Evaluation of potential oviposition deterrents for false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae)

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Abstract

There has been extensive research on the use of semiochemicals as deterrents or true repellents in insect pest management, particularly in push-pull strategies. Much of this research has focused on pests of medical and veterinary importance and has been limited for agricultural pests. This means there is an opportunity to study use of deterrents to manage pests of agricultural importance. No study has been conducted on deterrents for false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), a key phytosanitary pest in citrus orchards across South Africa. This study assessed FCM oviposition deterrence in botanicals (plants (n = 11) and essential oils (n = 15)), and some commercial pesticides (n = 7) used for FCM control in South Africa. All tested botanicals were selected based on an extensive literature review of plant compounds that have been reported to deter or repel lepidopteran pests. Choice and no-choice oviposition bioassays were conducted in complete darkness in a controlled environment room. Oranges treated with solutions/suspensions of potential oviposition deterrents were placed into a cage with gravid FCM females for four hours, with oviposition being recorded every hour. Of the 33 tested compounds, only eight significantly reduced FCM oviposition ($P < 0.05$) compared to the control in oviposition bioassays i.e. two essential oils (lavender and peppermint), two plant crude extracts (garlic and marigold), one fruit (Mango), and three commercial FCM insecticides (Delegate, Coragen, and Warlock). All identified oviposition deterrents, except for Mango, were further investigated for their ovicidal properties in concentration response bioassays, where all botanicals were identified to have dual action (both deterrent and ovicidal properties), as they significantly ($P < 0.05$) reduced FCM oviposition and egg hatch. Garlic was the most efficacious botanical whilst Warlock was the only commercial insecticide that did not show ovicidal activity ($F = 41.17$, $P = 0.0622$). Larval penetration of the host fruit was less than egg hatch for all tested compounds in concentration response bioassays. Oviposition, egg hatch, and larval penetration were all affected by concentration, with the higher concentrations being the most effective. The efficacy of these deterrent compounds should be further tested in semi-field and/or field trials, and they may have potential in FCM management as allomone dispensers or sprays. They can also be implemented in push-pull strategies where they can be used in conjunction with FCM attractants. Lastly, repellence studies should be conducted in absentia of the host fruit to determine whether oviposition deterrence was a result of true repellence or odour masking.

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List of Abbreviations

a.i	Active ingredient
ANOVA	Analyses of Variance
CA	California
CABI	Centre for Agriculture and Bioscience International
CE	Controlled environment
CGA	Citrus Growers' Association
cm	Centimetre
CRI	Citrus Research International
CrpeNPV	Cryptophlebia peltastica Nucleopolyhedrovirus
CSI	Chitin Synthesis Inhibitors
DAFF	Department of Agriculture, Forestry and Fisheries
DALRRD	Department of Agriculture, Land Reform and Rural Development
EAG	Electroantennography
e.g.	Exempli gratia (for example)
EOs	Essential Oils
EPF	Entomopathogenic Fungi
EPNs	Entomopathogenic Nematodes
EPPO	European and Mediterranean Plant Protection Organisation
et al	et alia (and others)
EU	European Union
FCM	False Codling Moth
g	Gram

GC-EAD	Gas Chromatography-Electroantennographic Detection
GC-MS	Gas Chromatography-Mass Spectrometry
GLM	Generalised Linear Model
h	Hours
ha	Hectare
HCN	Hydrogen Cyanide
H_2O	Water
HPLC	High Performance Liquid Chromatography
i.e.	Id est (namely)
IGRs	Insect Growth Regulators
IPM	Integrated Pest Management
ISCA	Integrated Pest Management for Sustainable Agriculture
kg	Kilogram
L	Litre
Ltd	Limited
mg	Milligram
min	Minutes
ml	Millilitre
mm	Millimetre
ODI	Oviposition Deterrent Index
ODPs	Oviposition-Deterring Pheromones
ppm	Parts Per Million
PPP	Plant Protection Products
Pty	Party
SANBI	South African National Biodiversity Institute

SEM	Standard Error Mean
SIT	Sterile Insect Technique
sp.	Species (singular)
spp.	Species (plural)
SPME	Solid-Phase Microextraction
SSR	Single Sensillum Recordings
UK	United Kingdom
USA	United States of America
WHO	Word Health Organisation
Xsit	X Sterile Insect Technique
&	And
~	Approximately
≥	Greater or equal to
°C	Degree Celsius
<	Less than
µl	Microlitre
%	Percentage
±	Plus/minus

Chapter 1,

General Introduction

1.1 THE SOUTH AFRICAN CITRUS INDUSTRY

1.1.1 Taxonomy of citrus

The genus *Citrus* was first established in 1753 by Carl Linneaus. Following its establishment, *Citrus* taxonomy and the number of species that belong to the genus has been widely debated (Nicolosi *et al*. 2000). There are currently two common classification systems of *Citrus* taxonomy: the Swingle & Reece (1967) system and the Tanaka (1977) system. Swingle & Reece's system described three groups of the subtribe Citrinae: Citreae (primitive citrus fruit trees), Aurantioideae (near-citrus fruit trees), and Rutaceae (true citrus fruit trees). The Rutaceae group consisted of six subgenera including *Clymenia* (Swingle), *Eremocitrus* (Swingle), *Microcitrus* (Swingle), *Fortunella* (Swingle), *Poncirus* (Raf.), and *Citrus* (L.). The *Citrus* (L.) genus consists of 16 species distributed in two subgenera: *Citrus* (consisting of 10 species) and *Papeda* (consisting of six species) (Nicolosi *et al*. 2000). Tanaka (1977) reported that the *Citrus* genus included a total of 159 species and 14 variant species distributed in two subgenera, *Metacitrus* (Tanaka) and *Archicitrus* (Tanaka). It has been long agreed that the *Citrus* genus consists of three ancestral species, namely the Mandarin *C. reticulata* (Blanco), the Citron *C. medica* (L.), and the Pummelo *C. maxima* (Merril) (Scora 1975; Barrett & Rhodes 1976; Moore 2001). However, as the years progressed following the establishment of the *Citrus* genus, chemical classification, morphological, and genetical analyses were introduced (Wu *et al*. 2018; Ollitrault 2020). As a result of the introduction of such technological advancements, it is now generally agreed that the *Citrus* genus consists of 10 fundamental species, which are, *C. glauca* (Lindl.) Burkill, *C. australasica* (F. Muell.), *C. australis* (A. Cunn. ex Mudie), *C. micrantha* (Wester), *C. mangshanensis* (MS), *C. ichangensis* (Swingle), *C. margarita* (Lour.), and the three 'ancestral' species mentioned above (Wu *et al*. 2018; Ollitrault 2020). It has been suggested that all other species were derived from hybrids, cultivars, or variant species which

comprise of some important commercial fruit including *C. paradisi* (grapefruit), *C. limon* (lemon), *C. aurantifolia* (lime), *C. aurantium* (sour orange), and *C. sinensis* (sweet orange) (Nicolosi *et al*. 2000). The main reasons for the complication of *Citrus* classification and phylogeny include apomixes (asexual reproduction) which leads to reproductive isolation as a result of limited gene exchange, long history of cultivation, and frequent bud mutations (Moore 2001).

1.1.2 The importance of the citrus industry

The production of citrus fruit is common practice in many countries across the globe. In South Africa, citrus was first introduced in 1654, with the first trees being planted in the Western Cape region (Citrus Growers' Association (CGA) 2022). Following its establishment, the South African citrus industry managed to grow exponentially with the first fruit being traded to Britain in the early 1900s (Mather & Greenberg 2003).

The South African citrus industry is currently the largest fruit producing industry in the country with large volumes of citrus fruit being produced and harvested annually (Table 1.1) (CGA Key Industry Statistics 2022). The industry is responsible for producing a variety of citrus fruit, such as soft citrus (mandarins), oranges, lemons, limes, and grapefruit (CGA Key Industry Statistics 2022). Citrus fruit is produced in many different regions across South Africa (Figure 1.1). The main production areas are located in the Limpopo (40 383 ha), Eastern Cape (25 226 ha), Western Cape (18 952 ha), and Mpumalanga (7 877 ha) provinces (Table 1.2) (Munro *et al*. 2016; CGA Key Industry Statistics 2022). Of the nine provinces in the country, only two provinces (Gauteng and the Free State) do not produce citrus (Figure 1.1) (CGA Key Industry Statistics 2020).

The difference in climatic conditions across the regions plays a significant role in the annual production of citrus. For example, the Western and Eastern Cape have cooler climatic conditions with production being mainly focused on Navel oranges and lemons, whereas the upper KwaZulu Natal and Limpopo regions consist of warmer climatic conditions selecting for the production of Valencia oranges and grapefruit (Munro *et al*. 2016). Oranges are the most produced citrus fruit in the country, with the Eastern Cape producing approximately 41% of Navel oranges and Limpopo producing approximately 59% of Valencia oranges (Munro *et al*. 2016; CGA Key Industry Statistics 2022).

South Africa is currently the second largest exporter of citrus fruit globally, behind Spain (largest exporting country) (CGA Key Industry Statistics 2022). A large percentage (77%) of the citrus produced is exported to overseas markets to boost foreign income and thus promote economic growth, as a result. South Africa is responsible for more than 60% of citrus exports in the Southern Hemisphere (CGA Key Industry Statistics 2022). The remaining citrus is either sold to local markets (5%) or sent for processing (18%) (CGA Key Industry Statistics 2022). There are seven major export destinations for South African citrus with Europe being the largest and accounting for 34% of the total exports, followed by the Middle East (18%), South-East Asia (14%), United Kingdom (UK) (10%), Russian Federation (8%), North America (8%), and Asia (7%) (CGA Key Industry Statistics 2022). Other smaller countries only account for 1% of the total exports. Because the export market is important and accounts for a large proportion of the total gross income, it is vital that the citrus industry produces high quality fruit that meets international trade standards (Grout & Moore 2015; CGA 2022). This means that the fruit must be free of pests, diseases and have very little to no chemical residue from pesticides. These phytosanitary regulations are applied on an international level and thus put pressure on the citrus farmers to produce high grade fruit in an environmentally friendly manner, or face risk of rejection by overseas markets (Moore 2002; Moore & Hattingh 2012; Dikilili & van Rooyen 2018).

Table 1.1: Harvesting period for different types of citrus in South Africa (CGA 2019).

Citrus	**Harvest Period**
Marsh Grapefruit	March to June
Star Ruby Grapefruit	April to September
Navel Oranges	March to July
Valencia Oranges	July to September
Mandarins/Tangerines	March to August
Lemons/Lime	February to September

Table 1.2: Number of hectares of citrus planted in each South African province (CGA Key Industry Statistics 2022).

Province	Area (ha)
Limpopo	40 383
Eastern Cape	25 226
Western Cape	18 952
Mpumalanga	7 877
KwaZulu-Natal	2 440
Northern Cape	1 838
North West	822

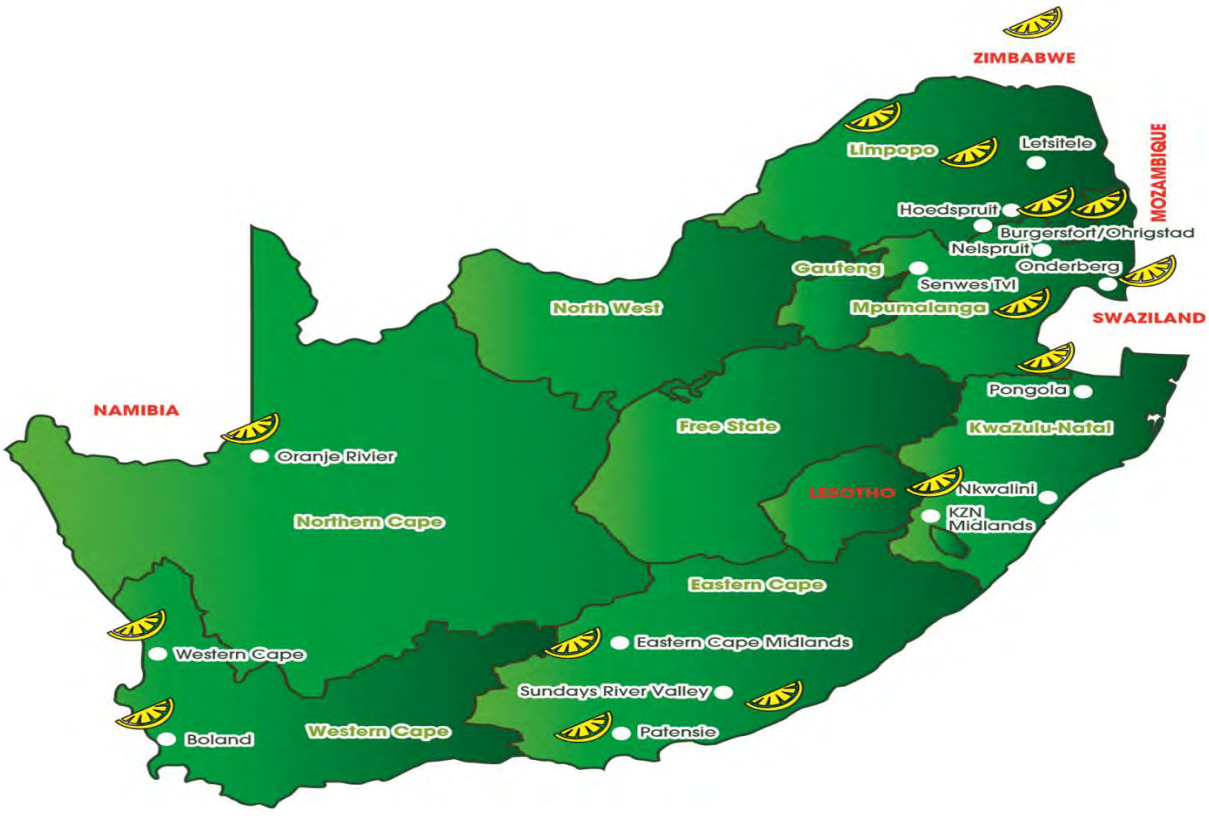


Figure 1.1: Citrus producing regions in South Africa (Map adapted from CGA Key Industry Statistics 2020).

1.1.3 Common South African citrus pests

Citrus pests (insects, pathogens, weeds etc.) pose a significant threat to production on a global scale. In simple terms, citrus pests can be described as organisms of mostly economic, cosmetic, or phytosanitary concern, which make citrus fruit less marketable. South Africa has recorded over 100 species of citrus pests over the years (Bedford 1998). It is important to note that not all pests have a significant impact on citrus production, as they are largely affected by varying climatic conditions (Munro *et al*. 2016). Warmer and more humid regions are more prone to citrus pests (Urquhart 1999; Smith & Peña 2002); as a result, some pests only obtain pest status during specific seasons when the climatic conditions are favourable (Bedford 1998).

South African citrus orchards consist of many insects and other organisms that can be regarded as pests; very few can be considered beneficial. Most of these pests belong to the order Hemiptera (~30-60%) with insects such as mealybugs and scale insects causing the most damage (Smith & Peña 2002). Next in line are lepidopteran insects (fruit borers, leaf miners, and leaf rollers). Furthermore, pests such as beetles, mites, flies, and thrips can also cause considerable damage (Smith & Peña 2002).

Pests are classified as being major or minor pests, based on the amount of damage caused and lack of marketability (Smith & Peña 2002). Major pests are regarded as pests that are constantly present throughout the season and cause the most damage to tree and fruit health or pests that are of significant cosmetic or phytosanitary concern. On the other hand, minor pests are pests that are not of major cosmetic or phytosanitary concern, cause little economic damage, and whose population size can be easily controlled (Smith & Peña 2002). In South Africa, a total of seven important major pests have been identified, and these are citrus thrips *Scirtothrips aurantii* (Faure) (Thysanoptera: Thripidae), citrus psylla *Trioza erytreae* (Del Guercio) (Hemiptera: Triozidae), California red scale *Aonidiella aurantii* (Maskell) (Hemiptera: Diapsidae), citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), Natal fruit fly *Ceratitis rosa* (Karsch) (Diptera: Tephritidae), Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), and false codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (Smith & Peña 2002).

False codling moth (FCM) is the topic of this thesis given that it is a key phytosanitary pest (Bloem *et al.* 2007; Moore & Kirkman 2008). This is because FCM is considered a quarantine pest for lucrative export markets. The mere presence of a single moth in consignments destined to these markets may result in the rejection of the entire shipment. For this reason, South Africa has a zero tolerance to this pest in fruit packed for export (Moore *et al*. 2015).

1.1.4 Citrus pest management

Majority of pest control strategies require monitoring before effective implementation. Monitoring of pest population dynamics helps to determine when a control strategy should be implemented to control the pest (Smith & Peña 2002). For this reason, monitoring forms the core of most control strategies utilised by the South African citrus industry to date (Ezzat *et al*. 2020).

Since its establishment in the early 1900s, the South African citrus industry has utilised chemical pesticides to control pests. Such pesticides included resin-wash, sulphur dust, lime sulphur, nicotine sulphate, miscible petroleum, oil emulsions and fumigation with hydrogen cyanide (HCN) (Bedford 1998). All chemicals and oils were reported to have no harmful effects to the environment and human health, except for fumigation, which involved the use of toxic substances harmful to humans (Bedford 1998). In 1948, most pesticides were replaced with parathion to enhance chemical control, which was fast gaining popularity at the time (Bedford 1998). However, in the 1970s, the continued use of parathion proved to have detrimental environmental impacts as it led to large scale pest outbreaks, which were driven by insect resistance and a decline in natural enemy populations (Bedford 1998). A large proportion of these scale insect outbreaks consisted of *A. aurantii* (Bedford 1998). Following these limitations, the use of pyrethroids was introduced as a control measure for different pest species (Newton 1998). However, similar results were achieved as it also led to pest resistance and outbreaks of non-target pests (Urquhart 1999; Charleston *et al*. 2003). Because of such limitations to chemical control methods, the South African citrus industry was forced to develop and/or implement different strategies that were less dependent on the use of chemicals. A movement to integrated pest management (IPM) took place in the late 1900s (Urquhart 1999) and has been in use ever since.

IPM can be defined as “a decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost/benefit analyses that take into account the interests of and impacts on producers, society, and the environment” (Kogan 1998). It involves implementation of various control strategies aimed at reducing pest populations and simultaneously keeping harmful environmental impacts at a minimum (Nwilene *et al.* 2008; Pretty & Bharucha 2015). The South African citrus industry

has achieved great success with IPM programmes. This is discussed in greater detail in Urquhart (1999). More importantly, IPM programmes have been reported to achieve greater success because of their use of combined control techniques such as cultural (e.g. orchard sanitation), behavioural (e.g. life-cycle disruption), genetic (e.g. sterile insect technique (SIT)) and biological (e.g. Cryptogran®) control (Moore *et al*. 2004; Moore *et al*. 2014). IPM programmes save citrus growers a lot of money as they are primarily natural and environmentally friendly (Way & Van Emden 2000).

1.2 FALSE CODLING MOTH

1.2.1 Classification and taxonomy

FCM is a key citrus pest native to southern Africa. It belongs to the Order Lepidoptera and Family Tortricidae. In South Africa, FCM was first reported as a citrus pest in the KwaZulu-Natal province by Fuller (1901) and classified as *Carpocapsa* sp. (Newton 1998). Following its classification, FCM was later discovered as the orange codling moth in the then Transvaal region (Newton 1998). It was first described by Meyrick in 1912 as *Argyroploce leucotreta* (Eucosmidae: Olethreutinae) and later transferred to the genus *Cryptophlebia* by Clarke (1958). The species was finally transferred to its current genus *Thaumatotibia* by Komai (1999). The morphological features of species belonging to the *Thaumatotibia* and *Cryptophlebia* genera are adequately distinguished in Venette *et al*. (2003).

1.2.2 Host range and distribution

FCM is endemic to sub-Saharan Africa. It is considered an extremely polyphagous pest with over 70 host plants recorded on a global scale in 40 different plant families (EPPO 2017; Moore 2019). A large proportion of FCM host range falls within South Africa because of the rich citrus production culture in the country. There are currently 24 cultivated plants that are considered potential hosts for FCM in South Africa (Navel oranges being one of the most susceptible) (Grout & Moore 2015; EPPO 2017; Bazelet 2020) (Table 1.3). However, it is important to note that many of these plants were recorded from laboratory observations and there is no guarantee that the same observations can occur in the wild.

