Heath *et al.* 2006).

The aim of this chapter was to investigate the possibility of inter-population pheromone variations between different FCM populations in the field as we were unable to do so in the laboratory (Chapter 2). This chapter focused on field trials and tested the attractiveness of a few regional pheromone blends, commercial pheromone blends and virgin females from a range of different populations to FCM males of a single population.

3.2 METHODS AND MATERIALS

3.2.1 Olfactory response of adult male FCM to virgin female FCM from different populations

There were five populations available to use as a source of virgin females: Old (mixed origin), Addo, Nelspruit, Marble Hall and Citrusdal, all housed at Rhodes University (Opoku-Debrah *et al.* 2014). These are the same populations used for the laboratory experiments reported in Chapter Two. For the trial, six treatments were tested. Virgin females were sourced from the five available populations for five of the treatments. The sixth treatment was a control with no female present to cover the possibility of the males being attracted to the trap itself. Pupae were sexed using guidelines from Timm *et al.* (2007). The male pupa has four independently moveable abdominal segments, while the female pupa has three (Timm *et al.* 2007). The females were transferred to small 10 cm x 3 cm stainless steel mesh cages with plastic lids until they eclosed (Figure 3.1). The age of the females used for the trials was between 1 and 2 days, in order to ensure maximum pheromone output (Daiber 1980; Delisle & Royer 1994). Each of the treatments were represented by two virgin females housed in a

single stainless steel mesh cage and suspended in the middle of a yellow delta trap (Insect Science, South Africa) (height = 110 mm; length = 280 mm and width = 200 mm; weight = 600 g) with sticky floor, replicated six times each. The reason for using two females per trap was to maximize the pheromone output.

Fig. 3.1 Metal cage that was used to house the female FCM within the delta traps.

The FCM males used in the trial where gamma irradiated (150 Gy) sterilized moths obtained from the Xsit FCM colony, originating from the Citrusdal area. The sterilized moths were transported at approximately 4°C in small cardboard boxes that contained approximately 10 000 moths each. The trial was conducted in a large lemon orchard in the Addo area (33°31'54.41" S 25°38'56.11" E) outside of the area in which SIT was conducted (Figure 3.2). Lemons have been shown not to be a host of FCM (Moore *et al.* 2015) and this ensured minimal numbers of wild FCM in the orchard in which the trials were conducted. The orchard had more than 42 rows, ensuring the six treatments and control could be replicated six times each within a single orchard (Figure 3.3). A single trap was placed in each row 50 m into the orchard from the southern side. The traps were placed at a height of approximately 1.8 m above the ground and suspended from the tree, facing in a north easterly direction. The traps were baited on the morning of the start of the trial. The order of the traps was randomized using a random number table. After counting 50 moths, weighing them and taking an average from five counts it was determined that the average weight of 50 moths was 1.35 g. The moths were pre-weighed into 42 petri dishes, at 5.4 g of moths per petri dish, equating to

approximately 200 moths. This was done at the Xsit facility located at the airfield in Addo. This ensured that an equal number of moths were released in each row, and the moths were still inactive during this process due to the cold temperature they were stored under. One petri dish of moths was released per row in the afternoon around 3 h before sunset on the northern side of each trap and 15 m away from the trap. The predominant wind direction in the area was south-westerly. After 7 days the recaptures were determined by counting the male moths caught in each trap. The sterile moths that were released were fed a larval diet in the rearing facility that contained a dye, Calco Oil Red® (Royce International, Sarasota, Florida, U.S.A.). The recaptured moths were squashed to reveal if their intestines showed any presence of the dark pink dye; this allowed differentiation between wild and sterile moths.

Fig. 3.2 Aerial photograph of the farm where the study was conducted. The lemon orchard (33°31'54.41" S 25°38'56.11" E) that was used for the trials is highlighted by a red border.

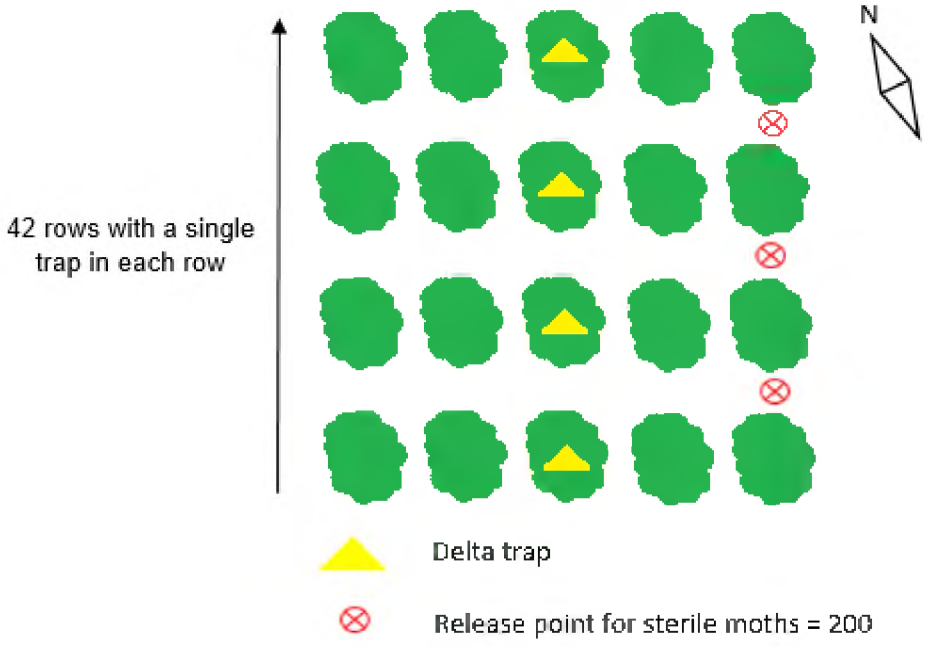

Fig. 3.3 Trap placement and sterile moth release points used for field trials.

A second virgin female trial was conducted in polyethylene tunnels, as the trap catches from the field trial were extremely low, even though logged data showed that temperatures during the evenings were high enough to allow for moth flight activity. The trial could be better controlled in the tunnels and as evening temperatures in the tunnels remained more moderate than outside the tunnels, the trial could be extended into the cooler months of autumn. The polyethylene tunnels also provided consistent controlled airflow and provided shelter from any environmental factors (rain, wind and frost). Two replicates were conducted per tunnel and two tunnels were used for the trial. The trial was replicated three times (29 June, 27 October and 13 December 2016). Figures 3.4 and 3.5 show the setup of the trial and the traps in one of the polyethylene tunnels. As per the first virgin female trial, six treatments were tested, each represented by two virgin females per cage per delta trap replicated six times each. The same populations were used for this trial i.e. Old Addo (mixed origin), Addo, Nelspruit, Marble Hall and Citrusdal, all housed at Rhodes University. The age of the females used in the trial was again between 1 and 2 days. The polyethylene tunnels used were situated in a citrus nursery and were 30 m long and 10 m wide. Each row of traps was one replicate. The traps were

spaced 4 m apart with a 5 m space between the trap and the side of the tunnel. The space between the traps of the two replicates was 6 m. Releases of sterile moths were made in-between the two rows of replicates (Figure 3.4). Releases were made every 4 m between the two replicates and in between the position of the traps. A total of 100 moths were released at the opposite ends of the tunnel and the rest of the releases were all 200 moths per release point. After 7 days the recaptures were determined by counting the male moths caught in each trap.

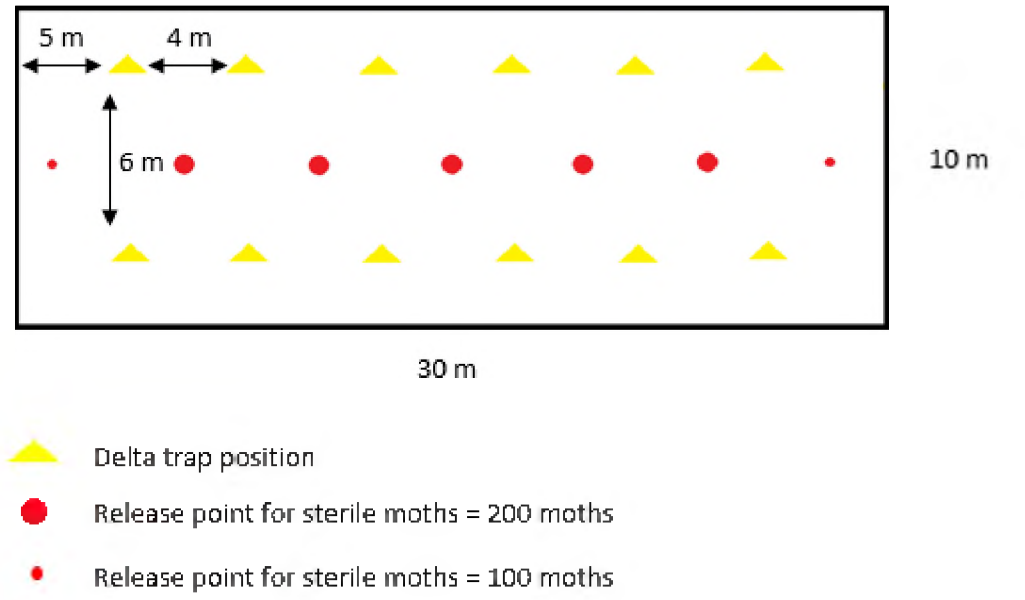

Fig. 3.4 Placement of traps and the release points of sterile male moths in the polyethylene tunnel.