FCM has a widespread distribution across southern Africa (Figure 1.2). It has also been reported to occur in some islands such as Mauritius and Madagascar (Daiber 1979). In the early 1980s, FCM was established in Israel as a pest of Macadamia nuts (Newton 1998). It was later intercepted in 2008 in a consignment in the United States of America (USA), However, following extensive observations, no population was found to have established in the USA during that period. Since 2008, FCM has been detected on rare occasions in countries such as the Netherlands, Sweden, and the UK (EPPO 2017). Population establishment cannot occur in any of these countries due to the cold climatic conditions that do not support permanent populations of FCM (EPPO 2017).

Table 1.3: Some potential cultivated host plants for false codling moth *Thaumatotibia leucotreta* in South Africa (Moore 2012; EPPO 2017).

Common name	**Scientific name**	**Family**
Navel orange	*Citrus sinensis*	Rutaceae
Olive	*Oleae europaea*	Oleaceae
Avocado	*Persea americana*	Lauraceae
Grape	*Vitis vinifera*	Vitaceae
Peach	*Prunus persica*	Roseaceae
Pomegranate	*Punica granatum*	Punicaceae
Litchi	*Litchi chinensis*	Sapindaceae
Macadamia	*Macadamia integrifolia*	Proteaceae
Coffee	*Coffea arabica*	Rubiaceae
Soursop	*Annona muricate*	Annonaceae
Common oak	*Quercus robur*	Fabaceae
Walnut	*Juglans sieboldiana*	Juglandaceae
Cotton	*Gossypium hirsutum*	Malvaceae
Okra	*Abelmoschus esculentus*	Malvaceae
Guava	*Psidium guajava*	Myrtaceae

Carambola	*Averrhoa carambola*	Oxalidaceae
Maize	*Zea mays*	Poaceae
Sorghum	*Sorghum halepense*	Poaceae
Nectarine	*Prunus persica variety nectarina*	Rosaceae
Grapefruit	*Citrus paradisi*	Rutaceae
Peppers	*Capsicum spp*	Solanaceae
Tea	*Camellia sinensis*	Theaceae
Jakkalsbessie	*Diospyros lycioides*	Ebenaceae
Castor bean	*Ricinus communis*	Euphorbiaceae

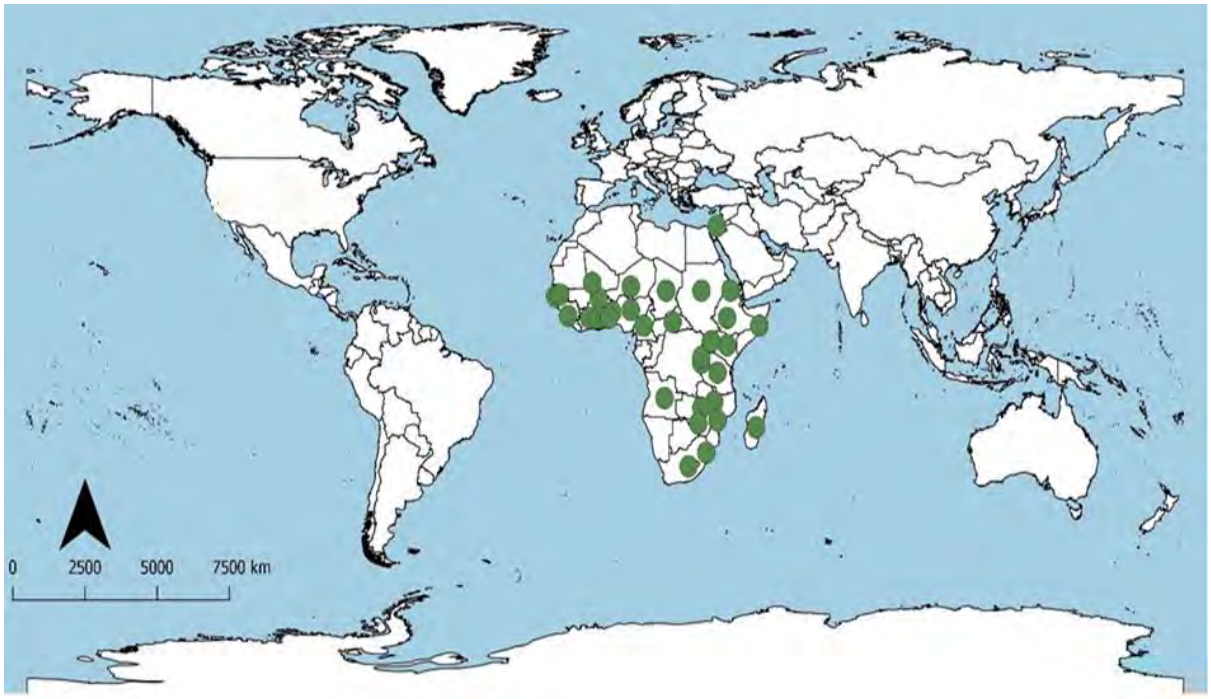


Figure 1.2: False codling moth *Thaumatotibia leucotreta* geographic distribution. The presence of FCM is represented by a green dot on the map (Map drawn by Bazelet (2020) using data from CABI (2017)).

1.2.3 Life cycle

FCM eggs are relatively small in size (approximately 0.9 mm long) and have a flat oval shape (Figure 1.3a) (Daiber 1979a). The duration of the egg stage is approximately nine to 12 days in winter and six to eight days in summer. This is because warmer climates select for shorter generation times (Terblanche *et al*. 2014). The larval stage consists of five instars with the final instar being the largest (approximately 15 mm long) (Figure 1.3) (Daiber 1979b). Early instars have a very pale and spotted appearance, whilst later instars have a bright pink appearance and can be distinguished from other species by their dark spiracle, eighth abdominal segment, and anal comb, which can be viewed under a microscope (Figure 1.3). The duration of the larval stage is 35-67 days in winter and 25-35 days in summer (Daiber 1979b). The larva is ready to pupate after the fifth instar. The pupae are approximately 11 mm long and are contained in a soil cocoon (Love *et al*. 2014). The prepupal stage is light beige/brown in appearance. This stage lasts 29-40 days in winter and 21-24 days in summer (Daiber 1979c). The final stage of the FCM life cycle is the adult stage, which lasts for approximately two to three weeks (Daiber

1980). The male adult has a wingspan of 15-16 mm, whereas the female adult has a longer wingspan of 19-20 mm (Daiber 1980). FCM adults can be easily recognised by a triangular marking on the edge of the wing which contains a c-shaped black spot above it (Figure 1.3). It is also easy to distinguish between the sexes as the male has a tuft of hair on the anus and hind wing used for sensing (Figure 1.4) (Daiber 1980). Additionally, the male hind tibia also has a tuft of elongated scales (Daiber 1980). The average female FCM can lay between 100-400 eggs.

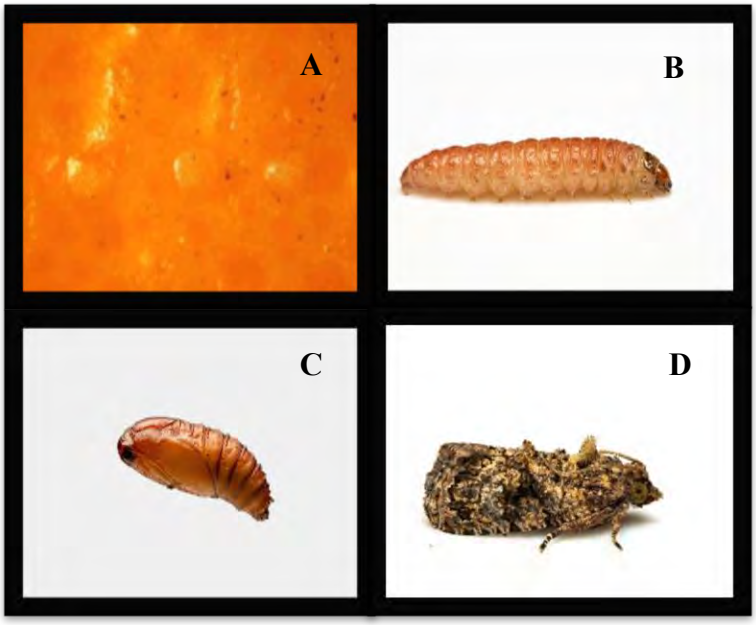


Figure 1.3: False codling moth, *Thaumatotibia leucotreta*, life stages: (A) eggs, (B) mature larva, (C) pupa, and (D) adult (Photographer: J.H. Hofmeyr 2006 (A); D. Taylor 2020 (B-D)).

Figure 1.4: Differences in adult male (left) and female (right) false codling moth *Thaumatotibia leucotreta* hindwings. The male hind wing has a scent organ indicated by the arrow (invasive.org; Bazelet 2020).

1.2.4 Brief overview of biology and life history

FCM has five to six overlapping generations per year with no winter diapause (Terblanche *et al*. 2014). The length of each generation is approximately five weeks to three months with a thermal development threshold of 11.6°C or lower (Terblanche *et al*. 2014).

The mating process of FCM is largely controlled by the female sex pheromone. However, in some cases, the male may also release its own pheromones to stimulate the female during courtship. Zagatti and Castel (1987) conducted a laboratory study on the role of pheromone releasing male androconia in courtship behaviour. The use of male androconia involves movement towards the head of the female with forewings flapping and hindwings spread horizontally exposing the androconia. The female then interacts with the exposed androconia using its antennae. It was reported that the use of FCM male androconia during courtship was not essential to mating success, however, it does provide a significant advantage to males as it increases the percentage of successful matings and decreases the mean number of attempts before copulation (Zagatti & Castel 1987). The adult female on the other hand, releases a sex pheromone at low concentrations to stimulate the male. The male then responds to the attractive

stimulus by sensing the pheromone from a distance using a specialised sensory gland located on the hind leg (El-Shafie & Faleiro, 2017). The attracted male flies up the plume to locate the female mate. Once the mate has been located, the male will begin the courtship process i.e. extension of genital valves, frequent vibrations of anal hairs, followed by physical contact with the female, and finally attempting to mate (El-Shafie & Faleiro 2017). Mating can be attempted numerous times before it is finally successful. Following mating, the female FCM generally begins to lay eggs two days after adult emergence. Failure to mate results in the female laying unfertilised eggs. Eggs are laid on fruit surfaces or leaves with preference given to ripened, damaged or rotting fruit (Catling & Aschenborn 1974; Hofmeyr *et al*. 2016).

Upon hatching, the first instars immediately bore into fruit, where they will complete larval development (Newton 1998). The larval stage is the only damaging stage of the FCM lifecycle (Newton 1998). Upon completion of the five larval instars, the final instar emerges from a perforated hole, carrying frass as it exits the fruit (Newton 1998). The larva then falls to the ground, where pupation occurs in a silk cocoon in topsoil (Georgala 1969; Newton 1988).

The extent of damage caused by FCM varies depending on the fruit. For example, it is rare for FCM to bore deep into the core of a citrus fruit, whereas in stone fruit, larvae bore deep into the core of the fruit and feed closer to the stone (Bazelet 2020). Citrus fruit usually drop within three to five weeks following larval penetration (Newton 1998). In South Africa, FCM life cycle length and damage caused differs between fruit types and regions (CGA 2019). Because of its extremely polyphagous nature and its ability to adapt to different environmental conditions and hosts, FCM is considered a quarantine pest in many parts of the world, especially in American, Asian, and European countries where establishment is likely to occur due to climatic conditions similar to those experienced by South Africa (Bazelet 2020).

1.2.5 Economic significance in citrus industry

FCM is a key economic pest of citrus in South Africa. Most of the crops on which the pest occurs are economically valuable commodities in the agricultural sector (Kirkman & Moore 2007; Moore 2019). In the early 1990s, citrus crop losses of 10-20% due to FCM infestation were reported in South Africa (Glas 1991). In addition, 28% losses were reported on late peach crops due to FCM damage in 1989. Citrus production forms a large proportion of the South African agricultural sector and contributes to the country's economic growth. FCM also has

quarantine pest status in many of the overseas markets (Moore 2002; Kirkman 2007). This is a huge cause for concern for the citrus industry, as the majority of fruit are exported to generate foreign income. For these reasons, it is mandatory to ensure that FCM populations are adequately controlled in South Africa to maintain export markets and continued income (Moore 2002; Moore & Hattingh 2016).

1.2.6 Common management strategies

FCM management is an important component of the South African citrus industry and involves a variety of pre-harvest control techniques, such as monitoring of pest populations, behavioural control, cultural control, chemical control, genetic and biological control. Many of these control strategies achieve higher efficacy when used in conjunction with one another (Moore & Hattingh 2016; Malan *et al*. 2018). FCM pre-harvest control mainly consists of three important components, the first being orchard sanitation which forms the foundation of all control programmes. Following this foundation is an areawide control technique, for example, sterile insect technique (SIT) or mating disruption. The last component involves the application of orchard specific control measures, such as insecticidal sprays or parasitoid releases (Moore 2021).

In IPM programmes, monitoring of pest populations is used as a tool to determine whether control measures need to be implemented in orchards and if so, when (Moore *et al*. 2016). Monitoring involves the combination of sex pheromone lures with a trap to measure the presence and abundance of pest species (Moore & Kirkman 2011). In the case of FCM in South Africa, commercial products such as (F.C.M PheroLure®) comprising of the synthetic female sex pheromone ((E)-7-Dodecenyl acetate (12.5 mg): (E)-8-Dodecenyl acetate (10.4 mg): (Z)-8-Dodecenyl acetate (2.1 mg)), are used in conjunction with a yellow delta trap to lure and trap male FCM (Munro *et al*. 2016). The trap is usually positioned at the top of citrus trees in order to allow for downwind movement towards the males. However, since FCM is now considered to be more of a phytosanitary pest rather than a regular economic pest, monitoring can no longer be used as a primary tool to determine the need for implementation of a control measure in orchards (Moore *et al*. 2008). This suggests that the consequences of FCM larvae being detected in the marketplace outweigh those of potential economic losses the pest could cause (Moore 2021). As a result, monitoring tools are now used to improve precision and timing of

treatments (Moore *et al*. 2008). South African citrus farmers utilise fruit infestation monitoring to determine whether infestation is sufficiently low in an orchard to allow for the export of fruit to markets which regard FCM as a phytosanitary pest (Hattingh *et al*. 2020).

Orchard sanitation is an old control method that plays a significantly important role in any FCM control programme (Newton 1998). It is often used in conjunction with biological control (occasional release of naturally occurring parasitoids) to prevent FCM outbreaks in South African citrus orchards. Orchard sanitation involves the regular collection and proper disposal of all infested and damaged fruit from orchard trees and floors (Moore & Kirkman 2009). Moore and Kirkman (2008) reported that six months (December to June) of weekly orchard sanitation reduced infestation by ~75% on average.

Chemical control remains one of the trickiest control measures of FCM due to the possibility of natural enemy population reductions resulting from chemical residues. Nonetheless, a large variety of chemicals are registered for use against FCM in South Africa. These are Alystin® (Triflumuron), Nomolt® (Teflubenzuron), Meothrin® (Fenpropathrin), Cypermethrin® (Cypermethrin), Delegate® (Spinetoram), Coragen® (Chlorantraniliprole), and Warlock® (Emamectin benzoate) (Moore & Hattingh 2012). The use of chitin synthesis inhibitors (Alystin® and Nomolt®) on fruit is prohibited in many export markets due to the requirements for submission of updated tox packages to retain usage tolerance and the unwillingness of manufacturers to do this, due to patent lapse (Kirkman 2007; Moore 2019). Pyrethroids (Meothrin® and Cypermethrin®) can also have a negative impact on a range of FCM natural enemies, hence these chemicals are not compatible for use in IPM programmes (Kirkman 2007; Moore & Hattingh 2012; Fullard & Hill 2013: Moore 2019). Delegate®, Coragen®, and Warlock® are the latest additions to FCM chemical control and have been reported to have less detrimental effects on natural enemy populations, mainly due to their eco-friendly approach (Moore & Hattingh 2012; Fullard & Hill 2013).

Mating disruption and the attract-and-kill technique focus mainly on pest behavioural manipulation. Mating disruption is a commonly used technique in IPM programmes. It is largely based on the manipulation of insect behaviour in a manner that selects for population reduction (Mitchell 1975; Moore & Hattingh 2012). In South Africa, mating disruption for FCM was first introduced in the 1990s with the first products consisting of the oriental fruit moth *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) pheromones. Although these products displayed some form of efficacy against FCM, it was not sufficient for

commercialisation of the products (Hofmeyr & Hofmeyr 1998). This led to trials in Navel orange orchards using Isomate® (Shin-Etsu, Japan), which comprised of synthetic female FCM sex pheromone (Moore 2021). This product was more successful reducing FCM fruit infestation by 55% in a heavily infested orchard between the months of December and April (Hofmeyr & Hofmeyr 2002).

During mating disruption, the FCM infested orchard is saturated with synthetic sex pheromones to confuse the males and make it difficult for them to locate their female mates (Figure 1.4) (Moore & Duncan 2017). It is important to note that mating disruption does not prevent mating completely, however, it has been reported that female fecundity may be reduced by up to 50% due to delayed mating (Baker 2009). Reduced fecundity means that females are not producing as many offspring as they would be under normal circumstances. For mating disruption to be successful, it needs to be applied early in the season before the FCM population builds-up. The synthetic pheromone dispensers also need to be placed high up in the trees, since pheromones are denser than the surrounding air (Moore & Hattingh 2012). Isomate® FCM, Checkmate® FCM-F (Suterra, USA), Splat-FCM (ISCA Technologies, Riverside, CA, USA) and X-Mate™ FCM (Insect Science, Tzaneen, South Africa) are currently the commercially available products for use in mating disruption (Moore & Hattingh 2012; Moore & Hattingh 2016; Moore 2019).

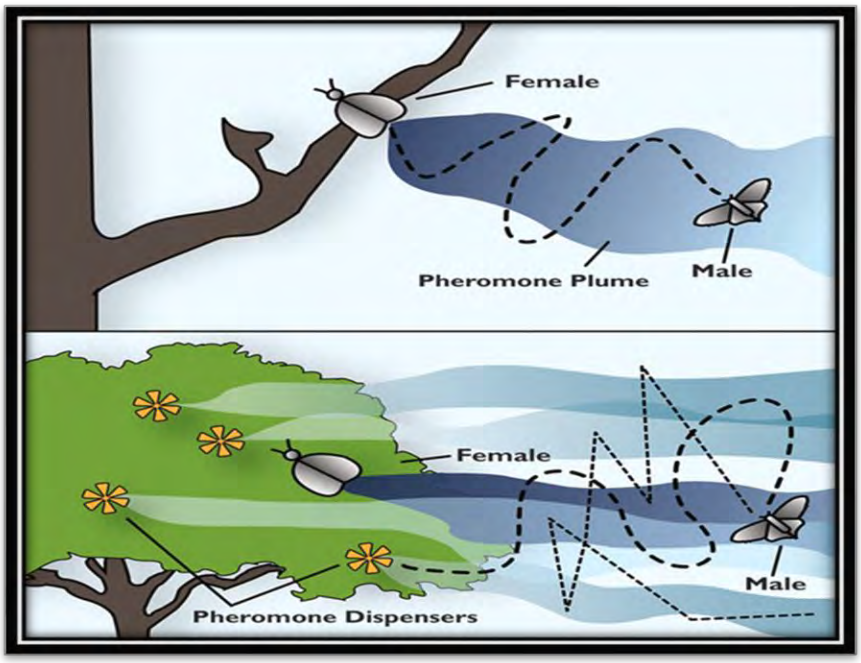


Figure 1.5: Pheromone dispensing makes it difficult for males to locate female mates during mating disruption (intermountainfruit.org).

The attract-and-kill technique makes use of a synthetic pheromone to lure FCM to a trap containing a 'killing agent' (insecticide, sterilant, or pathogen) that will either kill, sterilise or infect the moths (Charmillot *et al*. 2000). Attract-and-kill eventually leads to FCM population reduction by killing the target males that would otherwise mate with females (Campos & Phillips 2014). This technique differs from mating disruption in that it can be used successfully at lower pheromone concentrations when FCM infestation is low. Last Call^{TM} FCM (Insect Science, Tzaneen, South Africa) is the only commercially available product for use in attract-and-kill. It comes in the form a gel that is comprised of a blend of pyrethroid and a synthesized female sex pheromone (Moore 2021).

SIT is a widely used form of genetic control for FCM in South Africa since its commercialisation in 2007 (Hofmeyr *et al*. 2016). This strategy is currently implemented in over 18 000 ha in various regions within three provinces across the country (X Sterile Insect Technique (Xsit) 2018). It relies mainly on the ability of the sterile males to mate with as many wild females as possible, eventually outcompeting the wild males (Hendrichs & Robinson 2009; Hofmeyr *et al*. 2015). Sterilisation of FCM occurs through the irradiation of reproductive cells (Robinson 2005). It has been reported that sterile FCM males are released at infested areas at a ratio of at least 10:1 (sterile:wild) (Bloem *et al*. 2003; Barnes *et al*. 2015; Hofmeyr *et al*. 2015). SIT is currently conducted commercially by Xsit (Pty) Ltd, a company which has managed to reduce FCM population abundance by up to 95% since its registration in 2007 (Barnes *et al*. 2015; Hofmeyr *et al*. 2015; Nepgen *et al*. 2015). Xsit conducts SIT on FCM-susceptible cultivars in the Eastern Cape, in the Citrusdal and Breede River regions of the Western Cape and in the Northern Cape (Kirkman 2007; Hofmeyr *et al*. 2015).

Biological control (biocontrol) has long been considered an effective method for control of citrus pests and involves augmentation of a naturally occurring enemy in an infested area to reduce pest population. The natural enemy can be parasitic, predatory, or microbial. *Trichogrammatoidea cryptophlebia* (Nagaraja) (Hymenoptera: Trichogrammatidae) is a parasitoid of FCM eggs (Newton 1998). It occurs naturally in citrus orchards but is also commercially available to combat FCM infestations (Kirkman 2007). *Trichogrammatoidea cryptophlebia* has been reported to parasitise approximately 80% of FCM eggs in a given orchard under optimal conditions (Moore & Hattingh 2012). There are other FCM parasitoids that also occur naturally in South African orchards including a few species of wasp and fly larval parasitoids (Newton 1998; Moore 2002). *Agathis bishopi* (Nixon) (Hymenoptera:

Braconidae) is a species of wasp that has been reported to parasitise approximately 40% of FCM larvae in the Eastern Cape (Zimba *et al*. 2015; Zimba *et al*. 2016a; Zimba *et al*. 2016b).

Other natural enemies such as assassin bugs (Reduviidae), *Orius* bugs (Anthocoridae), and ants (Formicidae) are predators of larvae, eggs, and pupae of FCM, respectively (Newton 1998; Kirkman 2007; Malan *et al*. 2018). However, the presence of these predators in orchards is not always beneficial, especially without proper implementation of an IPM programme. For example, ant species such as *Anoplolepis custodiens* (Smith) and *Pheidole megacephala* (Fabricius) are regarded important pests in citrus trees due to them defending some citrus pests including *P. citri*, *Coccus hesperidum* (L.), *Ceroplastes brevicauda* (Hall), *Ceroplastes destructor* (Newst.), and *Pulvinaria aethiopica* (De Lotto), from their natural enemies in return of honeydew (Drinkwater 1982; Grout & Moore 2015). This suggests that control measures must be implemented to prevent ants from reaching citrus trees. Such measures include the barrier banding of citrus trees (Grout & Moore 2015; Moore *et al*. 2016), which is a difficult process to maintain but can be quite effective nonetheless (Moore 2021). More importantly, when adequately controlled, these predatory ants can be very beneficial on orchard floors as they prey on soil pupating pests including FCM. A study conducted by Bownes *et al*. (2014) reported high levels of predation on FCM pupae in control plots consisting of *A. custodiens* and *P. megacephala*.

Granulovirus products are widely used in South Africa for the control of FCM (Moore 2021). This can be largely attributed to their affordability, high efficacy (when implemented correctly), and simple application procedure (Moore 2021). The procedure only entails the use of simple pesticide spray equipment. Additionally, granulovirus products can be used in conjunction with other pesticides by simply mixing them in the same spray container/tank and still maintaining efficacy. There are three commercial granulovirus products in South Africa: Cryptogran® (River Bioscience, South Africa), Cryptex® and Gratham (Andermatt, Switzerland) (Moore & Jukes 2019). More recently, a nucleopolyhedrovirus was discovered and isolated from litchi moth *Cryptophlebia peltastica* (Meyrick) (Lepidoptera: Tortricidae) (Marsberg *et al*. 2018). Cryptophlebia peltastica NPV (CrpeNPV) is considered a unique virus with its virulence stretching beyond its homologous host (Marsberg *et al*. 2018). When tested in laboratory bioassays, CrpeNPV showed similar virulence against *C. pomonella*, FCM, and *C. peltastica* (Moore 2021). Additionally, CrpeNPV was also found to be significantly more virulent than the homologous granuloviruses of these species (Marsberg 2016; Marsberg *et al*. 2017). More importantly, CrpeNPV virulence has been tested against FCM in citrus field trials

and results showed ~ 90% infestation reduction from a single application in one of the tests (Hatting *et al*. 2019).

Three species of locally isolated entomopathogenic fungi (EPF) have been identified with pesticidal potential in laboratory bioassays against final instar FCM (Acheampong *et al*. 2020). These EPF species consist of two *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) isolates and one *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) isolate (Goble *et al*. 2011; Coombes *et al*. 2015), and were initially identified from citrus orchard soil samples (upper 5 cm) collected within the Eastern Cape Province of South Africa (Goble *et al*. 2010). More importantly, these isolates also showed good persistence when tested in semi-field conditions (Moore 2021). Similarly, eight species of locally collected entomopathogenic nematodes (EPNs) have been identified with high virulence in laboratory bioassays against FCM larvae at very low rates (Malan *et al*. 2011; Steyn *et al*. 2017). These include *Steinernema* sp., *Heterorhabditis bacteriophora*, *S. yirgalemense*, *S. khoisanae*, *H. zealandica*, *Heterorhabditis* sp., *Heterorhabditis baujardi*, and *Steinernema litchi* (Moore 2021). The *Heterorhabditis* species showed good persistence and high efficacy against FCM larvae in citrus orchard field trials (Malan & Moore 2016).

Many of the current control strategies for FCM can still be improved with more research. Alternative methods to incorporate different control techniques in IPM programmes are constantly being explored. Moreover, the study of natural plant and insect volatiles is fast gaining popularity, considering the limitations encountered with chemical control in the past. These natural volatiles are referred to as semiochemicals and have been utilised in various FCM control strategies such as monitoring, mass trapping, attract-and-kill, push-pull, and mating disruption (El-Ghany 2019).

1.3 SEMIOCHEMICALS

1.3.1 Brief overview

Insects and plants communicate by releasing volatile organic compounds known as semiochemicals (Ezzat *et al*. 2020). These compounds carry chemical messages between individuals, which induce behavioural, physiological, or ecological modifications (El-Shafie & Faleiro 2017; Ezzat *et al*. 2020). The individual releasing the semiochemical is called the

'emitter', and the individual that receives and responds to the chemical message is called the 'receiver'. Insects make use of semiochemicals to locate food sources, hosts, mates, and to avoid predation and competition (Norin 2007). In chemical ecology studies, semiochemicals from plants or insects can be assessed using a variety of analytical techniques, such as high-performance liquid chromatography (HPLC), gas chromatography coupled to mass spectrometry (GC-MS), solid-phase microextraction (SPME), single sensillum recordings (SSR), electroantennography (EAG), and gas chromatography coupled to electroantennographic detection (GC-EAD) (Pitts *et al*. 2014).

1.3.2 Classification

There are two major classes of semiochemicals (Figure 1.6), namely, pheromones and allelochemicals. Pheromones are chemical signals that facilitate communication between individuals of the same species (intraspecific), whereas allelochemicals are chemical signals that facilitate communication between different species (interspecific) (Pernaa & Aksela 2011; El-Shafie & Faleiro 2017). Depending on behavioural response time of the receiver, pheromones are categorised as either releaser or primer pheromones. Releaser pheromones induce an immediate change in the behaviour of the recipient, whereas primer pheromones induce a slower response and are most common amongst social insects (Pernaa & Aksela 2011). Releaser pheromones are further classified according to the interactions that they induce i.e. aggregation/sex pheromones (attract others to overwhelm a prey or mate), alarm pheromones (warn the population of danger), and food trail pheromones (indicate a path to food sources) (Pernaa & Aksela 2011; El-Shafie & Faleiro 2017; Ezzat *et al*. 2020). Allelochemicals on the other hand are subdivided into groups depending on how the emitter and/or receiver are impacted by the message i.e. allomones which are used in defence mechanisms and prey capture and benefit only the emitter, kairomones which are used to locate a food source and escape predation benefitting only the receiver, and lastly synomones, which benefit both the emitter and receiver (Pernaa & Aksela 2011; El-Shafie & Faleiro 2017; Ezzat *et al*. 2020).

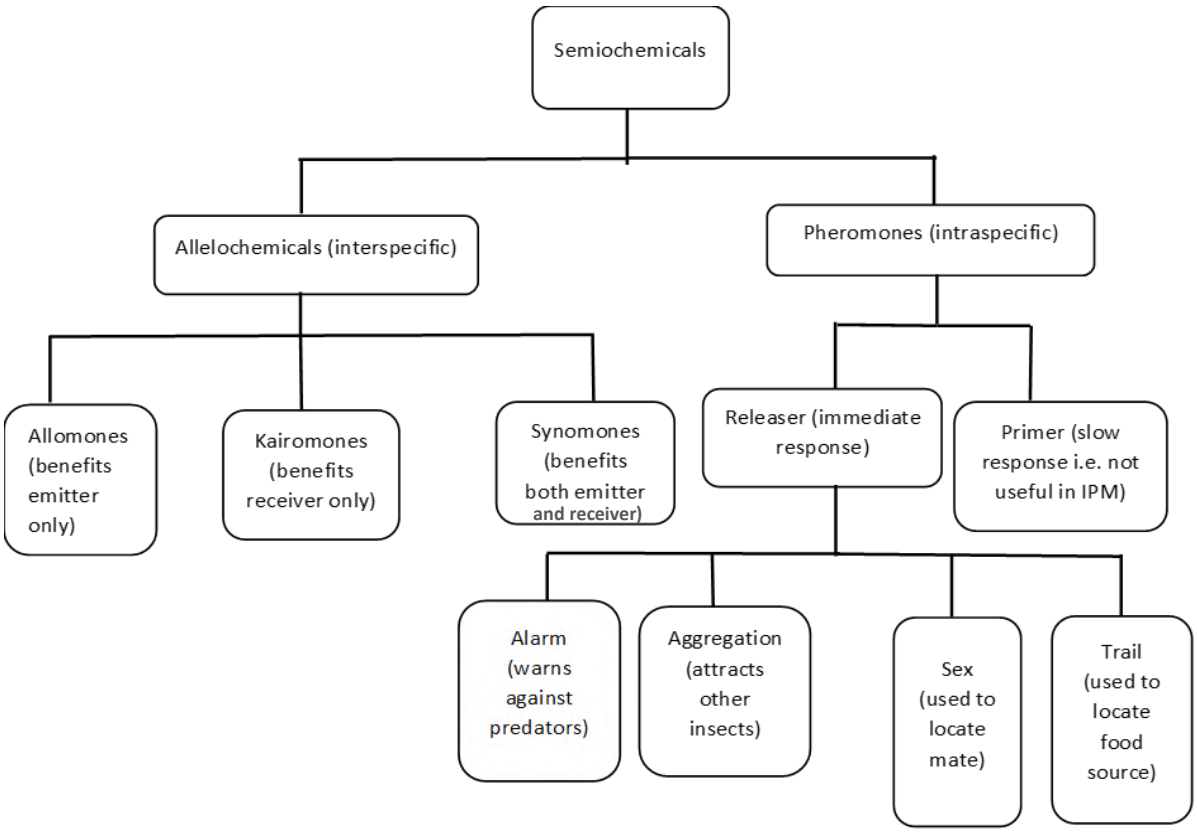


Figure 1.6: Schematic diagram representing semiochemical classification (adapted from El-Shafie & Faleiro 2017).

1.3.3 Role in insect pest management

Semiochemicals are often preferred in IPM programmes over other control measures, such as chemical control, because they are environmentally friendly and species-specific at low concentrations, therefore having a greater chance of eliminating pest infestations when used in conjunction with other control techniques such as biocontrol (Witzgall *et al*. 2010; Smart *et al*. 2014; El-Shafie & Faleiro 2017). They are useful in monitoring pest populations, determining if control is needed, and altering the behaviour of the insect pests (Reddy *et al*. 2005). Witzgall *et al*. (2010) reported that semiochemicals were applied to more than 10 million hectares of crop fields globally.

Kairomones are particularly of interest to this thesis because they consist of distinct plant odours which aid in the recognition of host plants by herbivorous insects (El-Shafie & Faleiro 2017). They also contain specific compounds that are produced by herbivorous insects to help predators and parasitoids locate or identify their prey/host (Murali-Baskaran *et al*. 2018). A

good example of kairomones used in IPM programmes is evident in the host-parasitoid relationship of *Trichogramma pretiosum* (Riley) (Hymenoptera: Trichogrammatidae) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Lewis *et al*. 1975; Murali-Baskaran *et al*. 2018). Volatile chemicals released by *H. zea* host plants can be utilised to manipulate host-seeking behaviour and thus increase field performance in *T. pretiosum* (Lewis *et al*. 1975). Significant quantities of the kairomones used by parasitoids have been discovered in food plants of host insects (Murali-Baskaran *et al*. 2018). This suggests that there is an opportunity for kairomones to be implemented in IPM programmes for the management of agricultural pests.

1.4 INSECT DETERRENTS

1.4.1 Brief overview

Studies have identified five different types of repellents according to the methodology applied to test for repellence (Bernier *et al*. 2007; Miller *et al*. 2009; Achee *et al*. 2012; Deletre *et al*. 2016): (1) true repellent (insect moves away from odour source without direct contact and in absentia of the host); (2) odour masking (reduction in host attractiveness by odour cue); (3) visual masking (reduction in host attractiveness by a visual cue); (4) contact irritancy (movement away from odour source after direct contact); and (5) deterrence (suppressant of insect behaviour after contact e.g. feeding or oviposition). Insect deterrents are the focus of this thesis and can be described as botanicals containing chemical properties that deter or inhibit insect pests from locating, feeding, or ovipositing on a preferred host species (Mafra-Neto *et al*. 2014). There has been an extensive amount of research on the use of semiochemicals in IPM programmes. Much of this research has been focused on pest species of veterinary and medical importance (Foster & Harris 1997; Isman 2006; Isman *et al*. 2011), such as *Anopheles* mosquitos (Meigen) (Diptera: Culicidae) which serve as vectors of malaria disease. For this reason, citronella oil which is derived from lemongrass is the most widely used commercial product to deter mosquitoes (Cook *et al*. 2007). Little research has been done on the potential use of insect deterrents in agricultural IPM programmes. Initial research on agricultural pest control was based on chemical pesticides as discussed earlier. However, with the rapid move to sustainable energy, the agricultural sector saw it worthwhile to explore environmentally friendly methods. Thus, several studies have been conducted to test for deterrence in natural

plants (Isman 2006; Alves *et al*. 2011). More importantly, several studies have reported repellent, deterrent, and/or antifeedant effects on known lepidopteran species of agricultural importance (Table 1.4) such as: *C. pomonella* (Landolt *et al*. 1999; Kovanci 2016) and diamondback moth *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) (Basukriadi & Wilkins 2014; Sangha *et al*. 2017). Crude extracts of plants with deterrent effects can be incorporated in IPM programmes as pest sprays or pheromone dispensers in orchards (Elsayed 2020). However, these deterrents are rarely applied on a practical level owing to the constant availability of cheaper and effective pest control alternatives, inadequate formulations for delivery, and regulatory challenges including registration (Isman 2006). In 2006, only a few botanical insecticides (plant essential oils and neem-based products) had been commercialised (Table 1.5) after three decades of published research on plant deterrents (Isman 2006). Insect deterrents are rarely used alone in IPM programmes, they are mostly used in conjunction with other attractants in "push-pull" management strategies against boring agricultural pests (Cook *et al*. 2007, Lopes *et al*. 2018, Midega *et al*. 2018).

Table 1.4: Plant extracts that have been reported to have a deterrent or repellent effect against species of Lepidoptera.

Pest	Plant(s)	Reference(s)
Coffee leaf miner, *Leucoptera coffeella* (Guérin-Mèneville) (Lyonetiidae)	1) Plantain, *Plantago lanceolata* (L.) (Lamiales: Plantaginaceae) 2) Bitter gourd, *Momordica charantia* (L.) (Cucurbitales: Cucurbitaceae)	Alves *et al*. 2011
Diamondback moth, *Plutella xylostella* (Linnaeus) (Plutellidae)	1) Yam bean seeds, *Pachyrhizus erosus* (L.) Urb. (Fabales: Fabaceae) 2) Cumaru seeds, *Dipteryx odorata* (Aublet) (Fabales: Fabaceae) 3) Asparagus, *Asparagus officinalis* (L.) (Asparagales: Asparagaceae) 4) Southern wormwood, *Artemisia abrotanum* (L.) (Asterales: Asteraceae)	Basukriadi & Wilkins 2014 Sangha *et al*. 2017

	5) Balsam fir, *Abies balsamea* ((L.) Miller) (Pinales: Pinaceae) 6) Black pepper, *Piper nigrum* (L.) (Piperales: Piperaceae) 7) Eucalyptus, *Eucalyptus polybractea* (Baker) (Myrtales: Myrtaceae) 8) Garlic, *Allium sativum* (L.) (Asparagales: Amaryllidaceae) 9) Thyme, *Thymus vulgaris* (L.) (Lamiales: Lamiaceae) 10) Tansy, *Tanacetum vulgare* (L.) (Asterales: Asteraceae)	
Potato tuber moth, *Phthorimaea operculella* (Zeller) (Gelechiidae)	1) Tickberry flowers, *Lantana camara* (L.) (Lamiales: Verbenaceae) 2) Blackberry nightshade fruits, *Solanum nigrum* (L.) (Solanales: Solanaceae)	Elsayed 2020
Cabbage looper, *Trichoplusia ni* (Hübner) (Noctuidae)	1) Garlic, *Allium sativum* (L.) (Asparagales: Amaryllidaceae) 2) Lemongrass, *Cymbopogon nardus* (L.) Rendle (Poales: Poaceae) 3) Patchouli, *Pogostemon cablin* (Blanco) Benth. (Lamiales: Lamiaceae)	Isman *et al*. 2011
Leafroller, *Choristoneura rosaceana* (Harris) (Tortricidae)	1) Thyme, *Thymus vulgaris* (L.) (Lamiales: Lamiaceae) 2) Patchouli, *Pogostemon cablin* (Blanco) Benth. (Lamiales: Lamiaceae)	Isman *et al*. 2011
Gypsy moth, *Lymantria dispar* (L.) (Erebidae)	1) Neem tree seeds, *Azadirachta indica* (A. Juss) (Sapindales: Meliaceae)	Kostić *et al*. 2008

	2)	Sweet basil, *Ocimum basilicum* (L.) (Lamiales: Lamiaceae)	
Codling moth, *Cydia pomonella* (L.) (Tortricidae)	1)	True cardamom seeds, *Elettaria cardamomum* (L.) Maton (Zingiberales: Zingiberaceae)	Kovanci 2016 Landolt *et al*. 1999
	2)	Red gum tree, *Eucalyptus tereticornis* (Smith) (Myrtales: Myrtaceae)	
	3)	Lavender, *Lavandula officinalis* (L.) (Lamiales: Lamiaceae)	
	4)	Pennyroyal, *Mentha pulegium* (L.) (Lamiales: Lamiaceae)	
	5)	Cypress, *Cupressus sempervirens* (L.) (Pinales: Cupressaceae)	
	6)	Rue, *Ruta graveolens* (L.) (Sapindales: Rutaceae)	
	7)	Garlic, *Allium sativum* (L.) (Asparagales: Amaryllidaceae)	
	8)	Patchouli, *Pogostemon cablin* (Blanco) Benth. (Lamiales: Lamiaceae)	
	9)	Tansy, *Tanacetum vulgare* (L.) (Asterales: Asteraceae)	
Fall armyworm, *Spodoptera frugiperda* (Smith) (Noctuidae)	1)	*Desmodium* spp. (Fabales: Fabaceae)	Midega *et al*. 2018
Spotted stalk borer, *Chilo partellus* (Swinhoe) (Crambidae)	1)	*Desmodium* spp. (Fabales: Fabaceae)	Khan *et al*. 2006
Apple fruit licker, *Spilonota lechriaspis* (Meyrick) (Tortricidae)	1)	Ageratum, *Ageratum houstonianum* (Miller) (Asterales: Asteraceae)	Song *et al*. 2014

	2)	French marigold, *Tagetes patula* (L.) (Asterales: Asteraceae)	
	3)	Summer savory, *Satureja hortensis* (L.) (Lamiales: Lamiaceae)	
Leafminer, *Tuta absoluta* (Meyrick) (Gelechiidae)	1)	Sweet basil, *Ocimum basilicum* (L.) (Lamiales: Lamiaceae)	Yarou 2018
	2)	Clove basil, *Ocimum gratissimum* (L.) (Lamiales: Lamiaceae)	

Table 1.5: Different botanicals approved for use in different countries across the globe (Pesticide Action Network 2004; Isman 2006; Isman 2020).