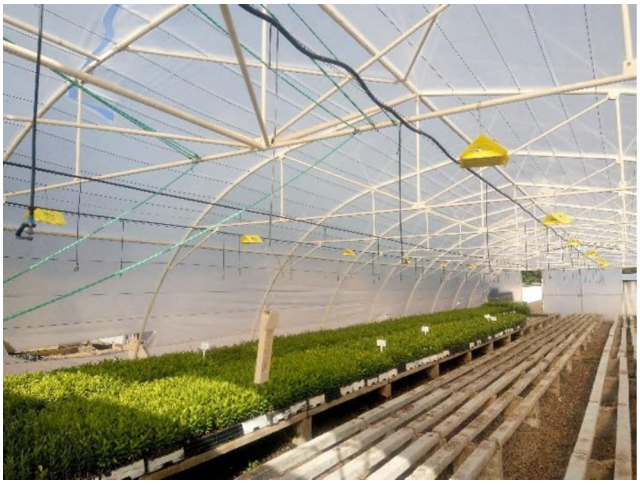

Fig. 3.5 Setup of the traps in polyethylene tunnel where male FCM were released.

3.2.2 Olfactory response of adult male FCM to different synthetic pheromone blends

For the synthetic pheromone trial the attractiveness of six known FCM female sex pheromone blends were used. These blends consisted of four commercial products, two used for monitoring and two used for mating disruption, and three others that have been identified as regionally specific blends. The FCM female sex pheromone consists of three isomers, including trans-8-dodecenyl acetate, cis-8-dodecenyl acetate and dodecyl acetate (Attygalle *et al.* 1986). The six isomer blends served as the six treatments for the field trial and a control with no pheromone was included as the seventh treatment.

The six treatments where:

Commercial Blends

- FCM Pherolure (monitoring lure, Insect Science (Pty) Ltd)
    - trans-8-dodecenyl acetate: cis-8-dodecenyl acetate: trans-7-dodecenyl acetate
    - (40%:10%:50%)
- Checkmate (mating disruption spray, Suttera LLC)

    - (trans-8-dodecenyl acetate: cis-8-dodecenyl acetate)
    - (78%:22%)
- Isomate (mating disruption dispenser, APS Biocontrol Ltd)
    - (trans-8-dodecenyl acetate: cis-8-dodecenyl acetate: trans-7/8-dodecenol)
    - (69.5%:29.5%:1%)

Regional blends

- Ivory Coast (trans-8-dodecenyl acetate: cis-8-dodecenyl acetate: dodecyl acetate) (69%:23%:8%) (Angelini *et al.* 1981)
- Malawi (trans-8-dodecenyl acetate: cis-8-dodecenyl acetate: dodecyl acetate) (32%:52%:16%) (Hall *et al.* 1984)
- South Africa (trans-8-dodecenyl acetate: cis-8-dodecenyl acetate: dodecyl acetate) (76%:10%:14%) (Attygalle *et al.* 1986)

The pheromone isomers were blended in the laboratory and used in the field trial. The commercial products were not used with their original dispensers as these could have differentially influenced dispersal rates. Eppendorf tubes (2 ml capacity) with the lid left open were used as the dispenser for all the pheromone blends. The Eppendorf tubes were filled with 20 mg of each synthetic pheromone blend. The trial was setup in the field following the method used for the virgin female field trial and in the same lemon orchard outside the SIT area that was previously used, and was replicated three times (5 April, 19 April and 10 May 2016) (Figure 3.1).

3.2.3 Temperature

Temperature (°C) readings were recorded in 30 min intervals using a temperature logger (iButton®, North America) for the field trials in the lemon orchard and polyethylene tunnels. The iButton was placed in a 50 ml plastic bottle to ensure no moisture reached it and placed in one of the trees in the middle of the lemon orchard used for the trials.

3.2.4 Statistical analysis

Tests for statistical significance included the Kruskal-Wallis H test for nonparametric data and a single factor ANOVA was used for the recapture data. If a significant relationship was found, Tukey Post Hoc analysis was conducted. A probability level (P) of < 0.05 was used in all cases.

3.3 RESULTS

3.3.1 Olfactory response of adult male FCM to virgin female FCM from different populations

The attraction of sterile FCM males to virgin FCM females from different populations across South Africa is shown below for the three replicates (Figures 3.6, 3.8 and 3.10). The recaptures from replicate 1 were too low to conduct any statistical analysis. The difference between recaptures of sterile males from Delta traps baited with the live virgin females from Citrusdal compared to the recaptures from all the other treatments was significant ($P < 0.05$) for replicate two and three (Figures 3.8 and 3.10). The Citrusdal females were the most attractive to the sterile males (also from Citrusdal). The least attractive females were those from the Nelspruit area for both replicate two and three (Figures 3.8 and 3.10).

Temperature data for replicate one and two are shown below (Figures 3.7 and 3.9). Unfortunately the temperature logger for replicate three was faulty and did not record any data. FCM is nocturnally active and require a minimum air temperature of between 10 - 15°C for flight activity, the required temperature for facility reared moths is above 15°C (Stotter & Terblanche 2009; Hofmeyr *et al.* 2015; Daniel 2016). Temperatures during the first seven evenings were far below 15°C for the duration of replicate one (Figure 3.7). For replicate two, the temperatures during the evening were just below or just above 15°C for all the evenings (Figure 3.9).

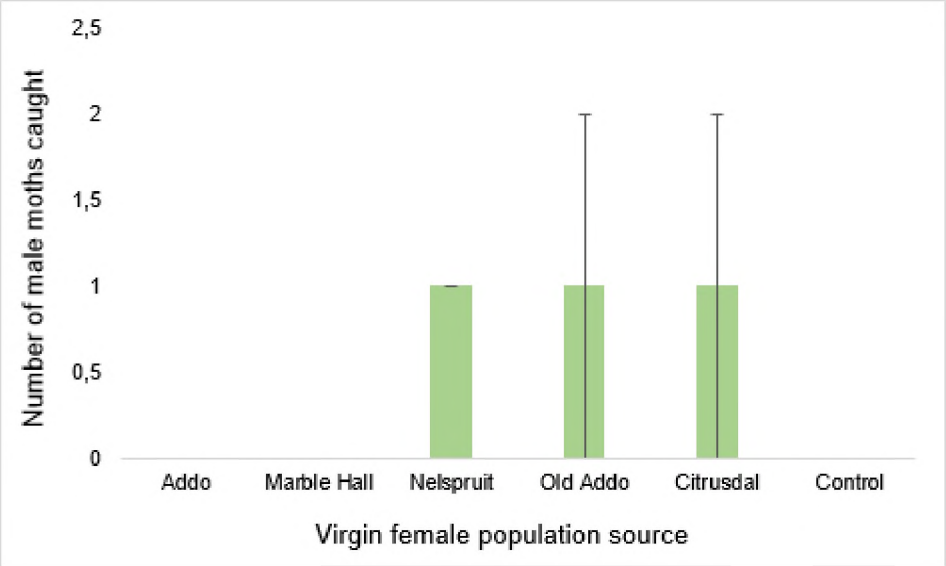

Fig. 3.6 Mean (± SE) number of sterile FCM males caught in delta traps using live virgin females from different populations as lures (Replicate 1 - 29/06/2016 - 6/07/2016).

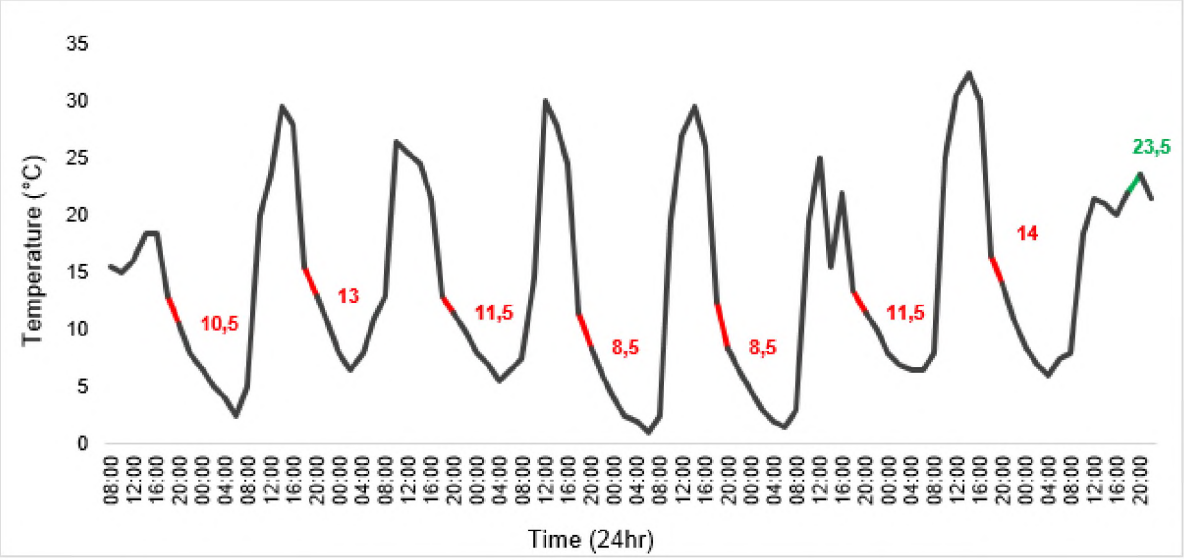

Fig. 3.7 Temperature data recorded in the lemon orchard that was used for the virgin female field trial. The temperature at 20h00 is displayed on the graph to indicate whether the minimum temperature for flight activity of at least 15°C was met during each evening (Replicate 1 - 29/06/2016 - 6/07/2016).