Country	Pyrethrum	Rotenone	Nicotine	Neem	Others
Australia	X	X	—	—	Citrus oils
New Zealand	X	X	—	X	
India	X	X	X	X	Ryania
Philippines	X	—	—	—	
Hungary	X	—	—	—	Quassia
Denmark	X	X	—	—	Lemongrass, clove, eucalyptus oils
Germany	X	—	—	X	
Netherlands	X	—	—	—	
United Kingdom	X	X	X	—	
South Africa	X	—	—	—	Tartar emetic, specified essential oils
Brazil	X	X	—	X	Garlic
United States	X	X	X	X	Specified essential oils, ryania, sabadilla
Canada	X	X	X	—	Specified essential oils
Mexico	X	X	—	X	Garlic, capsicum

Plant deterrents can be used in IPM as oviposition-deterring pheromones (ODPs). ODPs are compounds found in deterrent plants that prevent pests from ovipositing on host plants. ODPs have a great potential for use in push-pull strategies to control species that cause damage through oviposition (Pyke *et al*. 1987; Miller & Cowles 1990). Several botanical deterrents isolated from nonhost plants have deterred oviposition by pests in field and laboratory trials (Rice *et al*. 1985; Pyke *et al*. 1987; Martel *et al*. 2005). For example, a synthetic ODP of the European cherry fruit fly *Rhagoletis cerasi* (L.) (Diptera: Tephritidae), with chemical formula

[N-[15-(β-D-glucopyranosyl)-8-hydroxypalmitoyl]taurine], showed successful protection of cherry trees *Prunus avium* (L.) (Rosales: Rosaceae) when applied in field trials.

1.5 STUDY AIMS AND JUSTIFICATION

To the best of our knowledge, there are no past studies that have been conducted on potential oviposition deterrents to FCM. This means that there is an opportunity for a study to improve the management of FCM using oviposition deterrents. If a compound can be identified that deters FCM from ovipositing, it may be valuable for dispensing in an orchard or for spraying onto trees. Such a compound is likely to have a botanical origin, as there are certain plants known to have deterrent effects, such as those used in "push-pull" strategies (Cook *et al*. 2007; Sangha *et al*. 2017).

This research thesis aimed to: (1) determine if there were any treated fruit on which FCM would not oviposit; (2) investigate the efficacy of the identified compounds at different concentrations using fruit bioassays; and (3) determine if any of the identified compounds had dual action.

Chapter 2,

Deterrence trials against FCM oviposition

2.1 INTRODUCTION

The agricultural industry has sought to find alternative pest control strategies that take into consideration the health and safety of the environment and farm workers (Isman 2006; Isman 2014; Isman 2020). The introduction of synthetic pesticides has caused numerous problems for agriculture across the globe (Prakash & Rao 2018). These problems include pests becoming resistant against the pesticides, and chemical residues that are harmful to beneficial insects and humans (Ndakidemi *et al*. 2016). This means that there is a need for effective host specific and biodegradable pesticides that are suitable for use in IPM programmes. Natural plant extracts (botanicals) have been identified as potential pesticides and have greatly influenced modern agrochemical research (Isman & Grieneisen 2014; Benelli *et al*. 2016; Isman 2020; see chapter 1). Botanicals are classified into different categories according to their functions i.e. insecticides (control insects), herbicides (kill or inhibit the growth of unnecessary plants), fungicides (control fungal pathogens), nematicides (control nematodes), molluscicides (control molluscs) and rodenticides (kill rodents like mice, rats, and household pests) (Ortiz-Hernández *et al*. 2013). A thorough understanding of plant-insect interactions is essential in identifying potential botanical insecticides. Plants have evolved over many generations and have managed to persist by developing defence mechanisms against herbivorous insects. Such mechanisms involve the releasing of natural chemicals that possess deterrent or insecticidal effects (Isman 2015; Ivase *et al*. 2021). Knowledge of plants that are resistant to certain insects may provide direction for research into insect pests that may be controlled using the plants' natural extracts (Miresmailli & Isman 2014: Ivase *et al*. 2021). It is important to note that botanicals have different modes of action; some botanicals act as direct toxicants or sterilants whilst others act as behaviour modifiers or antifeedants/deterrents (Isman 2006; Isman 2015).

From a safety perspective, botanical insecticides are preferred over synthetic insecticides because they degrade much quicker in sunlight, air, and moisture, making them less persistent

in the environment (Isman 2006; Miresmailli & Isman 2014). Despite their inferior persistence, and hence efficacy, they are still beneficial when application is correctly timed and used in conjunction with biological control (Lengai *et al*. 2020). They are considered relatively friendly to the environment and less likely to kill beneficial insects (Gill & Garg 2014). The majority of botanicals usually degrade within a few days or hours, which means that they need to be applied more frequently than synthetic pesticides. Several crude extracts of plants and essential oils (EOs) have been identified with insecticidal and/or deterrent properties, however, only a few are available for commercial use due to numerous commercialisation issues discussed in the previous chapter (Isman 2006; Isman 2014; Isman 2020). The few that are commercially available primarily contain: (1) pyrethrin, which is an extract from *Chrysanthemum cinerariaefolium* daisies that causes rapid insect paralysis; (2) neem, from the neem tree *Azadirachta indica*, products which are insect growth regulators (IGRs), feeding and oviposition deterrents with azadirachtin as the active ingredient; (3) rotenone, which is a stomach poison in insects and is extracted from the roots of two tropical plants *Lonchocarpus* spp. and *Derris* spp., and lastly; (4) EOs, which are often used as attractants or deterrents in push-pull strategies (Isman 2006; Das 2014; Isman 2020; Ivase *et al*. 2021) (Figure 2.1).

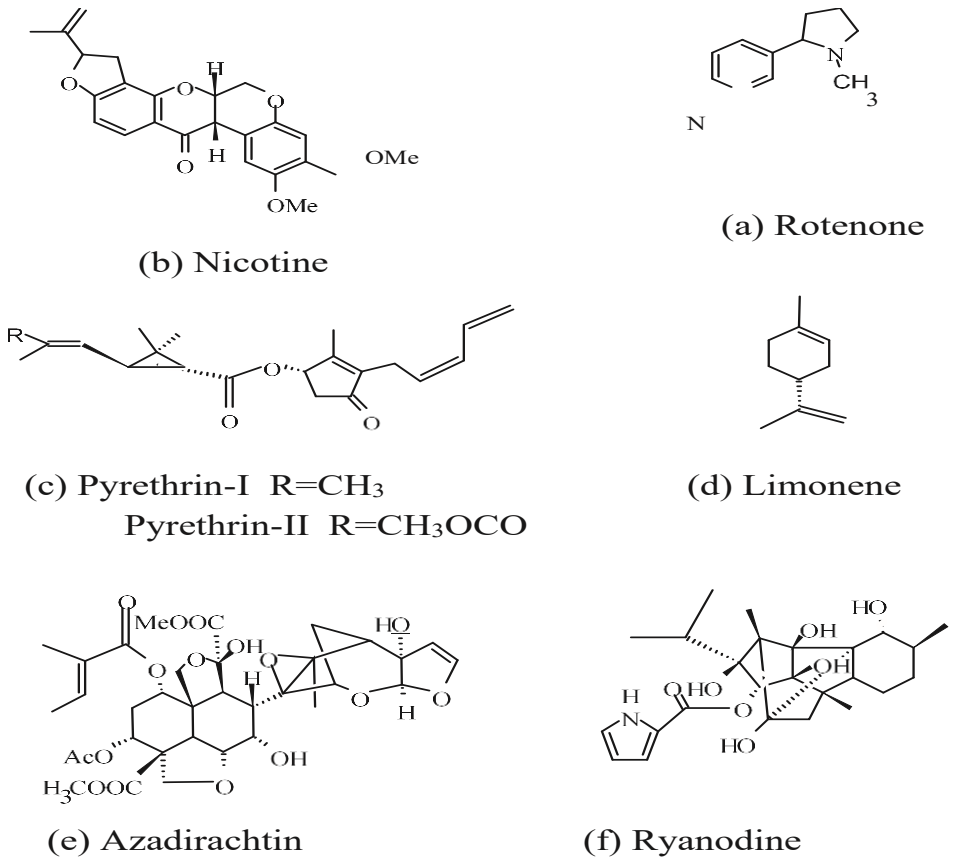


Figure 2.1: Chemical structures of some active ingredients of botanical pesticides from different plant sources, (a) rotenone, (b) nicotine, (c) pyrethrin I and II, (d) limonene, (e) azadirachtin and (f) ryanodine (adapted from Isman 2006).

This chapter focusses on the potential use of botanicals as oviposition deterrents against FCM. This is because the larval stage of FCM is responsible for causing fruit damage and thus deterring oviposition can be an effective control measure. Rice *et al*. (1985) reported that extracts of neem kernels significantly deterred oviposition by the sheep blowfly *Lucilia cuprina* (Wiedemann), whose larval stage causes myiasis on the animal skin, by up to 91%. Moreover, Yarou *et al*. (2018) reported that basil plants, *Ocimum gratissimum* L. and *O. basilicum* L. (Lamiaceae) and their associated EOs significantly reduced oviposition by *Phthorimaea absoluta*, which is one of the most important pests of tomato globally. Several other plants have been identified with deterrent effects against lepidopteran pests of agricultural significance (Khan *et al*. 2006; Song *et al*. 2014; Midega *et al*. 2018; Elsayed 2020). However, no plant extracts have been reported to deter FCM. Consequently, the aim of the study reported in this chapter was to determine if there were any deterrent solutions/suspensions (EOs and crude extracts), which would prevent or reduce oviposition by FCM on treated oranges. In addition to these botanical compounds, the oviposition deterrence of some commercial

insecticides (biological and chemical) that are registered or intended for registration against FCM, was also tested, as this was also unknown.

2.2 METHODS AND MATERIALS

2.2.1 Insect rearing

FCM pupae were reared through to adulthood in a constant environment room at the Department of Zoology and Entomology, Rhodes University, where the temperature was set to 26°C ± 2°C, 50-60% relative humidity, over a 12/12 h light/dark cycle. Pupae used in this study were from a 'mixed' culture, which was established by Moore (2002). The culture consists of field collected larvae from three different areas: Citrusdal in the Western Cape, Zebediela in Limpopo, and Addo in the Eastern Cape provinces of South Africa. The culture has been maintained for more than 300 generations on an artificial diet (Moore *et al*. 2014), which consists of maize meal, wheat germ, milk powder, brewers' yeast, nipagin and sorbic acid. Individuals used in trials were selected as pupae and each placed separately in plastic vials, to ensure that we started with only virgin males and females. Female/male pairs were established from the newly emerged adults and allowed to mate for 24 h in pill vials containing moistened cotton wool. vials were checked for presence of eggs after one to two days. Males were removed from vials in which females laid eggs, and females that had not laid eggs were discarded. Females that had laid eggs were assumed to be mated and were used in oviposition bioassays.

2.2.2 Identification and collection of potential deterrents

An extensive literature study was conducted for plant extracts and EOs (Table 2.1) that have been recorded to have some degree of repellence or deterrence to lepidopteran species, particularly those in the family Tortricidae. Commercially available chemical and microbial insecticides used for FCM control in the citrus industry were also tested (Table 2.2). Additionally, two fruit types (mangoes and lemons) were tested, as they have been reported to not support FCM development (Grove *et al*. 2012; Moore *et al*. 2015). Vanilla essence was also tested as it was previously used by some farmers who considered it to be a deterrent (Sean

Moore pers. comm). However, it is not clear what this assumption was based on and whether it was indeed effective or not.

Plants were collected in late summer from the Rhodes University botanical gardens and Sunnyside Garden Centre in Makhanda South Africa; pure EOs were all purchased commercially from eOil.co.za (Johannesburg, South Africa); samples of chemical and microbial insecticides were supplied by Citrus Research International (CRI) in Gqeberha South Africa, and lastly, vanilla essence (Robertsons, Durban South Africa) and mature fruit were purchased from Pick 'n Pay and the Farmbox Fruit and Veg store in Makhanda, respectively.

Table 2.1: Plant extracts and EOs identified with deterrent or repellent properties from an extensive literature review.

Common name	**Scientific name**	**Reference(s)**
Lavender oil	*Lavandula angustifolia*	Raman *et al*. 1987; Landolt *et al*. 1999
Patchouli oil	*Pogostemon cablin*	Landolt *et al*. 1999
Black pepper oil	*Piper nigrum*	Sangha *et al*. 2017
Cypress oil	*Cupressus sempervirens*	Landolt *et al*. 1999
Rosemary verbenone oil	*Rosmarinus officinalis*	Miresmailli *et al*. 2006
Basil oil	*Ocimum basilicum*	Sharaby *et al*. 2009; Yarou 2018
Sage oil	*Salvia officinalis*	Landolt *et al*. 1999
Lemongrass oil	*Cymbopogon flexuosus*	Isman 2006; Isman *et al*. 2011
Citronella oil	*Cymbopogon winterianus J.*	Isman 2006; Cook *et al*. 2007
Thyme oil	*Thymus vulgaris*	Ribeiro *et al*. 2015; Sangha *et al*. 2017
Clove bud oil	*Eugenia caryophyllata*	Ribeiro *et al*. 2015
Peppermint oil	*Mentha piperita*	Ribeiro *et al*. 2015
Eucalyptus oil	*Eucalyptus smithii*	Sangha *et al*. 2017
Bitter gourd	*Momordica charantia*	Alves *et al*. 2011
Clove basil	*Ocimum gratissimum*	Sharaby *et al*. 2009; Yarou 2018

Rue	*Ruta graveolens*	Landolt *et al*. 1999
Tomato	*Solanum lycopersicum*	Antonious & Snyder 2015
Marigold	*Tagetes minuta*	Song *et al*. 2014
Ginger	*Zingiber officinale*	Ribeiro *et al*. 2015
Garlic	*Allium sativum*	Samarasinghe *et al*. 2007; Ribeiro *et al*. 2015; Sangha *et al*. 2017
Gum tree	*Eucalyptus globulus*	Landolt *et al*. 1999; Sangha *et al*. 2017
Sage	*Salvia africana*	Landolt *et al*. 1999
African wormwood	*Artemisia afra*	Modise 2015

Table 2.2: Active ingredients and recommended concentrations of chemical and microbial insecticides registered or intended to be registered for use against false codling moth on citrus in South Africa.

Common name (manufacturer)	**Active ingredient**	**Recommended concentration**
Warlock (ADAMA, South Africa)	Emamectin benzoate	100 ml/ 100 L H_2O
Delegate (Corteva Agriscience, South Africa)	Spinetoram	20 g/ 100 L H_2O
Coragen (DuPont, South Africa)	Chlorantraniliprole	17.5 ml/ 100 L H_2O
Exirel (DuPont, South Africa)	Cyantraniliprole	50 ml/ 100 L H_2O
Runner (Dow Agrosciences, South Africa)	Methoxyfenozide	60 ml/ 100 L H_2O
Cryptogran (River Bioscience, South Africa)	CrleGV-SA[1]	10 ml/ 100 L H_2O
Litchi moth NPV	CrpeNPV[2]	80 ml/ 100 L H_2O

[1]Cryptophlebia leucotreta granulovirus (5×10^{10} occlusion bodies (OBs)/ml), [2]Cryptophlebia peltastica nucleopolyhedrovirus (8.82×10^{6} OBs/ml).

2.3 Preparation of solutions/suspensions

2.3.1.1 Plant extract suspensions

Plant extract suspensions were prepared at standard concentration (10 mg/ml), following methodology described by Landolt *et al*. (1999) and Elsayed (2020). Fresh plant material (leaves & flowers) was air-dried at room temperature for three weeks, ground to powder using a commercial blender (Philips HR2056/90) for approximately five minutes, weighed on a balance machine, and mixed with 500 ml of pure methanol (solvent) in a conical flask. The flask was then placed on a rotary shaker for two days. Following this, the mixture was filtered using suction filtration with a Büchner funnel containing filter paper attached to the flask. The solvent was evaporated in a fume hood (Alves *et al*. 2011, Elsayed 2020) and the resulting crude extract was weighed and mixed with distilled water in a plastic beaker to achieve the desired concentration (10 mg/ml) (Elsayed 2020).

2.3.1.2 EOs and vanilla essence solutions

EOs and vanilla essence solutions were also prepared at a standard concentration (10 mg/ml). Three grams of oil was weighed on a balance and then diluted to a total volume of 300 ml with 90% ethanol (Kostić 2008). Ethanol is used to solubilise EOs into water-based sprays so they can be diluted without separating, forming a homogeneous mixture (Moore 2017).

2.3.1.3 Commercial insecticide suspensions

All commercial products were used at the recommended concentration on the original packaging. The insecticides were simply dissolved in water to achieve the desired concentrations mentioned in Table 2.2 above.

2.3.2 Oviposition deterrence bioassays

Oviposition deterrence bioassays were conducted in complete darkness in a controlled environment (CE) room with the temperature set at 25°C (± 1.5°C) with low relative humidity (26%). Complete darkness was necessary because FCM adults are mostly active at night

(Stofberg 1954). For each treatment, a total of six oranges of the same cultivar and maturity level were placed one by one, into a cage (60 × 30 × 35 cm) with seven gravid FCM females. This was replicated five times for all tested compounds with new oranges and gravid females being used each time. All oranges used in this study were collected from orchards near Addo in the Sundays River Valley in the Eastern Cape Province of South Africa. Upon arrival, all fruit were thoroughly rinsed with distilled water, dried, and stored in a cold room to preserve their health. Oviposition on the oranges was tested in 'Choice' and 'No Choice' trials following methods described by Love *et al*. (2014). The main difference to Love *et al*. (2014) was the cage size, which consequently affected the number of females and oranges used in each treatment for this study.

In 'Choice' trials, three untreated oranges that served as the control and three treated oranges (dipped in potential deterrent solution/suspension) were placed equidistant from each other in a cage of gravid FCM females. Oviposition was measured every hour for four hours in another room with lighting, by counting the number of eggs laid on each orange. In 'No Choice' trials, the positive choice (untreated oranges) was removed, and six treated oranges were placed in a cage for four hours with oviposition being measured and recorded as before. Six untreated oranges were used for the control treatment and oviposition was measured as before. For lemons and mangoes, the actual fruit served as the treatment and untreated oranges served as the control.

All treatments were tested on different days to avoid mixing of volatiles in the CE room. Excessive oviposition on any of the treated oranges resulted in the elimination of that particular treatment from further investigations. If no/less than usual oviposition (relative to the control fruit) occurred on the treated oranges, then a further analysis of deterrence and ovicidal activity was conducted in concentration response bioassays.

2.3.3 Statistical analyses

All data for this study were analysed using R-STUDIO for windows, version 4.2.0 (2022-04-22). A Shapiro-Wilk normality test was conducted for all variables involved to determine the distribution of the data (package: 'stats', function: 'shapiro.test'; R Core Team, 2022). The test confirmed that the data were normally distributed in choice trials. T-tests for paired comparisons were used to test for significant differences between the treatments and controls.

Furthermore, oviposition deterrent indices (ODI), which are an indication of the strength of the deterrent effect were calculated using the following formula: $ODI = \frac{100(C-T)}{C+T}$ where C and T represent the average number of eggs laid on control and treated fruit, respectively (Huang *et al*. 1995). Positive ODI values are an indication of a deterrent effect whilst negative values are an indication of attraction. The data were not normally distributed in no-choice trials, thus, an independent two-group Mann-Whitney test was used to test for significant differences between the treatments and control. Only treatments with an ODI value of over 50 and significantly deterred oviposition in both choice and no-choice trials were selected for further analysis. These were the compounds with the best chance of achieving field efficacy.

2.4 RESULTS

2.4.1 Plant extract suspensions

Of the nine tested plant extracts, eight significantly deterred FCM oviposition on oranges in choice trials, i.e. marigold, ginger, garlic, gum tree, African wormwood, bitter gourd, rue, and tomato leaves ($P < 0.05$). Marigold had the strongest deterrent effect (ODI = 69.8) followed by garlic, bitter gourd, gum tree, ginger, rue, African wormwood, and tomato leaves, respectively (Table 2.3). In contrast, sage had no significant deterrent effect against FCM oviposition ($P = 0.062$) and had the lowest ODI value (Table 2.3).