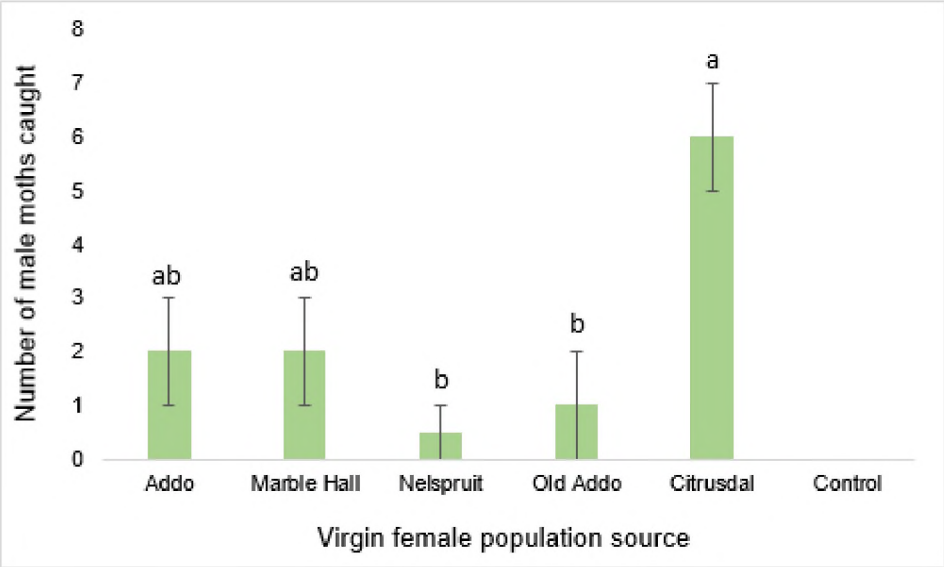

Fig. 3.8 Mean (± SE) number of sterile FCM males caught in delta traps using live virgin females from different populations as lures (Replicate 2 - 27/09/2016 - 04/10/2016).

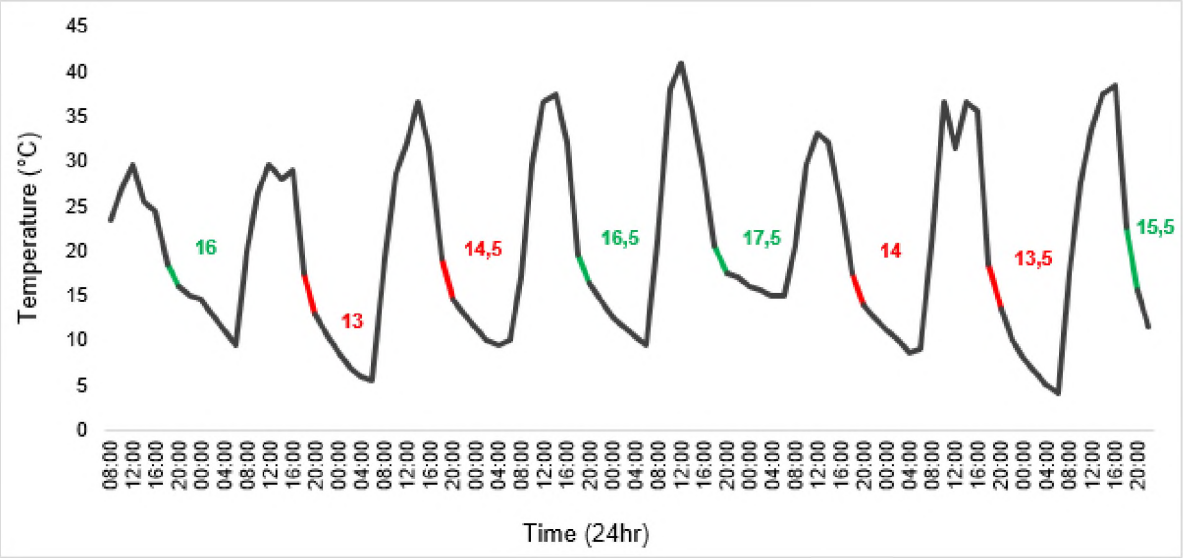

Fig. 3.9 Temperature data recorded in the lemon orchard that was used for the virgin female field trial. The temperature at 20h00 is displayed on the graph to indicate whether the minimum temperature for flight activity of at least 15°C was met during each evening (Replicate 2 - 27/09/2016 - 04/10/2016).

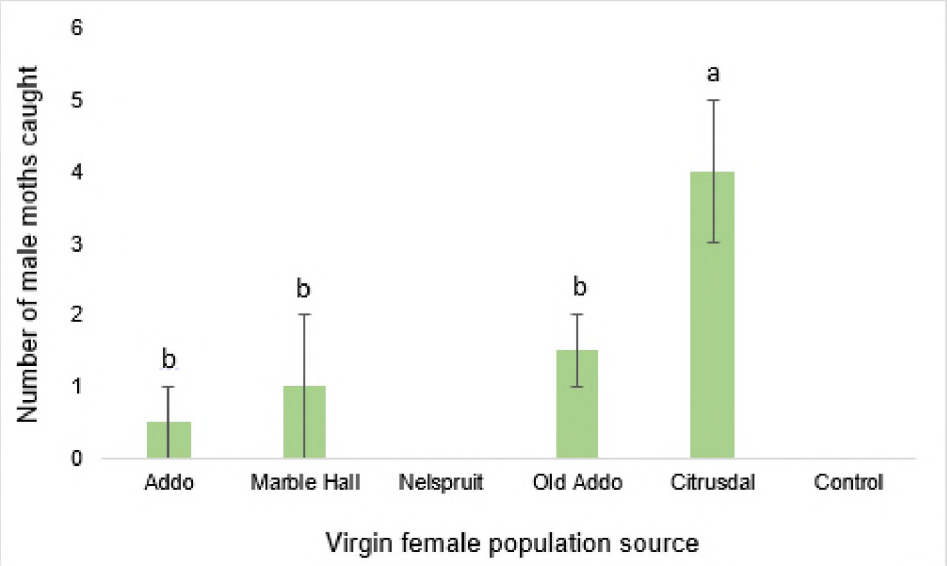

Fig. 3.10 Mean (± SE) number of sterile FCM males caught in delta traps using live virgin females from different populations as lures (Replicate 3 - 13/12/2016 - 20/12/2016).

3.3.2 Olfactory response of adult male FCM to different synthetic pheromone blends

The attraction of male moths to a range of synthetic pheromone blends consisting of commercial and regional blends is shown below (Figures 3.11, 3.12 and 3.14). The difference in recaptures between the range of pheromone blends used were significant for all the trials ($P < 0.05$). The trend in sterile male recaptures was very similar for all three of the replicates (Figures 3.11, 3.12 and 3.14). The South African regional blend had the highest number of recaptures of sterile male moths out of all the blended synthetic pheromones. The Malawi and Ivory Coast regional blends had the lowest number of recaptures of sterile male moths, followed closely by the Isomate commercial blend. Checkmate had the highest recaptures of sterile male moths out of all the commercial pheromone blends, followed by the Pherolure commercial blend.

Temperature data for replicate two and three of the trial are shown below (Figures 3.12 and 3.14). The temperature data for replicate one is not shown due to a faulty temperature logger which did not record any data. Temperature was recorded in half hourly intervals and the temperatures during peak hours of FCM activity are displayed on the graphs. Temperatures during peak hours of FCM activity were well above the 15°C threshold for the first five evenings during replicate two (Figure 3.13). During replicate three only evening 3, 5 and the last two evenings experienced temperatures above 15°C for an extended period (Figure 3.15).

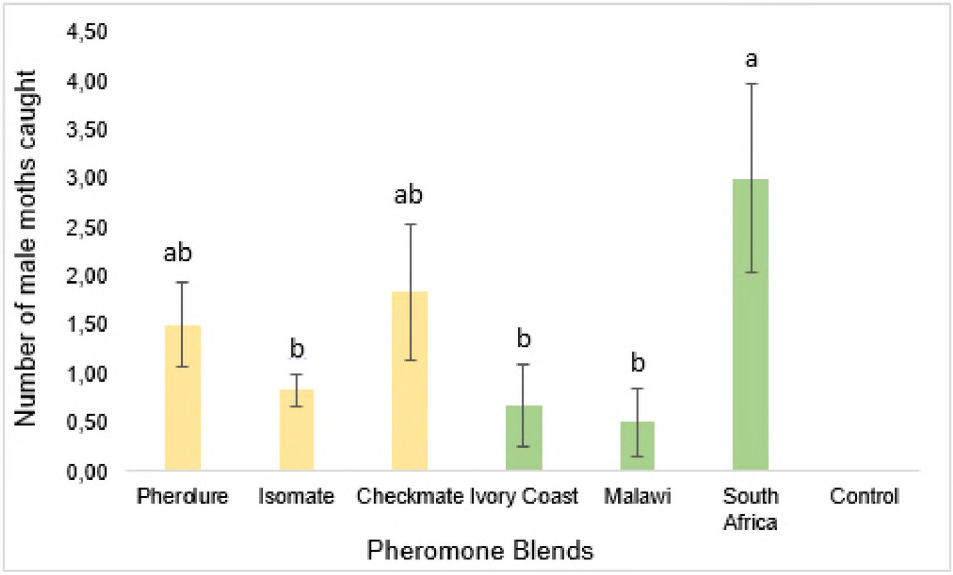

Fig. 3.11 Mean (± SE) number of sterile FCM males caught in delta traps using different pheromone blends (Replicate 1 - 05/04/2016 - 12/04/2016).

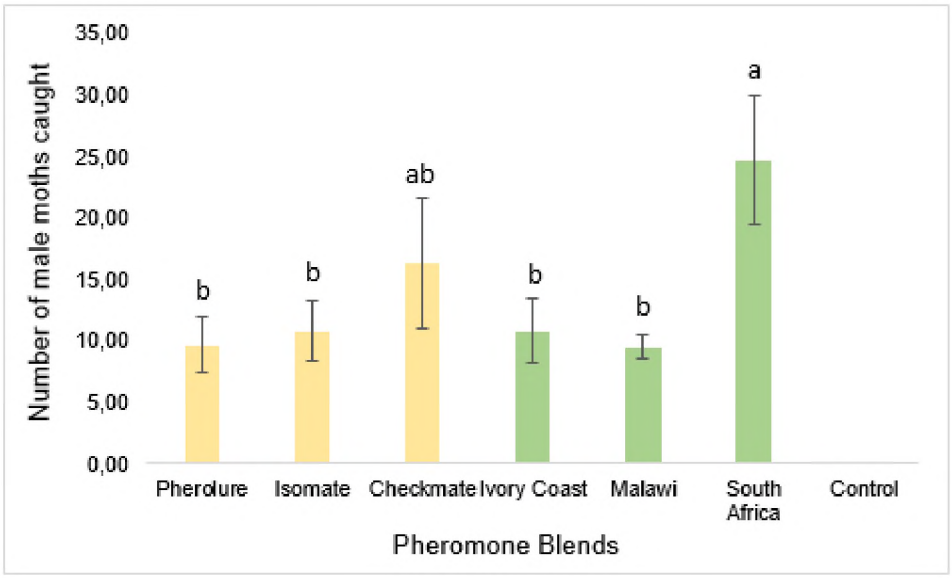

Fig. 3.12 Mean (± SE) number of sterile FCM males caught in delta traps baited with different pheromone blends (Replicate 2 - 18/04/2016 - 25/04/2016).

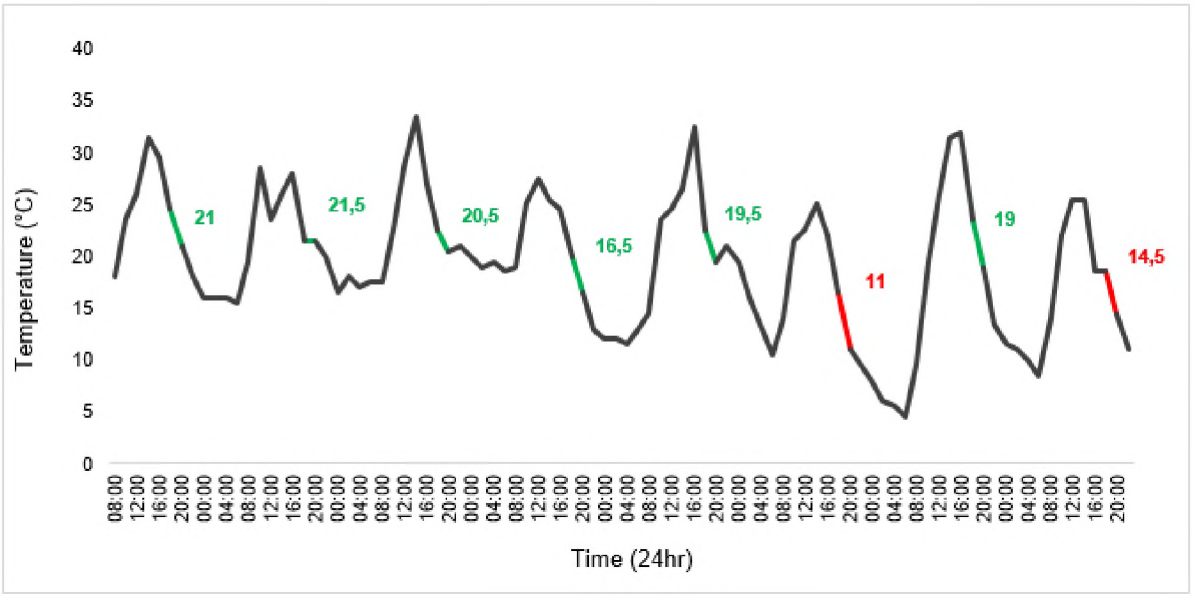

Fig. 3.13 Temperature data recorded in the lemon orchard that was used for the pheromone field trial. The temperature at 20h00 is displayed on the graph to indicate whether the minimum temperature for flight activity of at least 15°C was met during each evening (Replicate 2 - 18/04/2016 - 25/04/2016).

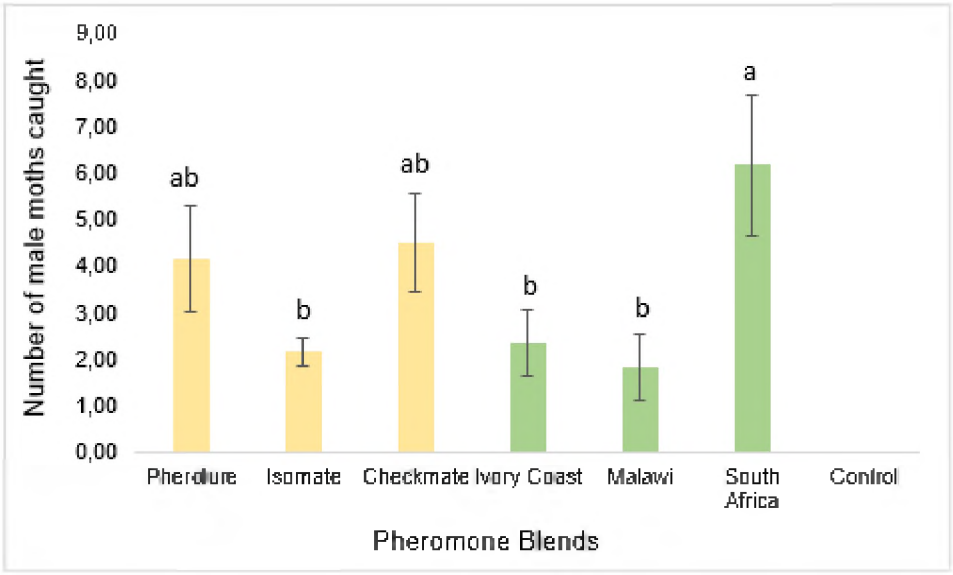

Fig. 3.14 Mean (± SE) number of sterile FCM males caught in delta traps baited with different pheromone blends (Replicate 3 - 10/05/2016 - 17/05/2016).

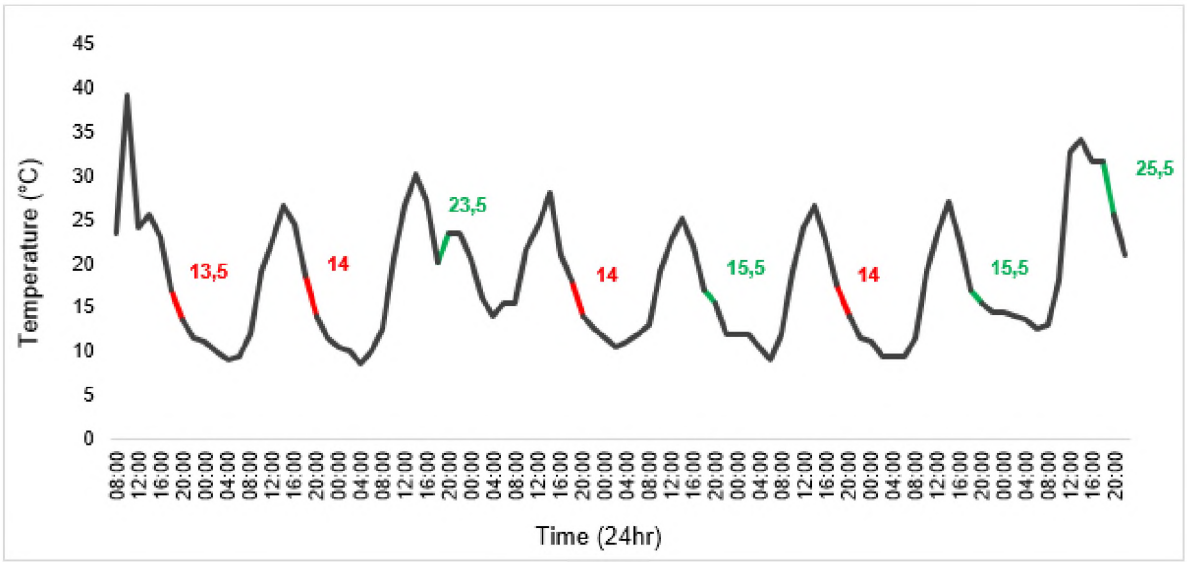

Fig. 3.15 Temperature data recorded in the lemon orchard that was used for the pheromone field trial. The temperature at 20h00 is displayed on the graph to indicate whether the minimum temperature for flight activity of at least 15°C was met during each evening (Replicate 3 - 10/05/2016 - 17/05/2016).

The highest number of sterile male moths were recaptured when the temperatures during the evening was above 15°C for an extended amount of time (Figures 3.13 and 3.15). For replicate two the temperature in the orchard for the first five evenings was well above 15°C (Figure 3.15). The mean recaptures for replicate two was the highest. For replicate three the temperature in the orchard was below 15°C for the first two evenings. The mean recaptures for replicate three was lower than replicate two and higher than replicate one. The temperature logger that was placed in the orchard for the extent of replicate one was faulty and did not record any temperature data. The mean trap catches, as an average of the entire trial, show that the South African regional blend and the Checkmate commercial blend are the most attractive to FCM males (Figure 3.16).

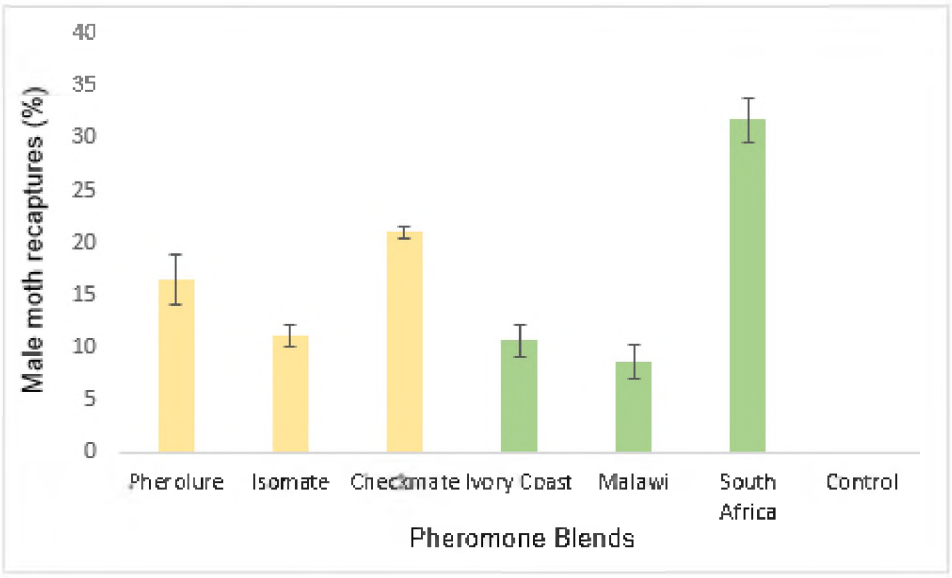

Fig. 3.16 Mean ± SE recaptures per pheromone blend as a percentage of the total recaptures for replicate one, two and three combined.