In no-choice trials, there were significantly fewer eggs oviposited in the marigold, garlic, gum tree, African wormwood (W = 0, $P < 0.005$), and rue (W = 2, $P < 0.05$) treatments compared to the control (Figure 2.2). Ginger, sage, bitter gourd, and tomato leaves treatments were not significantly different (W = 6, $P = 0.222$; W = 15, $P = 0.691$; W = 5, $P = 0.151$; and W = 6, $P = 0.126$, respectively) from the control (Figure 2.2).

Table 2.3: Oviposition by false codling moth on Navel oranges treated with plant extract suspensions (10 mg/ml) in laboratory choice bioassays.

Plant extracts	**Mean number of eggs laid (± SE)[a]**				**ODI[c]**
	Treated	**Control**	***t***	***P* values[b]**	
Marigold	29.4 ± 4.9	165.6 ± 9.3	-21.4	< 0.001	69.8
Ginger	49.2 ± 10.1	117.0 ± 9.5	-5.6	0.005	40.8

Garlic	30.8 ± 8.5	97.2 ± 6.1	-7.0	0.002	51.8
Gum tree	51.4 ± 6.1	125.8 ± 18.1	-5.6	0.005	41.9
Sage	76.4 ± 11.2	103.2 ± 8.5	-2.6	0.062	15.1
African wormwood	53.8 ± 7.5	140.4 ± 22.0	-3.5	0.024	36.9
Bitter gourd	59.6 ± 4.5	154.8 ± 11.8	-6.0	0.004	44.4
Rue	56.6 ± 14.0	129.6 ± 5.3	-4.5	0.011	39.2
Tomato leaves	66.2 ± 9.8	119.4 ± 14.8	-4.3	0.013	28.7

[a] Average from five replicates. SE represents the standard error. [b] Statistical tests refer to *t*-test for paired comparison between treated and control fruit. [c] ODI represents the oviposition deterrent index.

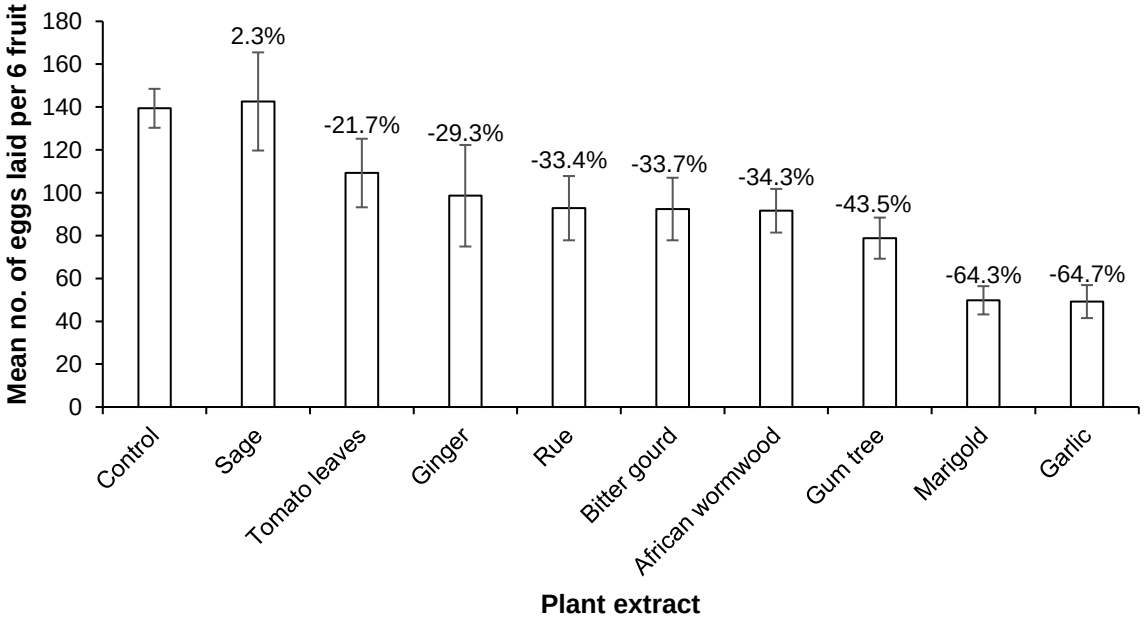


Figure 2.2: Average oviposition (± SEM) by false codling moth on Navel oranges treated with plant extract suspensions (10 mg/ml) in no-choice bioassays. Percentage reduction/increase relative to the control is indicated above the bars.

2.4.2 Essential oil solutions

In choice trials, lavender, black pepper, rosemary, basil, lemongrass, thyme, citronella, peppermint, eucalyptus, clove basil, and vanilla essence essential oils all significantly ($P < 0.05$) deterred FCM oviposition (Table 2.4). Lavender had the strongest deterrent effect (ODI = 64.3). On the other hand, patchouli ($P = 0.761$), cypress ($P = 0.68$), sage ($P = 0.470$), and clove bud ($P = 0.103$) had no significant deterrent effect against FCM oviposition. Patchouli had the weakest deterrent effect (ODI = -3.8).

In no-choice trials, significantly fewer ($P < 0.05$) eggs were oviposited on oranges treated with lavender, patchouli, peppermint, eucalyptus and basil essential oils (Figure 2.3). There were no significant differences in the number of eggs laid on black pepper (W =13, $P = 1$), cypress (W = 16.5, $P = 0.463$), rosemary (W = 6, $P = 0.222$), lemongrass (W = 3, $P = 0.056$), thyme (W = 13, $P = 1$), clove bud (W = 18.5, $P = 0.248$), citronella (W = 5.5, $P = 0.173$), clove basil (W = 7, $P = 0.290$), and vanilla essence (W = 7.5, $P = 0.2$) compared to the control. Moreover, there were significantly more (W = 25, $P = 0.008$) eggs laid on the sage treatment compared to the control (Figure 2.3).

Table 2.4: Oviposition by false codling moth on Navel oranges treated with essential oil solutions (10 mg/ml) in laboratory choice bioassays.

Essential oils	Mean number of eggs laid (± SE)[a]				ODI[c]
	Treated	Control	*t*	*P* values[b]	
Lavender	32.0 ± 8.9	147.2 ± 18.8	-6.3	0.003	64.3
Patchouli	54.4 ± 14.5	50.4 ± 9.7	0.3	0.761	-3.8
Black pepper	86.0 ± 15.4	185.8 ± 30.4	-6.1	0.004	36.7
Cypress	100.0 ± 6.1	114.4 ± 28.3	-0.4	0.680	6.7
Rosemary	66.0 ± 9.9	168.0 ± 15.7	-4.5	0.011	43.6
Basil	54.6 ± 14.9	149.8 ± 26.3	-3.4	0.026	46.6
Lemongrass	51.2 ± 11.5	195.0 ± 18.8	-9.5	0.001	58.4
Sage	92.2 ± 10.5	107.2 ± 13.4	-0.8	0.470	7.5
Thyme	82.2 ± 7.1	172.8 ± 13.6	-5.5	0.005	35.5
Clove bud	86.2 ± 13.5	137.6 ± 15.3	-2.1	0.103	23.0
Citronella	56.4 ± 9.3	166.0 ± 15.5	-4.6	0.010	49.3
Peppermint	31.4 ± 4.5	116.2 ± 19.0	-5.1	0.007	57.5
Eucalyptus	46.2 ± 9.8	136.8 ± 16.4	-4.1	0.014	49.5
Clove basil	68.6 ± 6.7	147.4 ± 16.6	-6.1	0.004	36.5
Vanilla essence	85.4 ± 11.5	152.8 ± 18.9	-5.6	0.005	28.3

[a] Average from five replicates. SE represents the standard error. [b] Statistical tests refer to *t*-test for paired comparison between treated and control fruit. [c] ODI represents the oviposition deterrent index.

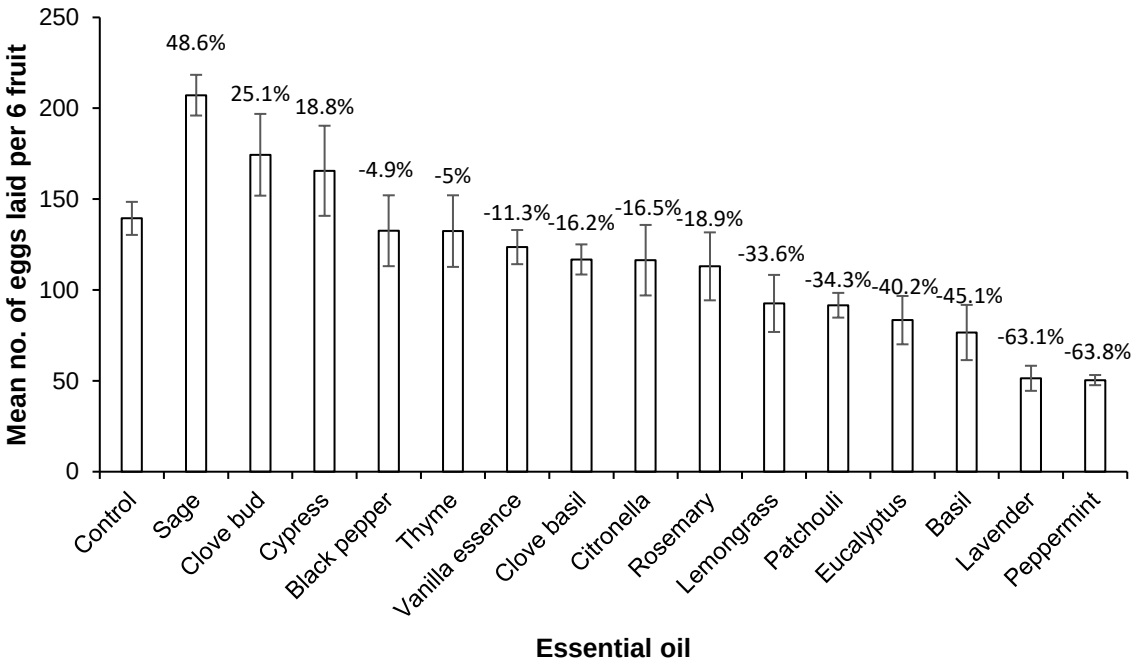


Figure 2.3: Average oviposition (± SEM) by false codling moth on Navel oranges treated with essential oil solutions (10mg/ml) in no-choice bioassays. Percentage reduction/increase relative to the control is indicated above the bars.

2.4.3 Lemon and mango treatments

In choice trials, significant ovipositional deterrence was shown by mango (Table 2.5), whereas no significant ovipositional deterrence was shown by lemon (P = 0.405). Consequently, mango's deterrent effect had an ODI of 23.6, whereas the ODI of lemon was only 14.8 (Table 2.5). In no-choice trials, FCM oviposited significantly fewer (W = 0. $P < 0.05$) eggs on mango compared to the control, and there was no significant difference (W = 9, P = 0.548) in the number of eggs laid on lemon compared to the control (Figure 2.4).

Table 2.5: Oviposition by false codling moth on mangoes and lemons in laboratory choice bioassays.

Fruit	Mean number of eggs laid (± SE)[a]				ODI[c]
	Treatment	Control	*t*	*P* values[b]	
Mango	60.8 ± 7.0	98.4 ± 9.4	-4.6	0.010	23.6
Lemon	76.2 ± 17.1	102.6 ± 13.5	-0.9	0.405	14.8

[a] Average from five replicates. SE represents the standard error. [b] Statistical tests refer to *t*-test for paired comparison between treatment and control fruit. [c] ODI represents the oviposition deterrent index.

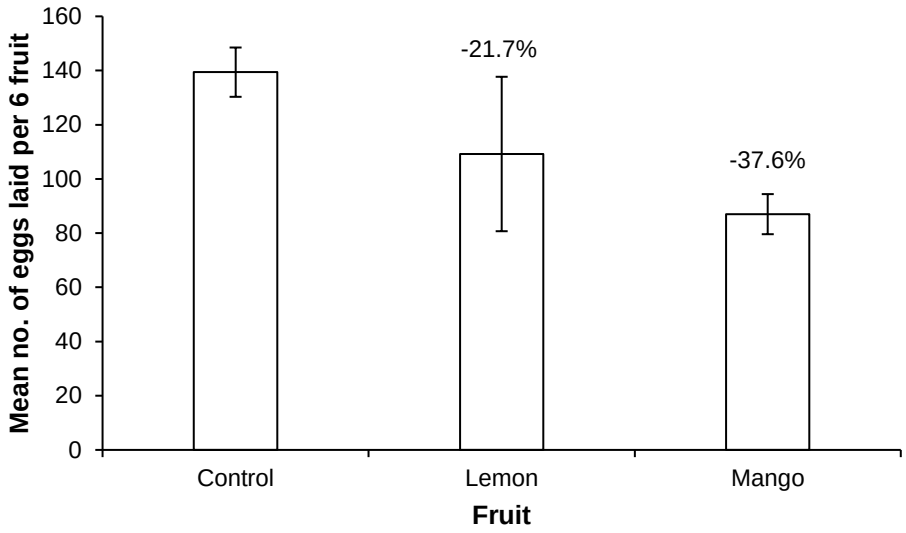


Figure 2.4: Average oviposition (± SEM) by false codling moth on mangoes and lemons in no-choice bioassays. Percentage reduction/increase relative to the control is indicated above the bars.

2.4.4 Insecticide solutions

In choice trials, Delegate, Coragen, Exirel, CrpeNPV, and Warlock significantly ($P < 0.05$) deterred FCM oviposition (Table 2.6). Cryptogran ($P = 0.392$) and Runner ($P = 0.06$) were the only commercial products that did not significantly deter FCM oviposition (Table 2.6). Coragen had the strongest deterrent effect (ODI = 72.7) whereas Cryptogran had the weakest (ODI = 2.3).

In no-choice trials, significantly fewer eggs were oviposited on oranges treated with Delegate, Coragen, and Warlock compared to the control (Figure 2.5). There were no significant differences in the number of eggs laid on oranges treated with Exirel (W = 4, $P = 0.095$), Runner (W = 3, $P = 0.056$), Cryptogran (W = 5, $P = 0.151$), and CrpeNPV (W = 4, $P = 0.094$) compared to the control (Figure 2.5).

Table 2.6: Oviposition by false codling moth on Navel oranges treated with commercial insecticide solutions/suspensions (recommended rate on package) in laboratory choice bioassays.

Insecticide	Mean number of eggs laid (± SE)[a]				ODI[c]
	Treated	Control	*t*	*P* values[b]	
Delegate	29.0 ± 10.2	113.8 ± 18.4	-4.9	0.008	59.4
Coragen	29.6 ± 3.1	187.4 ± 5.0	-25.0	< 0.001	72.7
Exirel	51.6 ± 10.7	124.2 ± 16.7	-4.2	0.014	41.3
Runner	70.8 ± 7.2	97.0 ± 3.8	-4.2	0.060	15.6
Cryptogran	98.2 ± 6.4	102.8 ± 3.7	-1.0	0.392	2.3
CrpeNPV	79.0 ± 10.5	119.6 ± 8.4	-3.0	0.038	20.4
Warlock	43.8 ± 5.9	148.2 ± 3.7	-17.3	< 0.001	54.4

[a] Average from five replicates. SE represents the standard error. [b] Statistical tests refer to *t*-test for paired comparison between treated and control fruit. [c] ODI represents the oviposition deterrent index.

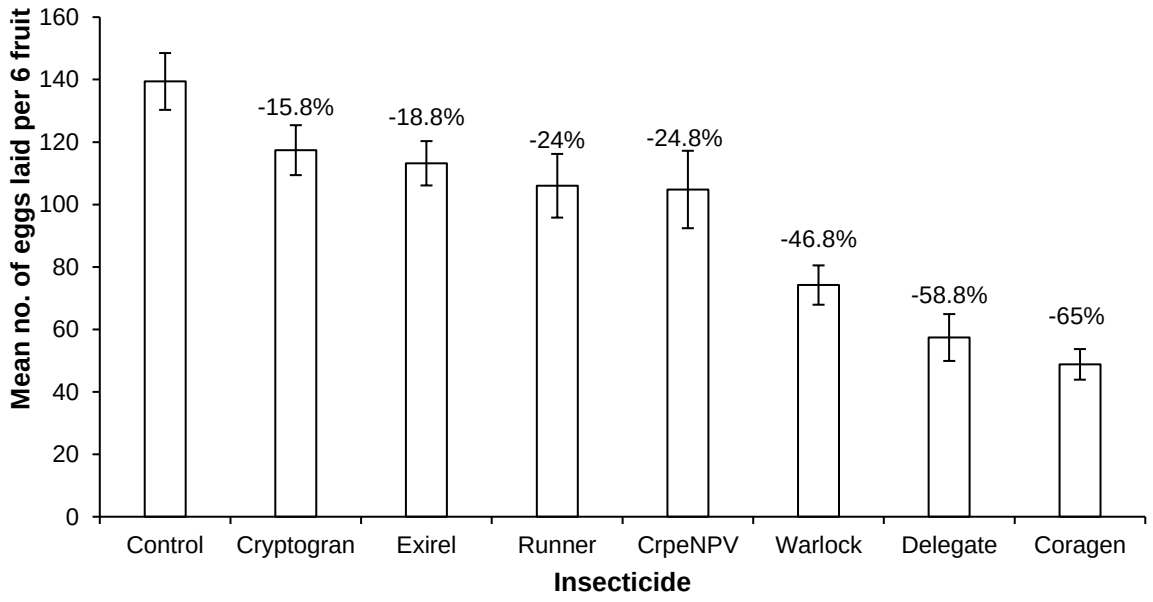


Figure 2.5: Average oviposition (± SEM) by false codling moth on Navel oranges treated with commercial pesticide solutions (recommended rate) in no-choice bioassays. Percentage reduction/increase relative to the control is indicated above the bars.

2.5 DISCUSSION

No previous study has documented the potential use of botanicals as oviposition deterrents against FCM adult females. However, the use of botanicals as oviposition deterrents of other insect pests (including those that belong to the order Lepidoptera) has been widely researched (Isman 2006; Isman 2011; Song *et al*. 2014; Isman 2020).

In this study, oviposition by FCM adults was deterred on oranges treated with marigold, ginger, garlic, gum tree, African wormwood, bitter gourd, rue, and tomato leaf extract in choice trials. Marigold and garlic had the strongest deterrent effect. This was expected, as both plants have been reported to deter oviposition by a wide range of insect pests (Landolt *et al*. 1999; Samarasinghe *et al*. 2007; Song *et al*. 2014; Sangha *et al*. 2017). Song *et al*. (2014) reported that intercropping marigold plants in apple orchards significantly reduced the densities of three pests of the Tortricidae family which were *Adoxophyes orana* Fischer, *Spilonota lechriaspis* Meyrick, and *Acleris fimbriana* Thunberg. Garlic has demonstrated deterrent activity against many species of Lepidoptera in laboratory bioassays (Machial *et al*. 2010; Yang *et al*. 2012; Ribeiro *et al*. 2015). Such species include *Anticarsia gemmatalis* Hübner, *Cydia pomonella* and *Plutella xylostella* (Landolt 1999; Ribeiro *et al*. 2015; Sangha *et al*. 2017). Additionally, Landolt *et al*. (1999) showed that rue, ginger, and eucalyptus (same genus as the gum tree used in this study) all deterred *C. pomonella* larvae in barrier assays. Alves *et al*. (2011) reported that bitter gourd extracts deterred *Leucoptera coffeella* oviposition in choice trials. Sage showed no deterrent effect on the oviposition of FCM. This result was in contrast with Landolt *et al*. (1999), where sage deterred *C. pomonella* larvae, a close relative of FCM. It is also possible that the different life stages of FCM react differently to plant odours. Adult females have been shown to be less dependent on plant odour for oviposition (Elsayed 2020). Moreover, plant allelochemicals influence the chemosensory behaviour of gravid female moths (Elsayed 2020).

Marigold, garlic, gum tree, African wormwood, and rue treatments showed deterrence in no-choice trials as significantly fewer eggs were oviposited by FCM compared to the control. However, ginger, sage, bitter gourd, and tomato leaf treatments were not significantly different from the control. This means that ginger, bitter gourd and tomato leaves had no deterrent effect when FCM females were not given a choice. Unfortunately, research on laboratory no-choice experiments of botanicals is very limited, as most studies use dual choice assays (Alves *et al*. 2011; Sangha *et al*. 2017). More studies need to include no-choice assays to allow for ease of comparison between studies. More importantly, no-choice bioassays are essential because they

give us an idea of how a deterrent will perform in the field where the insect will not be given a choice.