3.4 DISCUSSION

This chapter investigated the attractiveness of a range of synthetic and naturally produced FCM female pheromones to sterile male FCM. These male moths are reared and sterilised by Xsit for their SIT programmes. It is clear that sterile Citrusdal FCM males do not

respond equally to the range of pheromone blends that were tested. Out of all the pheromone blends tested, both commercial and natural (regional), the most attractive blend was the South African blend; the regional blends from Malawi and Ivory Coast were far less attractive to the males. Checkmate was the most attractive commercial blend. The South African blend may thus have application for mating disruption or the development of a commercial lure for use in South Africa. However, this may also differ from region to region.

The virgin female trial further tested attractiveness of virgin FCM females from different citrus growing areas across South Africa to sterile Citrusdal males. The sterile Citrusdal males found Citrusdal females the most attractive. The males were thus able to locate females from their own local population more effectively. False codling moth males rely on the pheromones produced by the females to locate a mate.

The results from the pheromone and virgin female trials support the possibility of inter-population pheromone variations in FCM, a phenomenon that has been studied and confirmed in other lepidopterans (Klun *et al.* 1973; Löfstedt & Van der Pers 1985; Glover *et al.* 1987; Linn *et al.* 1997; Kawazu *et al.* 2000; McElfresh & Millar 2001; Lassance *et al.* 2010). Recent publications on both the biology and genetics of geographically distinct FCM populations across South Africa also support this finding (Timm *et al.* 2010; Opoku-Debrah *et al*. 2014; Opoku-Debrah *et al*. 2016). Unfortunately only males from the Citrusdal population could be used for the field trials due to the number of moths required for the releases. The fact that Citrusdal males found Citrusdal females the most attractive was however a key factor in establishing if this study found any support for inter-population pheromone variations in FCM. Males from other populations may not be as sensitive to variations in the pheromone produced by females from other populations, but this will have to be investigated in future studies.

Timm *et al.* (2010) analysed the population genetic structure of two Tortricidae species, one being FCM, from different citrus growing areas across South Africa, using amplified fragment length polymorphism (AFLP) analysis. The study presented evidence that supported geographically structured FCM populations and in some instances it was possible to

distinguish between populations from different farms or even orchards. A number of factors can cause this genetic structuring on a geographic scale. This study considered host range, origin, dispersal ability, control practices and anthropogenic movement (Timm *et al.* 2010). Host range seems to account for some of the population genetic structure, but has not produced any host specific strains. Mgocheki & Addison (2016) also found no host specific strains in FCM, supported by mitochondrial DNA (*mt*DNA) analysis and mating studies. It is important to note that the authors highlighted the challenges this poses when trying to control FCM. Limited dispersal ability, insecticide use and anthropogenic movement were found to be the key drivers of population structuring in FCM (Timm *et al.* 2010).

Opoku-Debrah *et al.* (2016) reported a study on five geographically distinct FCM populations. The populations used were the same colonies used for this study, namely the Addo, Old Addo (mixed origin), Nelspruit, Marble Hall and Citrusdal colonies. These colonies are all maintained under the same laboratory conditions and reared on the same diet. The study found that the colonies differed in a number of ways regarding their biological attributes including pupal mass, pupal survival, female fecundity and egg hatch (Opoku-Debrah *et al.* 2016).

In conclusion strong evidence has been found that suggests FCM populations across South Africa may differ in the pheromones they produce. While only Citrusdal males were tested, results indicate that they were more efficient in locating females from their own geographic population. The experiments made use of choice test where there was competition between different pheromone blends. This ensured that the results have more significance because the males would have to select a pheromone source which is the most attractive. These results should be taken into consideration in future efforts to expand the SIT programmes implemented for FCM across South Africa's citrus growing areas, including areas where other susceptible crops are commercially farmed.

Chapter 4: Fertility and fecundity of combinations of *Thaumatotibia leucotreta* males and females from different geographic populations

4.1 INTRODUCTION

It has been shown that FCM populations across South Africa differ in their genetic structure and biology (Timm *et al.* 2010; Opoku-Debrah *et al.* 2016). The work done by Timm *et al.* (2010) has presented evidence that FCM populations can be traced to their area of origin or in some cases the exact orchard from which the samples were collected. The AFLP analysis used in the study by Timm *et al.* (2010) has some advantages compared to other molecular techniques, including low occurrence of artefacts and ease of generating a large number of high resolution markers (Mueller & Wolfenbarger 1999).

Opoku-Debrah *et al.* (2016) showed that different FCM populations differ in a number of biological attributes, including pupal mass, pupal survival, female fecundity and egg hatch. This study used the same FCM colonies as Opoku-Debrah *et al.* (2016). The results from Chapter Three support these two studies, as it was also found that the different FCM populations varied, i.e. it was found that males were less attracted to females from a different geographical population.

A recent study by Mgocheki & Addison (2016) used mitochondrial DNA (*mt*DNA) analysis and did not find any difference in group or clade between the different FCM populations that were analysed. The study also conducted cross-mating experiments between a number of different FCM populations that originated from different areas and hosts. The study found that all the FCM populations successfully mated except the male and female combinations where a Swellendam moth was involved. This was found to be a different tortricid and not FCM, but was not identified. All the crosses that mated produced viable offspring, and there was no significant difference in numbers of viable eggs, but there was a

difference only between number of pupae and eclosed adults (i.e. survival) (Mgocheki & Addison 2016). The study reported different area specific melanic forms of FCM, moths from Riebeek Kasteel had a lighter colour, while moths from other areas had the typical FCM colouring. This may be due to limited dispersal of moths from an area and supports the theory of possible inter-population variations. Individuals reared from olives were smaller than moths reared from other larger hosts (Mgocheki & Addison 2016).

The aim of this chapter was to conduct cross-mating experiments with FCM from different geographical populations across South Africa, the same populations used for Chapter Three, and determine any possible postmating isolation between the populations.

4.2 METHODS AND MATERIALS

4.2.1 Cross-mating trial

The FCM populations used for this study were the Old, Addo, Nelspruit, Marble Hall and Citrusdal populations, all housed at Rhodes University. These are the same colonies that were used for the laboratory experiments in Chapter Two and field trials in Chapter Three. The cross-mating experiments were conducted in petri dishes. A single virgin male and female were placed into a petri dish. The virginity of the moths was ensured by separating the males and females at the pupal stage. The male and female pupae were then kept separately until adult eclosion. The moths used were between 1-3 days old, to ensure the male and female moths were both sexually receptive. With the five available colonies, 25 crosses were possible and each cross was replicated five times (Table 4.1). A randomly selected male and female from specific regional colonies were placed in each petri dish and each pair was only used once. After a male and female combination was placed in a petri dish the lid was labelled with the date and the combination. After a total of five days the number of eggs laid by the female was counted. After the egg count, the eggs were left to hatch in the laboratory at room temperature (20°C) and the number of hatched eggs were then counted six days later. The majority of the eggs hatched five days after being laid (Figure 4.1).

Table 4.1 All the cross-mating combinations of FCM from the five available colonies that were conducted in petri dishes to determine fecundity and fertility.

	♀*OLD*	♀*ADD*	♀*NEL*	♀*MH*	♀*CD*
♂*OLD*	♀OLD x ♂OLD	♀ADD x ♂OLD	♀NEL x ♂OLD	♀MH x ♂OLD	♀CD x ♂OLD
♂*ADD*	♀OLD x ♂ADD	♀ADD x ♂ADD	♀NEL x ♂ADD	♀MH x ♂ADD	♀CD x ♂ADD
♂*NEL*	♀OLD x ♂NEL	♀ADD x ♂NEL	♀NEL x ♂NEL	♀MH x ♂NEL	♀CD x ♂NEL
♂*MH*	♀OLD x ♂MH	♀ADD x ♂MH	♀NEL x ♂MH	♀MH x ♂MH	♀CD x ♂MH
♂*CD*	♀OLD x ♂CD	♀ADD x ♂CD	♀NEL x ♂CD	♀MH x ♂CD	♀CD x ♂CD

- The acronyms used indicate the colony; Old colony (OLD), Addo colony (ADD), Nelspruit colony (NEL), Marble Hall colony (MH) and Citrusdal colony (CD).

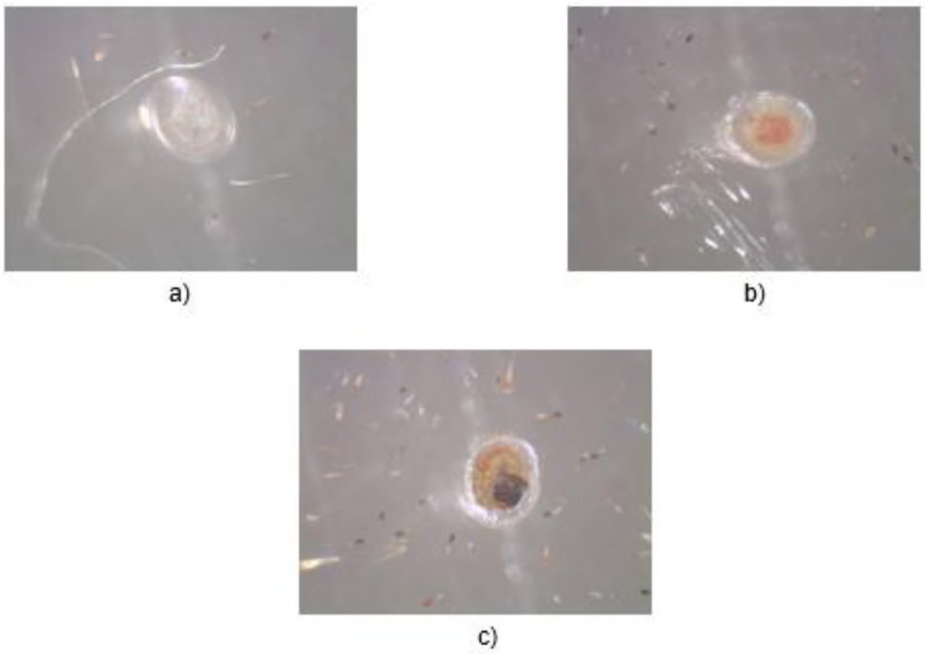

Fig. 4.1 Microscopic images taken of a) a newly laid egg, b) a three day old egg and c) a fully developed neonate larva about to hatch after five days.

4.2.2 Statistical analysis

Tests for statistical significance included the Kruskal-Wallis H test for nonparametric data and a single factor ANOVA was used for the egg count data. If a significant relationship was found, Tukey Post Hoc analysis was conducted. A probability level (P) of < 0.05 was used in all cases.

4.3 RESULTS

4.3.1 Cross-mating trial

All crosses produced eggs and larvae (Figure 4.2 and Table 4.2). In the case of the Addo, Marble Hall, Nelspruit and Old (mixed origin) populations, the FCM females laid the highest number of eggs when they were mated with males from their own population. However, the difference in the number of eggs laid between crosses was only significant for the Marble Hall females ($F = 3.050$; $P < 0.05$), but not for the Addo ($F = 2.802$; $P > 0.05$), Nelspruit ($F = 1.278$; $P > 0.05$) and Old Addo (mixed origin) ($F = 2.513$; $P > 0.05$) populations (Figure 4.2). The only population that did not have the highest number of eggs laid when the female mated with a male from the same population was the Citrusdal colony, but there was no significant difference in number of eggs laid between the crosses ($F = 0.177$; $P > 0.05$) (Figure 4.2).

There was only a significant difference in egg hatch with the crosses involving the Marble Hall females ($H=12.757$; $P < 0.05$) (Table 4.2). For these crosses only the cross involving a Marble Hall female and Addo male were significantly different to the Marble Hall female crosses with males from the other populations. For all the other crosses the origin of the male moth did not influence the percentage of eggs that hatched i.e. fertility (Table 4.2).

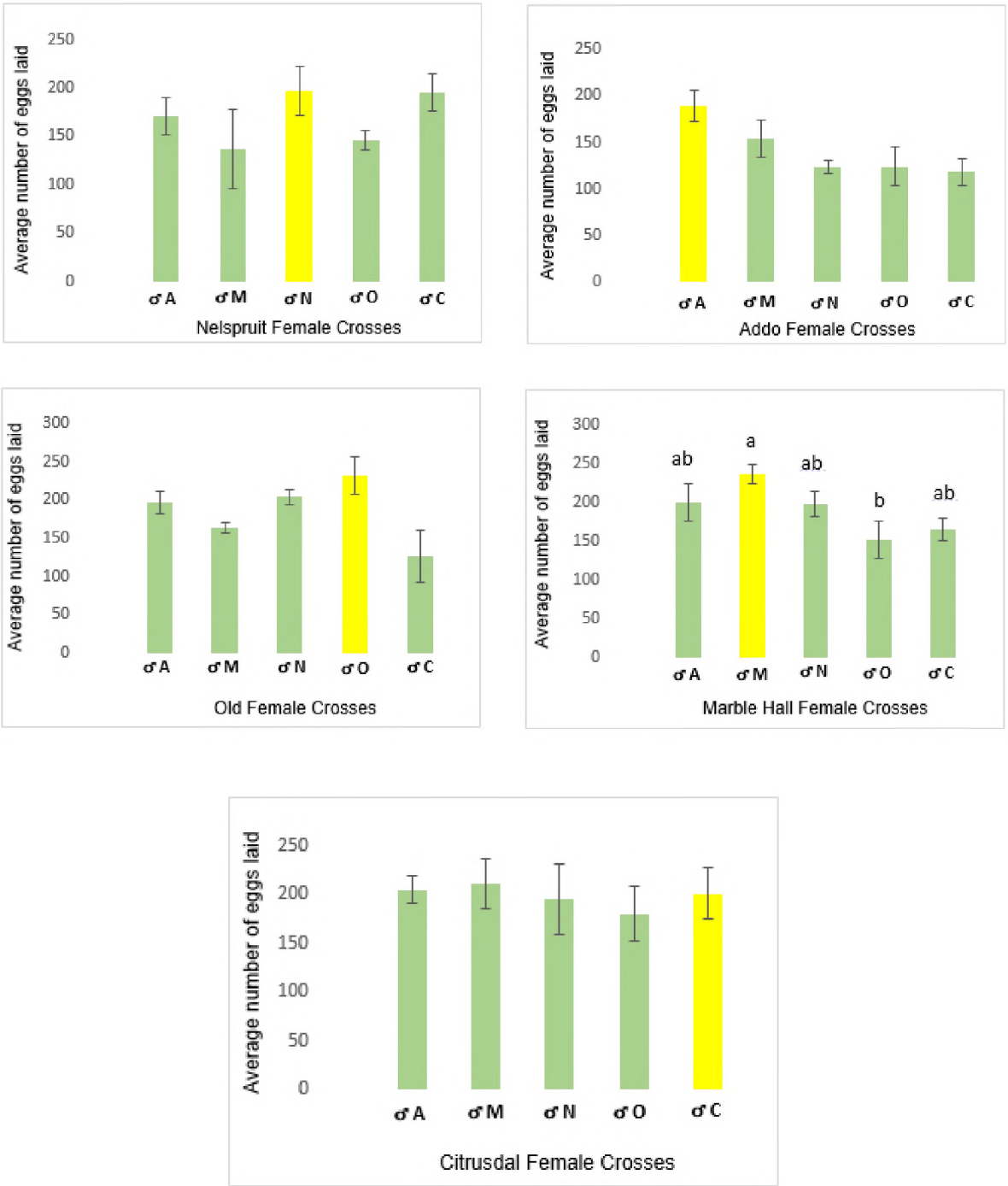

Fig. 4.2 Number of eggs laid by FCM females mated with males from different geographical populations across South Africa (the acronyms used indicate the colony; Old Addo (mixed origin) (OLD), Addo (ADD), Nelspruit (NEL), Marble Hall (MH) and Citrusdal (CD) population. Bars with different letters are significantly different ($P < 0.05$).

Table 4.2 Egg hatch results from cross-mating trial.

	Cross	Average egg hatch (%)	H	*P*
Addo ♀	♀ A ♂ A	71.53		
	♀ A ♂ M	61.30		
	♀ A ♂ N	79.19	0.575	0.9658
	♀ A ♂ O	77.75		
	♀ A ♂ C	67.01		
Marble Hall ♀	♀ M ♂ M	88.67		
	♀ M ♂ A	56.69		
	♀ M ♂ N	85.73	12.757	**0.0125**
	♀ M ♂ O	81.38		
	♀ M ♂ C	70.74		
Nelspruit ♀	♀ N ♂ N	64.22		
	♀ N ♂ M	72.70		
	♀ N ♂ A	69.29	4.767	0.3121
	♀ N ♂ O	66.05		
	♀ N ♂ C	68.45		
Old Addo ♀	♀ O ♂ O	78.15		
	♀ O ♂ M	75.32		
	♀ O ♂ N	69.18	5.100	0.2772
	♀ O ♂ A	76.62		
	♀ O ♂ C	75.88		
Citrusdal ♀	♀ C ♂ C	77.93		
	♀ C ♂ M	75.10		
	♀ C ♂ N	73.53	0.633	0.9593
	♀ C ♂ O	72.47		
	♀ C ♂ A	75.45		

4.4 DISCUSSION

This chapter investigated the likelihood of postmating isolation between different geographical populations of FCM. This will shed light on the efficacy of using sterilized males from a single origin for the use of SIT across South Africa to control FCM. Cross-mating studies have been used extensively to investigate the possibility of postmating isolation between

different populations of the same species or between different species that are closely related (Beevor *et al.* 1973; Mitchell & Tumlinson 1994; Laster & Hardee 1995; Qureshi *et al.* 2004)

Laster & Hardee (1995) conducted a study to test the intermating compatibility between *Helicoverpa zea* (Boddie) from Stoneville, Mississippi and *H. armigera* (Boddie) from Tashkent, Russia. The study reported a high percentage of females that produced viable offspring from the inbred crosses. The two species were found to have a high degree of genetic compatibility (Laster & Hardee 1995). Beevor et al. (1973) reported an example of cross-attraction between two species, *Diparopsis castanea* Hmps. and *D. waters*i (Roths.). The sex pheromones of these two species were found to be very similar, but cross-mating under laboratory conditions produced a very low egg hatch (Beevor et al. 1973).

For this study a cross-mating trial was conducted and the number of eggs and egg hatch produced by the different crosses was evaluated. It was found that all the crosses produced eggs and viable offspring. No support was found to indicate any form of postmating isolation between the evaluated FCM populations once an FCM male and female came into close proximity to one another. It was shown that females tend to produce more eggs when mated with males from the same population. This was the case for the Addo, Marble Hall, Nelspruit and Old (mixed origin) population, the Citrusdal population was the only exception. The only case in which this was found to be statistically significant was for the Marble Hall colony. Chapter Three did however show that FCM males may have a preference for the pheromone of females from their own geographical population, particularly so in the case of Citrusdal males, as this was the only population of males that could be tested. Although it has been shown that all the crosses mated, produced eggs and viable offspring, the males may not be able to efficiently locate females from a different population in the wild.

The study by Mgocheki & Addison (2016) also conducted a cross-mating trial with FCM. The moths they used originated from different geographical populations and from different hosts. The host plants included acorns, olives, and pomegranates. The areas of origin included Bonnievale, Bien Donne, Ceres, Elgin, Riebeek Kasteel and Nelspruit. The control

population used was reared from Citrusdal and maintained on an artificial diet (Mgocheki & Addison 2016). Five randomly selected males and females were placed in ventilated wooden boxes and left to mate and lay eggs for the cross-mating experiments. Unlike the trial that was conducted for this study the F_1 generation was also crossed to further asses the viability of the newly produced strain. The study also found that all the tested FCM crosses mated and produced viable offspring (Mgocheki & Addison 2016).