The deterrent effects of essential oils have been the most studied of all botanicals (Regnault-Roger *et al.* 2012). Lavender, black pepper, rosemary, basil, lemongrass, thyme, citronella, peppermint, eucalyptus, and vanilla essence essential oils all had a significant deterrent effect against FCM oviposition in choice trials. Lavender and peppermint had the strongest deterrent effect. This came as no surprise as both oils have been reported to deter several lepidopteran pests, such as, *A. gemmatalis*, *Phthorimaea operculella*, and *C. pomonella* (Raman *et al*. 1987; Landolt *et al*. 1999; Ribeiro *et al*. 2015; Elsayed 2020). Basil has been reported to reduce *P. operculella* and *P. absoluta* oviposition behaviour in dual choice experiments (Sharaby *et al*. 2009; Yarou 2018). Black pepper, thyme, and eucalyptus oils have been shown to deter *P. xylostella* oviposition (Sangha *et al*. 2017). Rosemary and lemongrass/citronella have been long reported to deter two-spotted spider mites *Tetranychus urticae* (Koch) (Acari: Tetranychidae) and mosquitoes *Aedes aegypti* (Meigen) (Diptera: Culicidae), respectively (Isman 2006; Miresmailli *et al*. 2006). No studies have been done on the deterrence of vanilla essence, however, it has been used by local farmers in an attempt to deter FCM in the past (Sean Moore pers.comm).

In contrast, patchouli, cypress, sage, and clove bud oils had no significant deterrent effect against FCM adults. This was expected for sage as the crude extract also showed no deterrent activity. The results for patchouli and cypress contradict those reported by Landolt *et al*. (1999) where both oils deterred codling moth larvae. This difference in oviposition deterrence can be attributed to the difference in FCM life stages as explained earlier in this discussion. Additionally, there might be a difference in FCM and *C. pomonella* chemosensory receptors as they are two different species.

Lavender, patchouli, basil, peppermint, and eucalyptus essential oils all showed deterrence in no-choice trials with significantly fewer eggs being laid on the oranges. However, black pepper, rosemary, lemongrass, thyme, citronella, and vanilla essence oils had no deterrent effect in no-choice trials. This means that the moths prefer untreated oranges in choice trials but would still oviposit comfortably on these treatments when not given a choice. It is possible that at higher concentrations, these essential oils would significantly deter FCM oviposition in no-choice trials. A study looking at concentrations higher than (10 mg/ml) might be worth conducting, however, we would then have to factor in the cost of this higher application in the

field. Moreover, there were significantly more eggs laid on the sage treatment compared to the control. This suggests that sage oil releases some odours that are attractive to FCM.

It is known that not all fruit types support the development of FCM larvae. Such fruit include mangoes and lemons (Grove *et al*. 2012; Moore *et al*. 2015). For this reason, it was worth investigating if these fruits had any deterrent effects towards FCM oviposition. Mango significantly deterred FCM oviposition in both choice and no-choice trials. This result supports Grove *et al*. (2012) where it was reported that no FCM eggs were found on any mango fruit in Limpopo and Mpumalanga orchards. The lack of FCM oviposition in the orchards was attributed to the presence of antixenosis (Grove *et al*. 2012). The latex on mangoes has been reported to serve as a chemical defence mechanism against herbivorous insects (Agrawal & Konno 2009). The latex is composed of two separate phases, an oil fraction and a protein-polysaccharide fraction (Loveys *et al*. 1992; Bezuidenhout & Robbertse 2004; Cronje 2005).

In contrast, lemon showed no deterrent activity in both tests. This suggests that FCM can comfortably oviposit eggs on lemons, however, the larvae fail to penetrate and infect the fruit (Economides 1979; Newton 1998; Moore *et al*. 2015), possibly due to the high oil and/or acid content of the fruit or gumming of the rind, as has been recorded to prevent successful penetration of the citrus flower moth, *Prays citri* (Milliere) (Moore & Kirkman 2014).

This study showed that Delegate, Coragen, Exirel, CrpeNPV, and Warlock significantly deterred FCM oviposition in choice trials. This result was expected for Delegate and Coragen because Fullard and Hill (2013) reported that the registered standard concentration of Delegate and Coragen caused FCM females to oviposit significantly fewer eggs than on the controls in fruit bioassay trials. Warlock insecticide is mostly used for the control of lepidopteran larvae and kills via contact or ingestion (ADAMA South Africa 2009). It is possible that the chemical odours from this insecticide deterred FCM adults through olfactory receptors, or a toxic effect was experienced by the moths which affected oviposition behaviour. Very little information is available on CrpeNPV, except that it infects FCM larvae (Marsberg *et al*. 2018). It is however probable that a host and its virus co-evolve over time, with it being beneficial for the virus to evolve an increased attractiveness to the host (this is seen clearly in certain insects e.g. bollworms, albeit in the feeding larvae) and on the other hand, to the benefit of the host to evolve a repulsion to the virus. However, in this case, either is questionable, as CrpeNPV is not the homologous virus of FCM, it is also questionable whether they would encounter one another in a citrus environment, where litchi moth (and hence probably the virus) is not present.

Moreover, the FCM used in this study is from a very old lab culture reared at Rhodes University.

Cryptogran and Runner were the only insecticides that did not significantly deter FCM oviposition in both trials. Cryptogran was isolated from FCM and relies on the ingestion of the virus by larvae, after which it attacks the digestive system (Moore *et al*. 2004). It is known that FCM adults are non-feeders and thus it is possible that the volatiles from Cryptogran were not registered as deterrents by the moths' olfactory receptors. The same can be assumed for the Runner insecticide which is also dependent on larval ingestion (Sabri *et al*. 2017).

Most of the botanicals and insecticides in this study significantly deterred FCM oviposition in choice trials. However, this deterrent effect was not exhibited in no-choice trials for most of them. This suggests that in a field setup where the moths would not be given a choice, they would still find the fruit suitable for ovipositing eggs, which defeats the purpose of a deterrent. For this reason, only botanicals and insecticides with strong deterrent effects that were evident in both trials were chosen for further analysis. The efficacy of these deterrents can be further tested in semi-field trials, and they may be valuable for dispensing in an orchard (through some volatile release mechanism), spraying onto trees, planting between rows of trees, or implementing in push-pull strategies. Results from this study have the potential to improve FCM control in current integrated pest management programmes.

Chapter 3,

Efficacy of identified deterrent compounds: Concentration response bioassays.

3.1 INTRODUCTION

The chemicals released by different plant species as a form of defence against herbivorous insects are diverse (Isman 2006; Miresmailli & Isman 2014). Some of these chemicals are constitutive (Wittstock & Gershenzon 2002), which means that they are constantly present, whereas others are only induced after attack (Karban & Myers 1989). Several plant compounds affect the herbivorous insect directly, whilst others attract natural enemies in a tri-trophic manner (Turlings *et al*. 1998). These chemicals are located across all plant tissues above and below ground: toxic terpenes and volatile infochemicals are released from plant foliage (Gershenzon & Dudareva 2007; Maffei *et al*. 2011); flowers carry behaviour-modifying floral scents (Schiestl & Dötterl 2012); phytotoxic root fluids are released from roots (Bais *et al*. 2006; de Kroon 2007); and toxic latex is released from the stem (Agrawal *et al*. 2012).

Plants can communicate with the natural enemies of herbivorous insects through chemical signals (Yoneya *et al*. 2009; Kappers *et al*. 2011; Pierre *et al*. 2011). Additionally, plants can also elicit a chemical response to herbivore oviposition before feeding damage occurs (Hilker *et al*. 2005; Hilker & Meiners 2011). This is of key interest to this study, as botanical insecticides have different modes of action. Of all commercially available botanical insecticides, EO-based products are the most diverse (Miresmailli & Isman 2014). This can be attributed to them being complex mixtures of low molecular weight and highly volatile secondary metabolites (Miresmailli & Isman 2014). Because of their natural diversity, they have been utilised in a variety of products as contact toxicants, fumigants, and behavioural modifiers (attractants and deterrents).

More importantly, the majority of neem-based botanical insecticides have been reported to have dual action properties, as they can act as both insect growth regulators (IGRs) and deterrents (Isman 2006; Pavela 2007; Mahmoudvand *et al*. 2011; Pavela 2016). IGRs are a

unique class of insecticides with selective effects on various life stages of some insect orders, including Lepidoptera (Mahmoudvand *et al*. 2011). They disrupt moulting by blocking the synthesis and release of moulting hormones (juvenile hormone or ecdysone mimics) at the physiological level (Isman 2006). Additionally, they also interrupt cuticle formation (chitin synthesis inhibitors) and affect the insect endocrine system (Dhadialla *et al*. 1998; Hoffmann & Lorenz 1998). Chitin synthesis inhibitors (CSI) are a group of IGRs, such as benzoylphenylurea (BPU), with high specificity, low toxicity to mammals, and fast degradation in the environment (Mahmoudvand *et al*. 2011). These compounds act on the immature life stages of insects, including the egg stage, meaning that they may have ovicidal properties (Mian & Mulla 1982; Reynolds 1987).

Several studies have reported dual action activity in botanicals, for example, the biocidal effects (insecticidal and deterrent) of *Ocimum* spp. have been well investigated, mainly on pests of stored products (Adeniyi *et al*. 2010; Koubala *et al*. 2013) and on malaria insect vectors (Belong *et al*. 2013; Akono-Ntonga *et al*. 2014).

Biological assays form the core foundation of any biological study (McLaughlin *et al*. 1998) and have long been used in deterrent studies (World Health Organisation 2013). This means that the efficacy (deterrence, effective dose, and duration of protection) of the identified compounds can be tested on fruit (or other substrates) in laboratory bioassays. Establishing concentration-response relationships in deterrent studies is important because it enables identification of appropriate doses and thus prevents overuse and lowers economic costs (WHO 2013). Therefore, the aim of this study was to determine if any of the deterrent compounds identified in Chapter 2 had dual action (both deterrent and ovicidal properties) when tested at different concentrations in fruit bioassays and to determine concentration-response relationships.

3.2 METHODS AND MATERIALS

3.2.1 Concentration response bioassays

Treatments that were identified to have the strongest deterrent activity in the oviposition deterrence trials were evaluated in no-choice bioassays (six treated oranges in each cage) at various concentrations (0.1, 1, 10, 50, and 100 mg/ml) for botanicals, and (0.25×, 0.5×, 1×, 2×,

and 4× the field rate) for insecticides, using the same methodology as before (Chapter 2, section 2.2.4). A total of 150 oranges (6 oranges per concentration × 5 concentrations × 5 replicates) were used for each deterrent treatment and a total of 30 oranges were used for the control treatment. To test for ovicidal properties, the oranges were transferred individually into clear plastic containers and held for seven days in a CE room at 26°C, after which the number of eggs that hatched and the number that failed to hatch was recorded. Eggs generally started hatching five days after oviposition. The oranges were then held for a further seven days to allow the newly emerged neonate larvae to penetrate. FCM larval penetration generally occurs shortly after the eggs have hatched (Bazelet 2020). The number of larvae that were still penetrating the external surface of the fruit was recorded at the end of the seven days. Following this, the oranges were cut in half and then into thin slices to inspect for larval penetration below the surface. The number of larvae that penetrated the fruit was recorded under a light microscope.

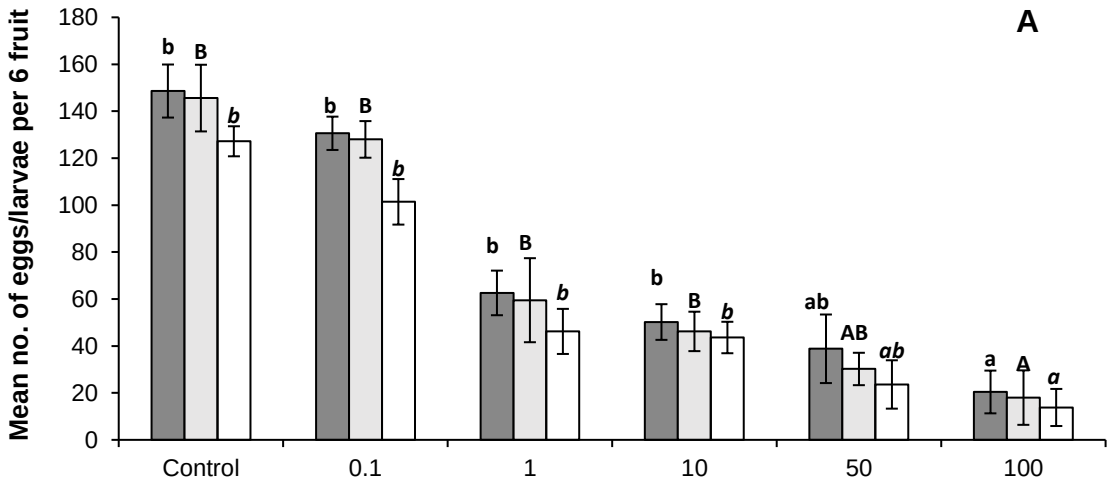
3.2.2 Statistical analyses

All data in this chapter were analysed using GraphPad Prism for windows, version 9.4.1 (2022-07-27). A Kolmogorov-Smirnov test was used to test for normality in the multiple concentration trials. The test confirmed that the data were not normally distributed ($P < 0.05$), therefore a non-parametric Kruskal-Wallis ANOVA was used in conjunction with Dunn's multiple comparisons test to determine significant differences between treatments and the control across concentrations. The difference in oviposition compared to egg hatch, and egg hatch compared to penetration within a given deterrent treatment was analysed using a one-way, repeated measures (based on GLM) analysis of variance (ANOVA), on square root transformed data. A Tukey HSD test was used for *Post Hoc* comparisons. The difference in oviposition, egg hatch, and penetration between different deterrent treatments was also analysed using a repeated measures ANOVA.

3.3 RESULTS

3.3.1 Botanicals

The concentration data were significantly different from the control treatment data for oviposition (P = 0.0005; P = 0.001; P = 0.0003; P = 0.0004), egg hatch (P = 0.0008; P = 0.0005; P = 0.0004; P = 0.0007), and larval penetration (P = 0.0004; P = 0.0005; P = 0.0009; P =0.0008), for marigold, garlic, lavender and peppermint, respectively (Figure 3.1A to D). Marigold had the weakest deterrent and ovicidal activity of the four botanicals, whilst garlic had the strongest (Figure 3.1). A mean (± SE) of 148.6 ± 11.3 eggs was oviposited per six fruit in the control treatment (Figure 3.1). There was a significant difference between the number of oviposited eggs compared to the number of hatched eggs in the marigold treatment (F = 14.23, P = 0.0443). However, there was no significant difference in egg hatch compared to penetration in marigold (P = 0.0574; Figure 3.1A). Significant differences were found in oviposition vs egg hatch (P = 0.0204; P = 0.0134; P = 0.0124) and egg hatch vs larval penetration (P = 0.0027; P = 0.0024; P = 0.0077) in garlic, lavender and peppermint treatments, respectively (Figure 3.1B to D). There was no significant difference in oviposition (F = 1.737, P = 0.2552, R^2 = 0.3028) and egg hatch (F = 3.359, P = 0.1330, R^2 = 0.4565) between different botanical treatments (Figure 3.1). A significant difference in larval penetration (F = 9.530, P = 0.0215, R^2 = 0.7044) was found between different botanical treatments (Figure 3.1).



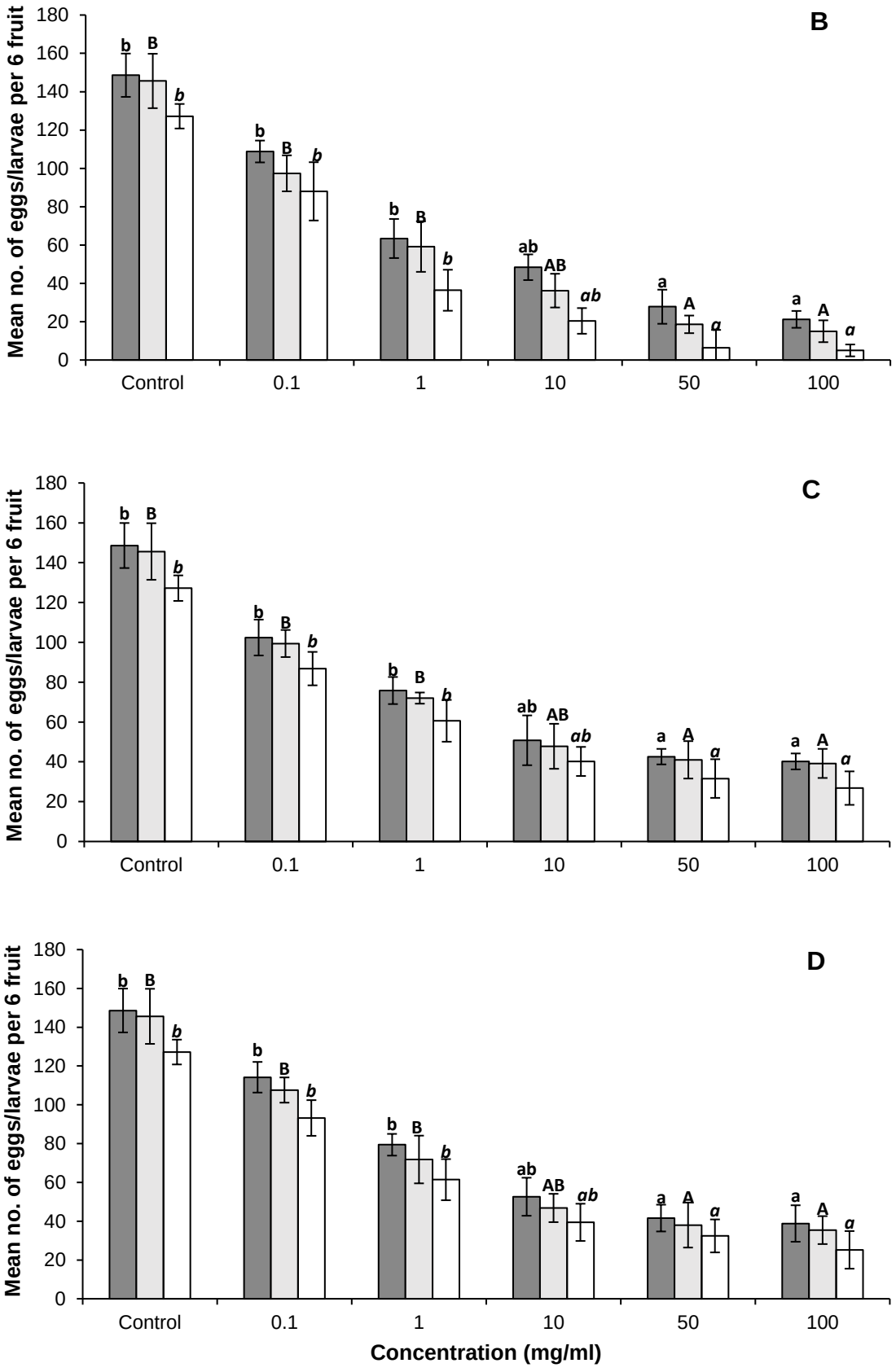

B
C
D
Mean no. of eggs/larvae per 6 fruit
0
20
40
60
80
100
120
140
160
180
Control
0.1
1
10
50
100
Concentration (mg/ml)

Figure 3.1: Mean oviposition (dark grey bars), egg hatch (light grey bars), and larval penetration (white bars) by false codling moth on oranges treated with various concentrations of marigold (A), garlic (B), lavender (C), and peppermint (D) in no-choice bioassays. Error bars denote standard errors of means. Different letters denote significant differences in oviposition (lower case), egg hatch (upper case), and penetration (italic) across all concentrations (multiple comparisons of mean ranks, $P < 0.05$).

3.3.2 Insecticides

FCM oviposition ($H = 20.92$, $P = 0.0008$; $H = 22.12$, $P = 0.0005$; $H = 22.93$, $P = 0.0003$), egg hatch ($H = 20.03$, $P = 0.0012$; $H = 22.49$, $P = 0.0004$; $H = 24.18$, $P = 0.0002$), and larval penetration ($H = 21.12$, $P = 0.0008$; $H = 21.60$, $P = 0.0006$; $H = 23.49$, $P = 0.0003$) were significantly reduced by Warlock, Coragen and Delegate, respectively, compared to the untreated control (Figure 3.2A to C). There was no significant difference between the number of eggs that were oviposited and the number of eggs that hatched in the Warlock treatment ($F_{7.336} = 41.17$, $P = 0.0622$; Figure 3.2A). However, the number of hatched eggs significantly differed from the number of larvae that managed to penetrate the fruit treated with Warlock ($P = 0.0008$). There were significant differences between the number of oviposited eggs and hatched eggs ($F_{8.006} = 28.27$, $P = 0.0208$; $F_{6.095} = 29.36$, $P = 0.0113$), and between hatched eggs and larvae that penetrated the fruit ($P = 0.0180$; $P = 0.0128$) in the Coragen and Delegate treatments, respectively (Figure 3.2B & C). There was no significant difference in oviposition ($F_{4.061} = 0.4317$, $P = 0.5495$, $R^2 = 0.0974$), egg hatch ($F_{4.204} = 0.0053$, $P = 0.9515$, $R^2 = 0.0013$) and larval penetration ($F_{4.180} = 0.0699$, $P = 0.8144$, $R^2 = 0.0172$) between different insecticide treatments (Figure 3.2A to C).