Chapter 5: General discussion

5.1 INTRODUCTION

False codling moth is considered to be the most important pest of citrus in South Africa. Controlling this pest is thus very important and optimizing available control methods as well as introducing new methods of control is key to the successful suppression of this pest. The aim of this study was to determine if there were differences in the attractiveness of commercial pheromone blends, regional pheromone blends and FCM females from different populations to FCM males. This was tested with trials conducted in the laboratory and in the field. These results have important implications for the monitoring and control using pheromone based methods throughout South Africa. This chapter will provide an interpretation of the significant findings and discuss the implications for FCM control through SIT in South Africa.

5.2 TESTING THE OLFACTORY RESPONSE OF *THAUMATOTIBIA LEUCOTRETA* MALES TO FEMALES FROM DIFFERENT POPULATIONS UNDER LABORATORY CONDITIONS

False codling moth females release a pheromone to attract males and it is this pheromone which consists of a blend of volatile isomers that the male moths use to hone in on a calling virgin female. Working in a laboratory with laboratory reared insects has considerable benefits over working with wild insects in the field (Huho *et al.* 2007). In order to do this it was decided to make use of a Y-tube olfactometer with which mate choice trials could be conducted. Laboratory studies using a Y-tube olfactometer are quite common, the majority of work being on the response of an insect to a plant volatile (Boff *et al.* 2001; Segura *et al.* 2012). As no previous studies have been successful in recreating the attraction of male FCM to female FCM, optimizing the setup of the Y-tube olfactometer was crucial. Male and female moths from the same population were used for this step in order to help get the response from males to virgin females from their own population as consistent and reliable as possible.

Unfortunately after changing the setup of the olfactometer and conducting trials under red light no consistent data were recorded.

Other studies have also found it difficult to recreate the attraction of male FCM towards virgin female FCM or FCM females towards a plant semiochemical in the laboratory (Smit 2004; 2005; Moore *et al.* (2013, unpublished data). Moore *et al.* (2013, unpublished data) also tested the attraction of FCM males towards virgin FCM females using both a Y-tube olfactometer and a flight tunnel, to develop a protocol for subsequent experiments. As in this study, changes were made to the design of the Y-tube olfactometer including the way in which the male was held in the Y-tube (10 mm diameter) and ultimately the substitution of a plastic tube (12 mm diameter) instead of a Y-tube. Experiments were conducted with a single male at one end and a single female at the other and finally with 25 females in a funnel at one end and 10 males on the other. As in the laboratory trials conducted in this study, the males showed a variable response and no consistent or repeatable results could be achieved (Moore *et al.* 2013, unpublished data).

There are a number of ways in which the reliability of these Y-tube experiments could be improved. In this set of trials, a positive response was regarded as a single male moving towards the virgin female within a set amount of time. Previous studies have viewed a positive response from a male moth towards a virgin female moth without setting a time limit (Baker *et al.* 1976). Baker *et al.* (1976) conducted a study on the redbanded leafroller, *Argyrotaenia velutinana* (Walker) (Lepidoptera: Tortricidae), to analyse the male's response to three components of the female produced sex pheromone. That study conducted a laboratory trial with an orientation tube and box olfactometer (Baker *et al.* 1976). For each treatment, 10 males were used. Wing-fanning and orientation towards the source of the pheromone was recorded as a positive response from the male. Using multiple males per treatment could be a more effective way to gauge a positive response towards a pheromone or calling female moth. Thus, if the majority of the 10 males show wing-fanning and orientated themselves towards the pheromone it was recorded as a positive response (Baker *et al.* 1976). However,

Moore *et al.* (2013, unpublished data) used this method of increasing the number of males per treatment in a flight tunnel trial on FCM, but here again it did not produce reliable and repeatable responses.

An alternative solution that may lead to more successful mate preference laboratory experiments with FCM may be to increase the complexity of the equipment used. The laboratory trials in this thesis relied heavily on visual observation and this may have hampered the data collection during the Y-tube olfactometer and flight tunnel trials. Electroantennograms (EAG) have been used in scientific studies for approximately 50 years and they are often used in combination with a Y-tube olfactometer or flight tunnel (Roelofs *et al.* 1971; Vickers & Thomas 1994; Soroker *et al.* 2004). With an EAG the physiological response of a mal'e moths antennae to a pheromone can be measured (Roelofs *et al.* 1971; Vickers & Thomas 1994; Soroker *et al.* 2004). The method involves removing the antenna from the insect and inserting two silver chloride wires to the ends. The voltage is then amplified between them and a pheromone is then introduced to see if a response can be measured. (Barnes *et al.* 1992). The insect can also be kept alive and a ground wire or a glass electrode is then inserted into the body and another wire to the antenna (Vickers & Thomas 1994). A way in which the flight tunnel trial could be made more viable would be to change it to a flying electroantennogram (EAG) design (Barnes *et al.* 1992; Vickers & Thomas 1994; Hansson 1995). With this method, the insects' physiological response and movement including orientation and wing fanning, after encountering the pheromone, can be synchronized (Vickers & Thomas 1994). This will eliminate any observer bias when gauging the response of the males to the pheromone of the calling virgin female. This equipment is expensive and although it is very popular for the screening of pheromones, it may not always be an affordable option for many studies (Cardé & Haynes 2004).

Zagatti and Castel (1987) conducted a study on the courtship behaviour of FCM. It was reported that FCM males possess three androconial areas. Androconia are specialized scales that release a pheromone to aid male lepidopterans in the attraction of mates (Zagatti & Castel

1987: Pivnick *et al.* 1992). These androconia have only been found to aid in short range attraction, leading to increased mating success (Zagatti & Castel 1987; Pivnick *et al.* 1992). Pivnick *et al.* (1992) conducted a study on *Thymelicus lineola* (Ochsenheimer) (Lepidoptera: Hesperiidae) and found that removing the androconia of males or covering the antennae of females led to a 30 to 40 % reduction in mating success. Zagatti and Castel (1987) reported that these structures are not always used when the male finds a potential mate, however if used by the male, they significantly increase the possibility of mating success. It was found that the courtship behaviour of FCM is one of the most complex found in any species of Lepidoptera (Zagatti & Castel 1987). There is a possibility that the androconia of males or the antennae of females were damaged during handling of the moths when they were prepared for the laboratory trials. This may be the reason for the difficulty in achieving consistency in the results from the trials.

5.3 FIELD TESTING THE OLFACTORY RESPONSE OF ADULT MALE *THAUMATOTIBIA LEUCOTRETA* TO DIFFERENT SYNTHETIC PHEROMONE BLENDS AND VIRGIN FEMALES FROM DIFFERENT POPULATIONS

The aim of Chapter Three was to test the attractiveness of a few regional pheromone blends, commercial pheromone blends and virgin females from a range of different populations to FCM males of a single population in the field. It was found that the Citrusdal males that were used do not respond equally to the different synthetic pheromone blends and virgin females. This is a significant finding for not only the FCM SIT programme in South Africa, but also for routine monitoring of the pest. The males showed a preference for the South African regional blend, Checkmate commercial blend and were most attracted to females also from the Citrusdal area.

The results from the field trials suggest that inter-population pheromone variations in FCM may be present across South Africa. This phenomenon has been well documented in other lepidopteran species (Klun *et al.* 1973; Löfstedt & Van der Pers 1985; Löfstedt *et al.* 1985; Glover *et al.* 1987; Linn *et al.* 1997; Kawazu *et al.* 2000; McElfresh & Millar 2001;

Lassance *et al.* 2010). For example, the turnip moth, *Agrotis segetum,* is an extreme example of inter-population pheromone variation (Löfstedt *et al.* 1985; Cardé & Haynes 2004). This noctuid can be found in Africa, Europe and Asia and its pheromone consists of four components: (Z)-5-decenyl, (Z)-5-dodecenyl, (Z)-7-dodecenyl, and (Z)-9-tetradecenyl acetates (Löfstedt *et al.* 1985). The ratio produced by the female varies between geographic regions and even within populations. This may lead to males from a specific area not being as attracted to a female from a different population as they are to females from their own population. Recent publications on both the biology and genetics of geographically distinct FCM populations across South Africa also support the results from the field trials (Timm *et al.* 2010; Opoku-Debrah *et al*. 2014; Opoku-Debrah *et al*. 2016).

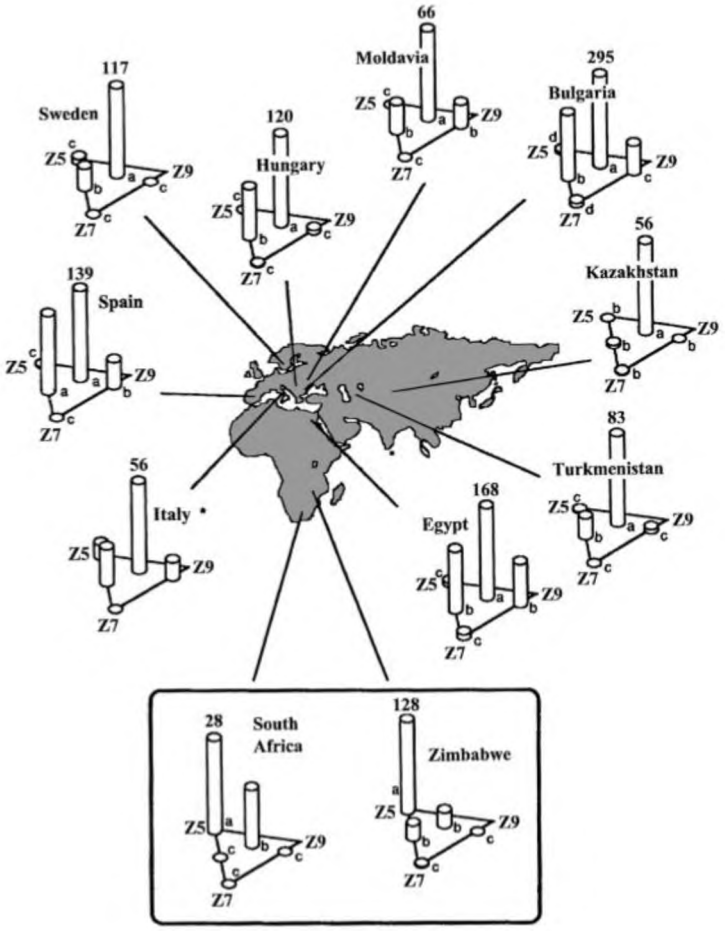

Fig. 5.1 Geographic variation in male attraction to pheromone in the turnip moth *Agrotis segetum* as percentage capture at 11 localities in Eurasia and Africa. The numbers above each column indicate the highest catch of the best. Z5, (Z)-5-decenyl acetate; Z7, (Z)-7-dodecenyl acetate; and Z9, (Z)-9-tetradecenyl acetate. Within each diagram, different letters indicate $P = 0.05$. (Tóth *et al.* 1992).