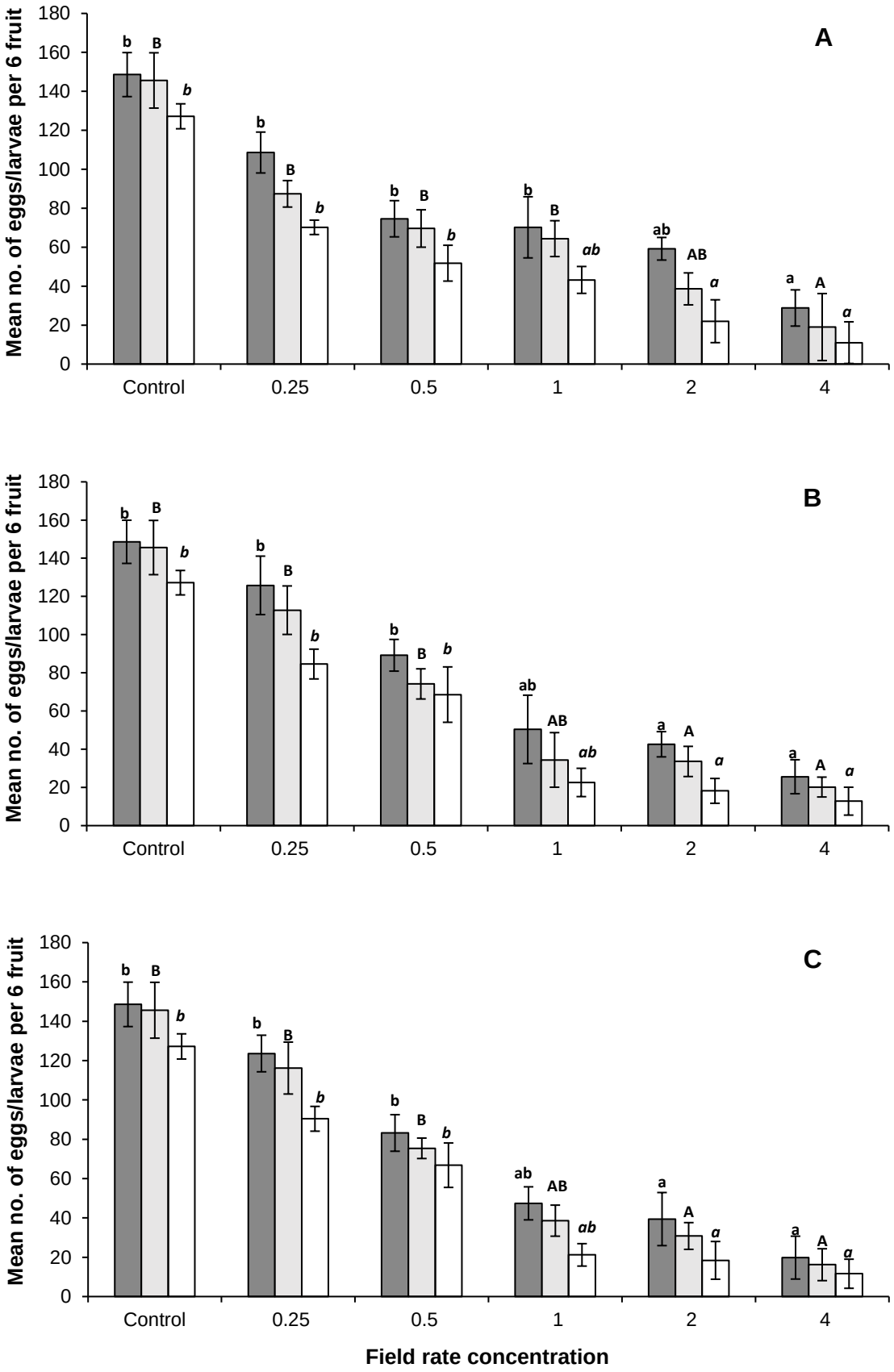

A
B
C
Mean no. of eggs/larvae per 6 fruit
0
20
40
60
80
100
120
140
160
180
Control
0.25
0.5
1
2
4
Field rate concentration

Figure 3.2: Mean oviposition (dark grey bars), egg hatch (light grey bars), and penetration (white bars) by false codling moth on oranges treated with various field rate concentrations of Warlock (A), Coragen (B), and Delegate (C) in no-choice bioassays. Error bars denote standard error of means. Different letters denote significant differences in oviposition (lower case), egg hatch (upper case), and larval penetration (italic) across all concentrations (multiple comparisons of mean ranks, $P < 0.05$).

3.4 DISCUSSION

In the context of this study, concentration-response bioassays are a representation of how FCM behaviour at different life stages is affected by concentration of different deterrent treatments. Botanical pesticides have long been considered as alternatives to synthetic pesticides for the control of harmful lepidopteran pests (Hussein *et al*. 2017). This study showed that all botanicals (both crude extracts and essential oils) significantly reduced FCM oviposition, egg hatch and larval penetration in a concentration-response manner. This was expected, as many concentration-response studies of agricultural pests have shown all three behavioural measures to be dependent on botanical treatment concentration (Landolt *et al*. 1999; Adeniyi *et al*. 2010; Elsayed 2020). Landolt *et al*. (1999) noted that the time taken by *Cydia pomonella* larvae to cross a barrier treated with garlic significantly increased with increasing concentration. The longest times recorded were 44.3 min and 60 min at 10 mg/ml and 100 mg/ml, respectively. Rafiee-Dastjerdi *et al*. (2013) reported that *Phthorimaea operculella* oviposition and larval penetration was significantly reduced on potato tubers treated with 5% methanolic extracts of lavender. None of the botanicals had a significant effect on all three measures of behaviour at the lower two concentrations in the present study. This supports the results achieved by Landolt *et al*. (1999) where it was recorded that concentrations higher than 10 mg/ml were most effective in controlling *C. pomonella* larvae.

The number of hatched eggs was significantly lower than the number of oviposited eggs in all botanical treatments, suggesting that all tested botanicals have ovicidal properties. This can be attributed to plant allelochemicals that can penetrate the eggs and prevent the embryo from developing (Elsayed 2020). Sangha *et al*. (2017) reported that *Plutella xylostella* larval emergence was significantly lower in eggs treated with garlic essential oil at 2.5% and 5% concentration. Marigold leaf extracts at 5% significantly reduced *Spodoptera litura* (Fabricius)

(Lepidoptera: Noctuidae) egg hatch (Kulkarni *et al*. 2016). Ahmad *et al*. (2020) reported that ethanol dilutions of lavender oil caused hatching inhibition of *Callosobrucus chinensis* (Linn.) (Coleoptera: Bruchidae) eggs at 0.25, 0.5, and 1% concentration. Moreover, Jeyasankar *et al*. (2013) noted that lavender and peppermint showed significant ovicidal activity against *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) at 3, 12, and 24 µl/L. It was also reported in Jeyasankar *et al*. (2013) that ovicidal activity was dependent on botanical concentration.

Larval penetration compared to egg hatch was significantly reduced in all botanical treatments, except marigold. This result was not expected for marigold, as chemical volatiles are known to affect larvae more than adults in moths (Elsayed 2020). Additionally, Salinas-Sánchez *et al*. (2012) noted antifeedant, larval and pupal mortality effects against *Spodoptera frugiperda* in African marigold at 500 ppm. However, botanical compounds are known to have low persistence and can be affected by temperature and relative humidity even in laboratory conditions (Lengai *et al*. 2020). It is possible that marigold had the lowest persistence, thus by the time the eggs hatched, the chemical volatiles had been diluted. Investigating larval penetration at different times post application thus warrants further investigation.

Evidence of larval upwind movement arrest and deterrence of neonate *C. pomonella* larvae was reported by Landolt *et al*. (1999) for lavender, garlic, and peppermint. Moreover, Ribeiro *et al*. (2015) reported that garlic and mint essential oils deterred feeding by *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Erebidae) larvae. No significant differences were found in oviposition and egg hatch when the botanicals were compared to each other. This suggests that they affected female oviposition and egg hatch behaviour in the same pattern, as a result of changing concentration. Larval penetration differed significantly between botanicals with the lowest number of larvae being recorded in the garlic treatment. This was expected as there are many past studies that have demonstrated deterrent, ovicidal, and larvicidal properties in garlic extracts and essential oils (Landolt *et al*. 1999; Chaubey 2017; Sangha *et al*. 2017). Additionally, garlic was the fifth most used botanical active ingredient for the control of agricultural pests across California in 2016, with a total of 385 kg being utilised throughout the year (Isman 2020).

The major chemical active ingredients in botanicals are responsible for the deterrent and ovicidal properties shown in the results (Isman 2020). Garlic contains allyl methyl trisulfide and diallyl trisulfide as major components (Chaubey 2017). Lavender is composed of linalool and linalyl acetate (Soković *et al*. 2010). Peppermint contains menthol, menthone,

isomenthone, neomenthol, acetylmenthol, pulegone and methyl acetate (Soković *et al*. 2010; Al-taweil 2014). Lastly, marigold consists of limonene, (*Z*)-ocimene, dihydrotagetone, (*E*) & (*Z*)-tagetone, and (*E*) & (*Z*)-tagetenone (Walia & Kumar 2020). It is important to note that the quantity of the active compounds often differs depending on the geographical location of the plants (Lengai *et al*. 2020). This type of variation is also evident in sex pheromones of lepidopteran females, where females from different regions have different pheromone compositions (Kawazu *et al*. 2009).

Chemical insecticides are neurotoxins that operate on a wide range of biochemical or molecular sites. However, studies on the deterrent properties of FCM chemical insecticides have been limited. Knowledge on pest and insecticide relationships can help improve understanding of pest behaviour in orchards treated with insecticides. In this study, all three insecticides significantly reduced FCM oviposition, egg hatch, and larval penetration when compared to the control treatment in the higher concentrations. More importantly, the registered field rates of all tested insecticides significantly affected FCM behaviour. This result was expected for Coragen and Delegate as Fullard and Hill (2013) achieved similar results. Additionally, Knight & Flexner (2007) reported that topical application of chlorantraniliprole, the active ingredient of Coragen, significantly affected reproductive output in *C. pomonella*. It is no surprise that Warlock also achieved the same results because all three insecticides have similar modes of action through ovicidal and/or larvicidal activity (ADAMA South Africa 2009; Miletić *et al*. 2011; Corteva Agriscience 2022).

The number of eggs that hatched did not differ significantly from the number of eggs that were oviposited in the Warlock treatment. Ioriatti *et al*. (2009) recorded low ovicidal activity (30%) in emamectin benzoate (the active ingredient of Warlock) against *C. pomonella*, irrespective of applied concentration in field trials. No ovicidal effects were found in emamectin benzoate when tested against *Cydia molesta* (Busck) (Lepidoptera: Tortricidae) in laboratory and field conditions (Ioriatti *et al*. 2009). The number of larvae that penetrated the fruit significantly differed from the number of eggs that hatched in the Warlock treatment. This suggests that Warlock only has strong deterrent and not ovicidal properties. Mangrio & Sahito (2022) reported that emamectin benzoate significantly deterred *Papilio demoleus* (Linnaeus) (Lepidoptera: Papilionidae) larvae in lemon orchards when applied at the field rate.

On the other hand, Delegate and Coragen significantly reduced egg hatch when compared to oviposition, and larval penetration when compared to egg hatch. This suggests that these

insecticides have both ovicidal and deterrent properties. These results are supported by Fullard and Hill (2013), where reduced egg hatch was attributed to direct toxic effects on the eggs that were laid directly on the treated fruit (Kirkman 2007). Moreover, Sisay *et al*. (2019) showed that Coragen 200 SC and Radiant 120 SC (same active ingredient as Delegate) significantly reduced leaf damage by *S. frugiperda* at the recommended field rate in laboratory, greenhouse, and field conditions. There were no significant differences in oviposition, egg hatch, and larval penetration between different insecticide treatments. This suggests that all insecticides affected FCM behaviour in a similar way across all concentrations, with oviposition, egg hatch and larval penetration decreasing with increasing concentration.

In conclusion, concentration affected FCM behaviour in all deterrent treatments with the highest concentrations being the most effective. This was expected as chemical volatiles are the strongest at higher concentrations (Lengai *et al*. 2020). All deterrent treatments, except Warlock, had dual action (both deterrent and ovicidal properties). Considering the results obtained in this study and other past studies, it is evident that dual action is common amongst botanicals (Landolt *et al*. 1999; Sangha *et al*. 2017; Elsayed 2020).

More studies need to be done on the deterrent effects of botanicals against FCM oviposition, followed by field trials to confirm the results in a natural environment. It was very difficult to establish an effective concentration amongst botanicals in past field studies (Lengai *et al*. 2020; Ngegba *et al*. 2022). Most botanical pesticide studies are laboratory based and the few that conduct field experiments vary in terms of applied concentration. Applied concentrations can range from 1% to 50% in field experiments (Agboka *et al*. 2009; Reddy *et al*. 2012; Forrer *et al*. 2014; Naqvi *et al*. 2018). This shows that there is a gap in literature for field trials investigating botanical pesticides at standard field concentration. It is very important to establish clear guidelines on standard preparation and application methods of botanicals in both laboratory and field conditions (Ngegba *et al*. 2022). This will help secure a sustainable future for botanical pesticides in the agricultural industry.

Chapter 4,

General Discussion

4.1 INTRODUCTION

There are a multitude of factors that can lead to reduced fruit yields and/or market rejections in export citrus in South Africa (CGA 2022). One common factor is the constant exposure of fruit to phytosanitary herbivorous insect pests such as FCM. This has forced farmers to rely on traditional pest management strategies, which often involves the use of synthetic pesticides (Lengai *et al*. 2020). However, with export markets such as the European Union (EU), which is imposing increasingly strict restrictions on the usage and permissible residues of plant protection products (PPP), and pests constantly developing resistance to synthetic pesticides, it has become vital to search for alternative control strategies that can be implemented in IPM programmes (Isman 2014; Lengai *et al*. 2020; Isman 2020; CGA 2022). This has led to the successful use of semiochemicals (organic compounds) in several pest management technologies, such as monitoring, mating disruption, attract and kill, and push-pull strategies (Blassioli-Moraes *et al*. 2019; El-Ghany 2019). Plant semiochemicals in push-pull strategies can either be used as insect deterrents or attractants, based on their mode of action (Byres & Levi-Zada 2022).

The use of botanicals as deterrents has been widely studied in medical and veterinary pests, such as the female *Anopheles* mosquitoes, which have been successfully deterred by lemongrass derived products (Foster & Harris 1997; Isman 2006; Isman *et al*. 2011). On the contrary, research on the potential use of botanical deterrents for agricultural pests has been limited. This study aimed to, firstly determine if there were any botanicals or commercial insecticide treatments, which could deter FCM oviposition. Oviposition deterrence was assessed for nine plant crude extracts, 15 essential oils, two fruit, and seven insecticides (Chapter 2). All tested botanicals were selected based on an extensive literature review on lepidopteran deterrent compounds, whereas the insecticides were selected based on what is currently used for FCM control in the South African citrus industry. Two plant extracts

(marigold and garlic), two essential oils (lavender and peppermint), and three chemicals (Warlock, Coragen, and Delegate) were identified with deterrent effects against FCM oviposition in the initial deterrence trials (Chapter 2).

The second aim of this study was to test the efficacy of the identified deterrent compounds in concentration-response detached fruit bioassays. The deterrent compounds identified in Chapter 2 were tested for their efficacy at five different concentrations in these detached fruit bioassays (Chapter 3). FCM behaviour was affected by concentration in all tested compounds, with the highest concentrations being the most effective (≥ 10 mg/ml for botanicals and ≥ 1× the field rate for insecticides). The third and final aim of this thesis was to determine if any of the identified compounds had dual action (deterrent and ovicidal properties). All tested botanicals were identified to have dual action, whereas only Coragen and Delegate were identified to have dual action in the insecticide treatments (Chapter 3).

This chapter focuses on placing these key findings in the context of the global literature on the topic, discussing their potential integration into current FCM programmes, especially the push-pull strategy, their impact on the economy, and lastly, providing some recommendations for the industry and for future research.

4.2 GLOBAL PERSPECTIVE

Plants produce secondary chemical compounds as a mechanism of defence against different insect pests (Isman 2011; Lengai *et al*. 2020; Byres & Levi-Zada 2022). These chemical volatiles have different modes of action, however, this thesis focused on the deterrent and ovicidal effects of potential botanical pesticides. Botanical pesticides can be applied as plant extracts and/or essential oils that have been mixed with a relevant solvent e.g. methanol or ethanol (Lengai *et al*. 2020; Byres & Levi-Zada 2022). Further, they can be derived from different plant parts such as leaves, flowers, roots, bark, cloves, seeds, and stems (Lengai *et al*. 2020). The most suitable plant parts to use are those that contain most of the bioactive compounds as secondary metabolites that are responsible for affecting insect behaviour, in most cases the leaves and flowers (Isman 2020). Some botanical compounds that have been commercialised globally include pyrethrum, neem, rotenone, and nicotine (Isman 2020). All four of these compounds have been successfully commercialised in countries such as the United States of America (USA) and India (Isman 2006; Isman 2020). However, nicotine

products, for example nicotine sulphate, have been reported to have toxic effects on some vegetables and ornamentals (Lengai *et al*. 2020). For this reason, nicotine pesticide use has seen a decline in the global market and is no longer commercially available in many countries (Lengai *et al*. 2020). Plant essential oils from citrus and eucalyptus have also been commercialised as pesticides in Australia, Denmark, USA and Canada (Isman 2006; Isman 2020). More importantly to the present study, garlic has been commercialised as a botanical pesticide in Brazil, Mexico, and the USA (Isman 2020). This means that there is potential for the registration and commercialisation of garlic as a botanical pesticide for FCM in South Africa. The overall use of pesticides in the African context is very low when compared to the global market. Moreover, pesticide use in Sub-Saharan Africa is approximately 3% lower than in other African countries (Sola *et al*. 2014).

In South Africa, only a few botanical compounds have been approved for commercial use, and these are pyrethrum, azadirachtin, various plant oils, Spinosad and Tartar Emetic SP (Universal Crop Protection (Pty) Ltd, Kempton Park) (Grove *et al*. 2000; Isman 2006; Sola *et al*. 2014). Synthetic pyrethroid pesticides, based on the natural pyrethrum plants, have been reported to have a negative impact on FCM natural enemies, thus making them not compatible for integration into IPM programmes (Kirkman 2007; Moore & Hattingh 2012; Fullard & Hill 2013: Moore 2019). Pyrethroids generally have low toxic effects on mammals, however, they have a broad spectrum and often kill pest natural enemies (Ndakidemi *et al*. 2016). They have been shown to be toxic to invertebrates and fish in previous studies (Taskin *et al*. 2014; Ndakidemi *et al*. 2016; Fernández-Grandon *et al*. 2020). However, natural pyrethrum has a much shorter residual efficacy than synthetic pyrethroids and should be far less detrimental to FCM natural enemies (Lengai *et al*. 2020). Although citronella has been successfully utilised in many pesticide products as an active ingredient to deter mosquitoes, it did not significantly deter FCM oviposition in the present study. Tartar Emetic is a salt derived from the fermented product of citrus and grapes and is used for the control of citrus thrips, *Scirtothrips aurantii* (Faure) (Thysanoptera: Thripidae) in South Africa (Grout & Richards 1992). However, this pesticide did not achieve much success in field experiments in the Mpumalanga province of South Africa, as the number of thrips on mangoes treated with the pesticide did not significantly differ from the control (Grove *et al*. 2000). Based on the limited use of botanicals in South Africa and the evidence of their wide application in other countries (Isman 2020), it is evident that there is an opportunity for the implementation of new botanical pesticides in the country.

This study showed the potential use of marigold, garlic, lavender, and peppermint as botanical pesticides for FCM control.