Temperature played an important role in the field trials in this study. Stotter and Terblanche (2009) have shown that laboratory reared FCM require a slightly higher temperature than their wild male counterparts in order to fly. This is confirmed by Daniel (2016). This was once again shown in the field trials of this study. When temperatures dropped below 15°C the flight activity of sterile, laboratory reared males decreased significantly, while wild males were still able to fly at a minimum temperature of 10-15°C (Stotter & Terblanche 2009). The results of the pheromone and virgin female tunnel trials highlighted the influence of temperature on sterile FCM recaptures. If temperatures were consistently above 15°C during the peak time of FCM activity for the first few evenings the recaptures were high in all instances. It was therefore crucial to keep updated with weather forecasts, in order to determine when a replicate for the trials could be attempted with a reasonable probability for success.

Recently, Daniel (2016) showed that larval diet additives (known insect cryoprotectants) could improve FCM male flight at lower temperatures (Daniel 2016). In that study, the diet of FCM larvae was augmented with different cryoprotectants over five generations and the flight ability of the resulting adults was tested in the laboratory and the field. Laboratory trials showed that male moths from augmented diets could fly at a temperature below 15°C, while control moths could not (Daniel 2016). Both the use of trehalose and cholesterol to augment the larval diets for improved flight ability was also shown to be successful in the field. In conclusion the study reported that cryoprotectants show great potential to improve the flight ability of laboratory reared moths at lower temperatures (Daniel 2016), thus potentially making released sterile moths more competitive and an SIT programme more effective.

Further, Boersma *et al.* (2017) also conducted a study with the aim of improving the performance of laboratory reared FCM through larval thermal acclimation. After acclimation at different temperatures the cold tolerance, fecundity and longevity of the adult moths was tested. It was shown that the larval acclimation did not have an effect on the cold tolerance of adult moths. The fecundity and longevity of adult moths was affected, the results were also

sex-dependant (Boersma *et al.* 2017). Interestingly the influence of larval acclimation was reported to be much greater on the female moths (Figure 5.2). These results suggest that sterile female FCM may have more significance in a SIT programme than which was previously believed. Further investigation is required to fully understand what the implications might be for a SIT programme (Boersma *et al.* 2017).

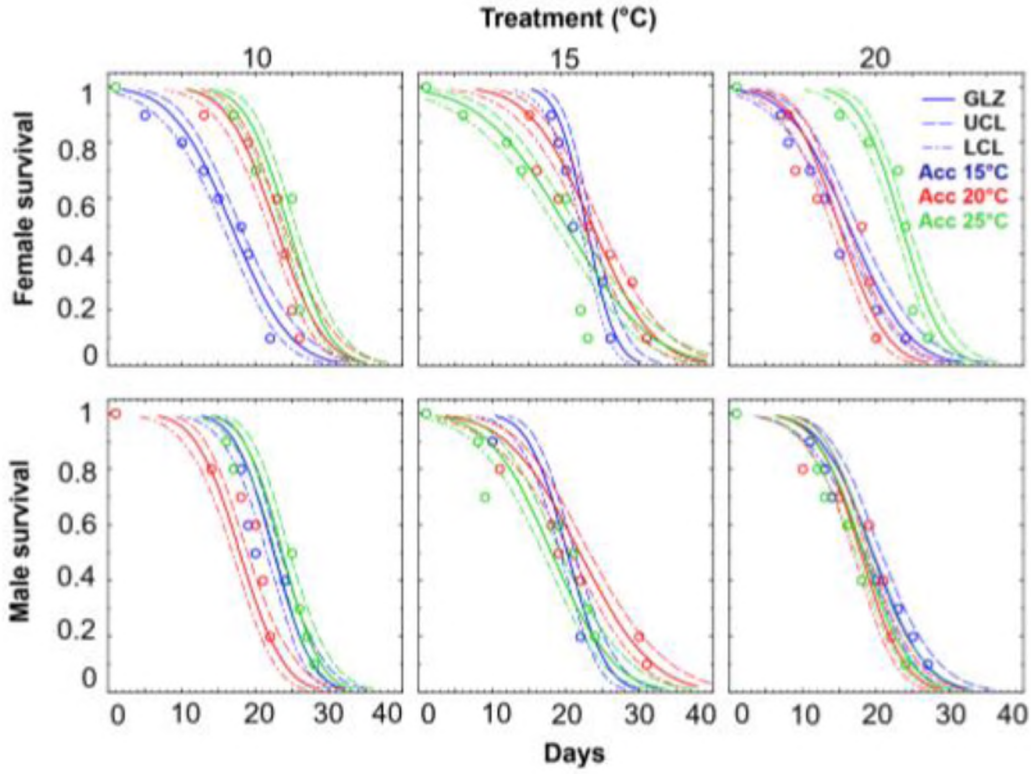

Fig. 5.2 Influence of sex, thermal acclimation (Acc) and temperature treatments on the survival time (longevity) of adult *Thaumatotibia leucotreta* with each point representing a replicate of raw data. The generalized linear model (GLZ) is displayed along with the predicted upper and lower 95% confidence limits (UCL and LCL), whereas the different colours represent the different thermal acclimation groups (Boersma *et al.* 2017).

5.4 FERTILITY AND FECUNDITY OF COMBINATIONS OF *THAUMATOTIBIA LEUCOTRETA* MALES AND FEMALES FROM DIFFERENT GEOGRAPHIC POPULATIONS

The aim of Chapter Four was to determine if any postmating reproductive isolation occurred between different populations of FCM, once a male and female were in close proximity to one another. So, although the Citrusdal males did show some differences in attraction to females of other populations, once this barrier was overcome, were inter-regional population mixes were viable. There was no indication of postmating isolation between the evaluated FCM populations, even though it was shown that females tend to produce more eggs when mated with males from the same population. This was the case for the Addo, Marble Hall, Nelspruit and Old (mixed origin) populations. The only case in which this was found to be statistically significant was for the Marble Hall populations. Mgocheki & Addison (2016) also found that there was no incompatibility between FCM populations from different geographic areas. This study even went further to test the viability of crosses by allowing the subsequent F_1 generation from the crosses to mate and produce offspring (Mgocheki & Addison 2016).

With a large number of lepidopteran species, there is a degree of inter-population pheromone variation (Klun *et al.* 1973; Löfstedt & Van der Pers 1985; Löfstedt *et al.* 1985; Glover *et al.* 1987; Linn *et al.* 1997; Kawazu *et al.* 2000; McElfresh & Millar 2001; Lassance *et al.* 2010). Some closely related species may even share some morphological features, while males are only attracted to the pheromone of conspecific females. *Arehips argyrospilus* (Walker) (fruittree leafroller) and *Archips mortuanus* Kearfoot are morphologically indistinguishable. However, there are subtle differences in the pheromones released by the female of each species (Cardé *et al.* 1977). This form of premating isolation seems to be the stronger driver for reproductive isolation in many tortricine moth species (Cardé *et al.* 1977: Presgraves 2002).

5.5 CONCLUSION

False codling moth is a pest that needs to be controlled as efficiently as possible. In order to do so, research is needed to better understand this pest. Better understanding of the pest will lead to an optimized approach when controlling and monitoring FCM populations with the methods that are currently available. The results from this study may lead to improvements in both the control and monitoring methods that utilize the pheromone communication between the male and female. These control methods include mating disruption, attract-and-kill and SIT. The results from this study unearthed the possibility for increasing the efficacy of these methods of control by tailoring them for a specific area, including a pheromone blend for a particular area or releasing SIT moths that originate from the same area. The virgin female trial highlighted the importance of the sterile males used for a particular SIT programme. Males from Citrusdal found the females from their own population more attractive and females from Nelspruit were the least attractive. This may be crucial for the future of SIT programmes across the rest of South Africa. The establishment of colonies for the production of sterile moths for a particular citrus growing area may help improve the efficiency of SIT and thus the control of FCM in an area. The South African synthetic pheromone blend showed considerable potential to be incorporated into a commercial lure, mating disruption or attract-and-kill product that can possibly be tailored to a particular citrus growing area. The synthetic pheromone trial and virgin female trial were only conducted with sterile Citrusdal males and future studies should therefore look at males from different populations in order to establish if males from other areas are also more attracted to females from their own population.

A research question that this thesis highlighted is the possibility of using sterilized males from a laboratory colony that originates from different populations to improve the efficacy of a SIT programme for FCM. The hypothesis is that with a mixed population the specificity may be reduced leading to optimized control in areas that share origin with the mixed population. Males from the Old (mixed origin) population can thus be used in a field trial to test this hypothesis. If this is the case there will be no need to have separate colonies for specific

growing areas when wanting to tailor SIT for a particular area. One population of mixed origin may be all that is required to optimize the SIT programme for FCM in South Africa.

This study once again showed the impact of laboratory rearing on the fitness of FCM used for SIT in South Africa, in this case the flight ability at lower temperatures. Daniel (2016) and Boersma *et al.* (2017) have already initiated this research effort and continued research in this area is crucial. Alternative control methods are already showing great promise and in the near future, effective control of all the stages of the FCM life cycle is going to be the key factor in reaching the optimum suppression of this pest in the field.

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