4.3 INTEGRATION INTO IPM

Despite their generally environmentally friendly profile, the use of botanical pesticides in IPM programmes has been rather limited. There are many reasons for their lack of practical application in IPM that continue to be discussed in literature, the main one being lack of residual efficacy. Isman (1997) and Regnault-Roger & Philogène (2008) reported that low efficacy in large scale field applications has made it difficult to commercialise botanical pesticides, and thus most botanicals do not make it out of the laboratory. Moreover, the few that have managed to show efficacy in the field such as neem products, still face obstacles regarding standardisation of extracts, synthesis of active compounds, and regulatory approvals (Regnault-Roger & Philogène 2008; Isman 2020). The botanical compounds identified in this study were subjected to the same methods of extraction as crude products. If these compounds can achieve efficacy in the field, it would mean that there is potential for a standard method of extraction and application for FCM botanical pesticides.

It is important to note that the above-mentioned challenges regarding the use of botanical pesticides in IPM programmes are not sufficient to disqualify research on improving field efficacy. The advantages associated with the use of botanicals outweigh the challenges that currently limit their use. For example, sources of botanical pesticides are readily available in the environment, and they currently have multiple uses in the medical, veterinary, and food industries (Isman 2006; Isman *et al*. 2011; Lengai *et al*. 2020; Byres & Levi-Zada 2022). This means that they should be inexpensive and thus suitable to be incorporated for use in the agricultural industry. Though not without risk to non-target organisms, botanical pesticides that are available commercially have been shown to be less harmful to non-target organisms, such as insect pollinators (Lengai *et al*. 2020). This means that botanicals have potential for use in sustainable agricultural practices. Their fast biodegradability, low persistence, and low mammalian toxicity in the environment give them advantages over synthetic pesticides (Figure 4.1). However, low persistence does mean that application must be more frequent and carefully timed to save on costs. Botanicals may be applied at sunset when there is less heat and humidity. This means that botanical pesticide application is likely to be more effective in cooler

regions. The chemical interaction between botanical pesticides and fruit is completely natural, which means that insect pests are less likely to develop resistance (Amoabeng *et al*. 2014; Lengai *et al*. 2020). More importantly, botanical pesticides can also be used in conjunction with other control techniques in IPM, such as attract and kill, augmentation of natural enemies (predators and parasitoids), microbial pesticides such as Cryptogran (CrleGV), and IPM compatible chemical insecticides such as Warlock, Delegate, and Coragen.

The attract and kill technique can be used in a push-pull strategy in conjunction with oviposition deterrents, such as the ones identified in this study. This combination of control techniques was demonstrated by Wallingford *et al*. (2017) in a study to reduce *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) oviposition. The combination of mass trapping (pull) and oviposition deterrent 1-octen-3-ol (push) significantly reduced *D. suzukii* oviposition when compared to either technique alone (Wallingford *et al*. 2017).

Currently, biological control as a standalone technique cannot achieve 100% control of FCM populations. However, when used in conjunction with botanical pesticides that are less detrimental to natural enemy populations than synthetic insecticides, there is great potential for improving overall efficacy. This suggests that the botanical compounds identified in this study can be used in conjunction with *Trichogrammatoidea cryptophlebia* (Nagaraja) (Hymenoptera: Trichogrammatidae), a known highly effective parasitoid of FCM eggs (Newton 1998). Similarly, they can also be used in the presence of FCM predators such as assassin bugs (Reduviidae), *Orius* bugs (Anthocoridae), and ants (Newton 1998; Kirkman 2007; Bownes *et al*. 2014; Malan *et al*. 2018). However, this does not mean that natural enemy toxicity studies using the identified compounds should be ignored. Granulovirus products can be used in conjunction with other pesticides by simply mixing them in the same spray container/tank and still maintaining efficacy. This suggests that botanical pesticides can be mixed with products such as Cryptogran® (River Bioscience, South Africa), Cryptex® and Gratham (Andermatt, Switzerland), and the Cryptophlebia peltastica nucleopolyhedrovirus (Marsberg *et al*. 2018; Moore & Jukes 2019). The combined deterrent effect of the botanical and the insecticidal effect of the virus may have a positive overall effect i.e. the virus potentially killing the small portion of the pest population that is not adequately deterred. The identified botanicals had ovicidal properties and most likely have larvicidal properties as well. Several studies have shown larvicidal activity in botanicals against different agricultural pests (Sangha *et al*. 2017; Murfadunnisa *et al*. 2019; Tran *et al*. 2022). Because of their quick biodegradability, it is also

unlikely that botanical pesticides will interfere with entomopathogenic fungi (EPF) used against FCM through surface runoff.

The use of botanical pesticides in conjunction with IPM compatible synthetic pesticides is not uncommon. A study by Ouma *et al*. (2014) showed that the combined use of biological and synthetic pesticides reduced the population of three different species of thrips *Megalurothrips sjostedti* (Trybom), *Frankliniella schultzei* (Trybom), and *Frankliniella occidentalis* (Pergande) by more than 60% and increased yield by up to 50% in field experiments. When compared to the use of synthetic pesticides alone, their combined use was shown to be the most cost-effective (Ouma *et al.* 2014). Two chemical insecticides used in the current study (Delegate and Coragen) were identified with deterrent and ovicidal properties, thus their combined use with botanical pesticides warrants further investigation. Dual action in both botanicals and insecticides is beneficial for the control of FCM, as it is unlikely that all moths will be deterred in the field. Thus, ovicidal activity is essentially a safety net that will improve efficacy.

This thesis only focused on the deterrent and ovicidal properties of the botanical compounds against FCM. However, several other studies have looked at factors such as pest mortality at different life stages and larval penetration at different times post application (Moawad *et al*. 2013; Sangha *et al*. 2017; Sohrabi *et al*. 2019). Knowledge of such measures of behaviour would have contributed positively to this thesis in terms of insecticidal properties (larval and adult mortality) and persistence of the botanicals. Botanical compounds have shown insecticidal activity in past studies, for example, natural extracts of *Pegaum harmala*, *Ajuga iva*, *Aristolochia baetica*, and *Raphanus raphanistrum* showed insecticidal activity against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Jbilou *et al*. 2006). Essential oils from garlic, rosewood, and thyme demonstrated significant larvicidal effects against *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) in laboratory bioassays (Sangha *et al*. 2017).

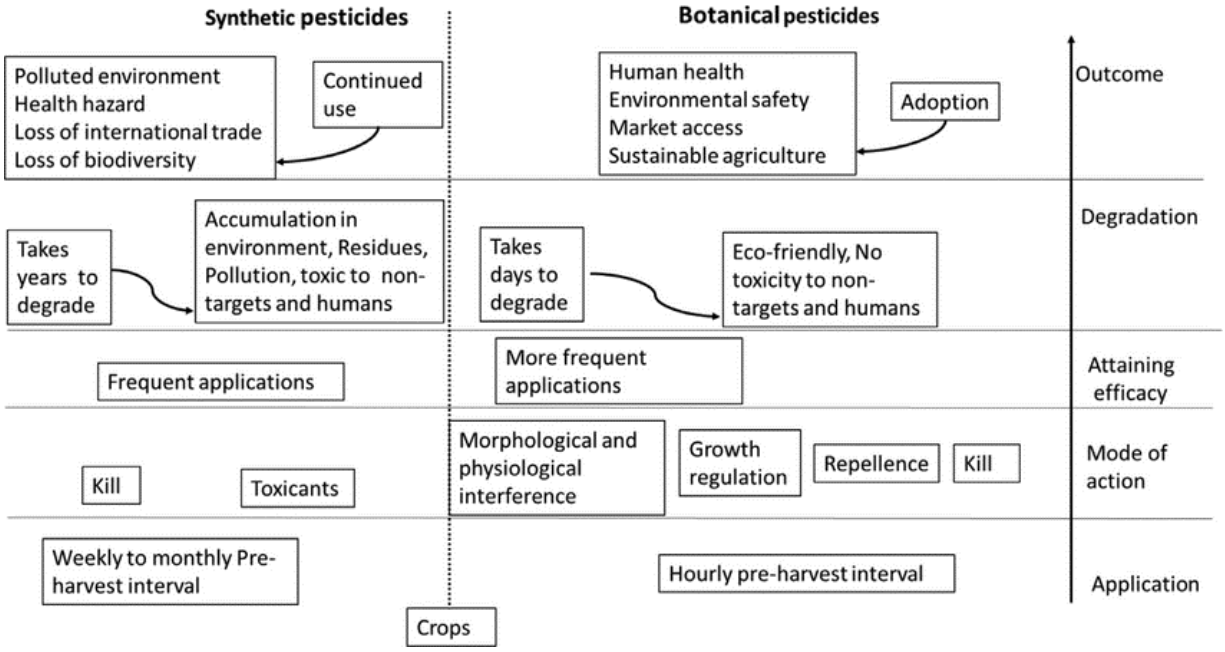


Figure 4.1: Differences between botanical and synthetic pesticides (Lengai *et al*. 2020).

4.3.1 Role in push-pull strategies

The push-pull strategy as we know it today is a combination of deterrent (push) and attractive (pull) stimuli to control pest populations by killing or trapping techniques. Over the years, it has been shown that the push-pull strategy requires thorough understanding of chemical ecology, insect biology, and natural enemy interactions with host plants (Cook *et al*. 2007; Hassanali *et al*. 2008; Khan *et al*. 2011; de Franca *et al*. 2013). For this reason, researchers are continuously looking for ways to improve push-pull strategies in IPM. Cook *et al*. (2007) investigated the potential use of alarm pheromones as deterrents. Alarm pheromones are usually released to warn pests of predators and their use as deterrents has been successful in the control of many species of aphids (Herrbach 1992; Zhang *et al*. 2017).

Botanical deterrents in push-pull strategies can either be used as whole plants (planting between rows) or extracts (spraying directly onto trees) together with attractants such as pheromone dispensers or actual trap plants. Possible attractants for male FCM include the female sex pheromone, which induces mating behaviour (Balaso *et al*. 2019). The lepidopteran sex pheromone has been widely studied in the past and has been successful in reducing pest populations (Balaso *et al*. 2019; Petkevicius *et al*. 2020). Pheromone traps such as the yellow delta trap can be used to attract FCM when deterred from a tree in an orchard. Moreover, this

study showed that sage oil releases volatiles that are attractive to FCM (Chapter 2). This means that sage can potentially be incorporated into a push-pull strategy as a botanical attractant to FCM adults. Analytical studies must be conducted to identify and quantify the attractive compounds in sage.

The push-pull strategy has been successfully implemented in the control of various agricultural pests (Table 4.1). In the citrus industry, the push-pull strategy has been widely studied for the control of the Asian citrus psyllid *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllidae), which is a vector of C*andidatus* Liberibacter asiaticus that causes citrus huanglongbing (HLB) (Yan *et al*. 2015; Goerge & Rao 2019; de Andrade *et al*. 2021). Additionally, Cui *et al*. (2022) reported that *Bactrocera minax* (Enderlein) (Diptera: Tephritidae) fruit infestation rate was reduced from 75.5% to 7.6% in Navel oranges and 100% to 5.6% in satsuma mandarins, when tested in a push-pull strategy using *Xanthium sibiricum* leaf extract as the deterrent and food traps as the attractants. Designing a model for a push-pull strategy in an orchard can be tricky because of the large size of some orchards. However, studies have shown traps to be effective when placed along a 1 ha area periphery as a barrier or in a grid pattern (Yan *et al*. 2015; Byres & Levi-Zada 2022). Deterrents need to be applied to each tree or planted between each row.

Table 4.1: Push-Pull strategy used for insect pest management in agricultural crops (Adapted from Balaso *et al*. 2019).

Insect Pest	**Crop**	**Components**		**Reference**
		Push	**Pull**	
Chilo partellus *Busseola fusca*	Maize and Sorghum	Molasses grass (*Melinis minutiflora*), silverleaf desmodium (*Desmodium uncinatum*)	Napier grass (*Pennisetum purpureum*) or Sudan grass (*Sorghum vulgare sudanense*)	(Khan & Pickett 2004)
Meligethis aeneus	Oilseed rape	Perimeter turnip rape trap crop	Cultivars of oilseed rape with low proportions of alkenyt glucosinolates	(Cook *et al*. 2004)
Frankliniella occidentalis	Chrysanthemum	Volatiles of the non-host plant rosemary	Polygodial (extracted from *Tasmannia stipitata*)	(Bennison *et al*. 2001)
Helicoverpa armigera	Cotton	Neem seed kernel extracts to the main crop	Trap crop, either okra or pigeon pea (*Cajanus cajan*)	(Njihia *et al*. 2014)

Chilo partellus	Maize	Silverleaf desmodium (*D. uncinatum*)	Napier grass (*Pennisetum purpureum*)	(Khan *et al*. 2008)
Eldana saccharina	Sugarcane	Molasses grass (*M. minutiflora*)	Bt maize and indigenous wetland sedges *Cyperus papyrus*	(Cockburn *et al*. 2014)
Spodoptera frugiperda	Maize	Greenleaf desmodium, *Desmodium intortum*	Brachiaria cv Mulato II	(Midega *et al*. 2018)

4.4 ECONOMIC SIGNIFICANCE

Most farmers in South Africa practice agriculture on a small scale and often do not have the means to afford chemical pesticides. For this reason, it is important to find ways to produce cheaper pesticides that are affordable to both the commercial and subsistence farmer. The use of readily available plant material combined with biological control offers an alternative and affordable control strategy to chemical control (Lengai *et al*. 2020). The South African citrus industry aims to maximise profits by increasing the quantity of exported fruit each year. In 2021, the highest number of total citrus exports was recorded compared to any other year ever in history (CGA Key Industry Statistics 2022). It would be ideal to keep improving each year, however, this is threatened by export markets continuously amending maximum residue levels. For example, Imidacloprid (ADAMA South Africa), which is an IPM compatible insecticide for the control of citrus psylla, was excluded as an orchard treatment option by EU in 2021 (CGA 2022). This further supports the need for cost effective control strategies such as the use of botanicals.

Amoabeng *et al*. (2014) showed that pest management methods using locally available botanicals in Ghana were cheaper and more economical when compared to synthetic insecticides. This was quantified by a higher cost to benefit ratio achieved by botanicals. The cost to benefit ratio gives an indication of relative economic performance in a treatment (Aziz *et al*. 2012). Amoabeng *et al*. (2013) reported that Siam weed and tobacco extracts were more economically viable than Attack® (permethrin (25 g/L) + pirimiphos-methyl (475 g/L), Nufarm Technologies (Pty) Ltd, New Zealand), when rainfall was high. However, the opposite was true in lower rainfall seasons. Charleston (2004) reported that syringa tree extracts were successful in controlling *P. xylostella* in the Limpopo Province of South Africa in areas where rainfall was high. Mkenda *et al*. (2015) reported that three botanical insecticide extracts of

Tephrosia vogelii, Vernonia amygdalina, and *Tithonia diversifolia* resulted in lower costs and higher profits when compared to a synthetic insecticide Karate (lambda-cyhalothrin (pyrethroid), Syngenta SA (Pty) Ltd, South Africa). It is important to note that although some studies show botanicals to be cheaper than synthetic insecticides, efficacy of botanicals has been shown to be variable in certain studies, influenced by factors such as rainfall.

There are many factors that influence the cost of botanical pesticides such as type of plant material used (crude or refined) and economic value of the extracted plant (Amoabeng *et al*. 2014). The labour cost and equipment involved in the extraction process may also influence the overall cost of commercialisation of a botanical pesticide (Amoabeng *et al*. 2014). The crude products used in this study were extracted in methanol using a rotary shaker. However, any other method that can thoroughly mix the powder with the solvent is also appropriate (Gupta *et al*. 2012; Amoabeng *et al*. 2013). The essential oils were purchased commercially but can also be extracted from the plant material through steam distillation (Isman 2020). Traditional methods of steam distillation are also appropriate for this extraction process, it is not mandatory to use expensive laboratory equipment (Lengai *et al*. 2020). This means that these botanicals can be beneficial to both small and large-scale farmers when standard protocols and quality control testing have been implemented.

The cheapest option would be to use readily available plants that are widely distributed across South African soils to avoid high purchase costs especially for small-scale farmers. Lavender is cultivated in the Mpumalanga, Free State, Gauteng, KwaZulu Natal, Western and Eastern Cape provinces of South Africa (DAFF 2012). Marigold is a biannual plant native to South and North America but has managed to spread across South Africa and neighbouring areas (SANBI 2017). Peppermint is cultivated in the higher altitudes of KwaZulu Natal, Gauteng, Eastern Free State, escarpment of Mpumalanga, and low areas of Western and Eastern Cape (DAFF 2012). Garlic is produced in the North West, Gauteng, Limpopo (Polokwane plateau), Free State, part of KwaZulu Natal, part of the Northern Cape, and Western Cape (Karoo) provinces (DALRRD 2020). It is evident that the identified botanicals are widely distributed across South Africa and thus should be easily accessible to farmers. However, it is important to note that regional differences in plant material may affect product efficacy (Lengai *et al*. 2020). This therefore suggests that variability in performance may be observed in the same plant species cultivated in different regions of the country.

4.5 RECOMMENDATIONS AND FUTURE RESEARCH

The citrus industry is fast moving towards limited use of chemical insecticides with all the chemical residue restrictions that have been put in place to date. Although synthetic pesticides are bound to enable production of higher yields of exportable citrus fruit, due to their generally superior efficacy, future research must focus on finding ways of improving the efficacy of botanical pesticides to save on both environmental and economic costs. Recently, research efforts have been directed at improving longevity of botanical pesticides using nanotechnology (Lengai *et al*. 2020). This formulation technique has shown positive results against agricultural pests through dispersion of active compounds in field conditions (Luiz de Oliveira *et al*. 2018; Monteiro *et al*. 2021). There is great potential for nanoscale formulations of botanicals and research must be directed at exploring and improving this new avenue.

The current thesis research showed deterrent and ovicidal effects in four botanicals (crude extracts and essential oils) and two chemical insecticides used for FCM control (Chapter 2 & 3). One insecticide only had deterrent properties i.e. Warlock. The recommended concentration for the botanical pesticides is 10 mg/ml and recommended field rate on label for the insecticides. However, there are still a few more experiments that need to be conducted on the botanicals to make sure that they are compatible for use in IPM programmes. The first step is to further test for their efficacy in semi-field/field trials, not only as extracts but also as whole plants in a push-pull strategy. This will ensure that the botanicals can be used by all farmers. The field efficacy of the botanicals should be tested in different seasons and at different times of the day, as past studies have reported variable results under different environmental conditions (Amoabeng *et al*. 2013; Mkenda *et al*. 2015).

The identified deterrents should also be tested in different hosts of FCM apart from citrus (Chapter 1, section 1.2.2). This will also provide a general idea of which host is mostly preferred by FCM. Laboratory bioassays should be conducted to test for the effects of the botanicals on FCM natural enemies. A negative impact on natural enemy populations would mean that the botanicals are not compatible for use in IPM. Attempts should be made to encourage standardised extraction techniques and the registration of botanical pesticides in South Africa. There is currently a backlog of PPP registrations which can be improved by the appointment of more staff members (CGA 2022). Studies investigating true repellence in the identified compounds should be conducted. This will help determine whether the achieved deterrent effect was a result of true repellence or odour masking.

Large volumes of plant material are needed for the production of botanical pesticides. Therefore, to avoid competition with important agricultural crops, the identified botanicals could be planted in areas that are not suitable for arable agriculture. Alternatively, they can also be planted in forests without interfering with forest trees as they are relatively short. The prices of the solvents used in the extraction process of botanicals should be compared and studies using cheaper solvents should be explored. If there are no significant differences in botanical pesticide performance with regards to the solvent used, then the use of cheaper solvents would be beneficial for small-scale farmers. This would encourage all farmers to adopt safer methods of pest control without the deterrent of affordability. Lastly, close collaborations and partnerships are encouraged between researchers and scientists in the chemistry and biology fields to produce high quality research that will benefit the citrus industry and horticultural crop production in general. Researchers also have the duty of raising awareness of the benefits of botanicals to all farmers.

4.6 CONCLUSION

This thesis has demonstrated deterrent and ovicidal activity against FCM in botanicals and registered insecticides under laboratory conditions. These results will form the basis for field and laboratory experiments to follow, on the control of FCM using botanicals. This is an important step in the right direction for the citrus industry, which extensively relies on the export market to generate foreign income. If the identified compounds can be found to be compatible with other control strategies in IPM at the recommended concentrations, it would mean that there is great potential for the registration of more botanical pesticides in South Africa. Consumers generally prefer organically cultivated fruit on the perception of lower risk to human health. This means that these compounds will provide reassurance to consumers that the produced fruit do not contain toxic residue. The application of these compounds also has minimal health risks compared to synthetic pesticides, which when not used correctly can result in health hazards (Lengai *et al*. 2020). This is especially a problem for small-scale farmers in developing countries. The identified botanical compounds would be most beneficial to all citrus growers when used in a cost-effective push-pull strategy.

Chapter 5,

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