Evaluating the nature and mode of action of deterrents of false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae)

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Abstract

Research on the use of semiochemicals as repellents against agricultural insect pests is still limited. As a result, Dambuza (2022) conducted a study evaluating potential repellents for false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), a key phytosanitary pest of citrus in South Africa. He identified eight deterrents of FCM including two essential oils (EOs) (lavender oil and peppermint oil), two plant crude extracts (marigold and garlic extracts), one fruit (mango) and three chemicals (Delegate, Coragen and Warlock). This study assessed if these compounds were true repellents of FCM. Choice and no-choice repellent trials were conducted in complete darkness in a controlled environment room. In choice trials, gravid FCM females were presented with a choice between untreated (control) and treated (with deterrent solutions or suspensions) artificial oranges in a Y-olfactometer setup. Oviposition and choice behaviours were recorded after 24 hours. In no-choice trials artificial oranges treated with deterrent solutions/suspension were placed into a cage with gravid FCM females for four hours, with oviposition being recorded every hour. Of all the eight compounds tested, only three were true repellents ($P < 0.05$) of FCM, two essential oils (lavender and peppermint) and one chemical (Delegate®). We also identified three odour maskants, two crude plant extracts (garlic and marigold) and one chemical (Warlock®). All the initial tested compounds were also evaluated in the presence of host volatile organic compounds (VOCs) using real fruit. The plant crude extracts were the best performers in the presence of VOCs preventing FCM oviposition the most. Lavender oil and Coragen failed to perform with similar oviposition numbers on real treated and artificial treated fruit. Various essential oils (castor oil, clove bud oil, cypress oil, and sage oil) were also evaluated as potential attractants for female FCM in choice trials. None of the tested essential oils significantly attracted FCM females ($P > 0.05$), castor oil and sage oil showed slight attraction. The efficacy of the true repellents and odour maskants can be further tested in semi-field trials, as they have the potential to be used as standalone treatments or in conjunction with attractants in push-pull systems. Even though there was no significant attraction of FCM females by the tested EOs castor oil and sage oil may hold some promise and require further testing. This study provides a foundation for more botanicals to be evaluated as potential attractants for FCM and development of push-pull systems.

Keywords: False codling moth, Botanicals, Semiochemicals, Repellents and Attractants

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List of Abbreviations

AOs	Artificial Oranges
CA	California
CABI	Centre for Agriculture and Bioscience International
CE	Controlled Environment
CM	Codling Moth
cm	Centimetres
CGA	Citrus Growers' Association
CRI	Citrus Research International
DEET	N, N-Diethyl-meta-toluamide
EFSB	Eggplant Fruit and Shoot Borer
e.g.	Exempli gratia (for example)
EOs	Essential Oils
EPPO	European and Mediterranean Plant Protection Organisation
et al	et alia (and others)
EU	European Union
FCM	False Codling Moth
g	Gram
GLM	Generalised Linear Model
h	Hour(s)
ha	Hectares
i.e.	Id est (namely)
IGR	Insect Growth Regulators
IPM	Integrated Pest Management

IQR	Interquartile Range
L	Litre
LRT	Likelihood Ratio Test
mg	Milligram
ml	Millilitre
mm	Millimetre
MRL	Maximum Residue Level
NPV	Nucleopolyhedrovirus
OR	Oregon
RH	Relative Humidity
SIT	Sterile Insect Technique
SS220	1S,2'S)-2-methylpiperidinyl-3-cyclohexene-1-carboxamide
UAE	Ultrasound-Assisted Extraction
USA	United States of America
USDA	United States Department of Agriculture
VOCs	Volatile Organic Compounds
&	And
%	Percentage
°C	Degree Celsius
<	Less than
>	Greater than
±	Standard error
×	Times by

Chapter 1: General Introduction

1.1 CITRUS IN SOUTH AFRICA

1.1.1 Citrus Taxonomy

Until the 1970s, citrus taxonomists based their classifications and identification of citrus fruit only on morphological and geographical data (Moore 2001). This led to many disagreements regarding the classification of species within the subgenus *Citrus* (Moore 2001). Swingle (1943) described a taxonomic system for *Citrus* where about 10 species were accepted. Later, Tanaka (1954) was able to describe 147 different species. The Swingle and Tanaka systems differed in how mandarins were treated (Moore 2001). Swingle (1943) placed all mandarins, except *C. tachibana* and *C. indica*, as *C. reticulata*, whereas in the Tanaka (1954) system, mandarins were classified into 36 species (Moore 2001). Therefore, the difference in the number of species recognised in the two systems made it difficult to know what differences constituted a species.

Modern techniques (chemical classification and genetic analyses) and technological advancements have been instrumental in solving the confusion in citrus taxonomy (Wu et al. 2018). It is now generally agreed that the genus *Citrus* consists of 10 fundamental species: *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), Mandarin *C. reticulata* (Blanco), Citron *C. medica* (L.) and Pummelo *C. maxima* (Merril) (Moore 2001, Wu et al. 2018). Therefore, all the other species come from hybrids, cultivars, or variants. Sweet oranges such as Navels, Valencia and Midknight oranges are more common is South Africa.

1.1.2 Citrus production and trade: Economic Importance

The agricultural sector plays a crucial role in South Africa's economy, as it provides jobs for thousands of people and generates considerable foreign income through exports. In South

Africa, the biggest fruit-producing industry is the citrus industry, with South Africa being the second largest citrus exporter in the world, behind Spain. Citrus produced in the country are oranges (Valencia and Navel), lemons, mandarins (soft citrus), grapefruit, and limes (Citrus Growers Association (CGA) Industry Statistics 2022). The production areas are Limpopo (40 383 ha), Eastern Cape (25 226 ha), Western Cape (18 952 ha), Mpumalanga (7 877 ha), KwaZulu-Natal (2 440 ha), Northern Cape (1 838 ha), North West (822 ha) and Free State (12 ha), accounting for a total area of 97 550 ha of citrus (CGA Industry Statistics 2022). Due to variations in climatic conditions across these production areas, different cultivars, which mature at different times of the year, are produced. In the provinces considered to be cooler, Western Cape and Eastern Cape, production is focused on growing Navel oranges and lemons, as well as easy-peeling cultivars like clementines and satsumas (Mather 2003, Mather & Greenberg 2003). In the warmer provinces like Mpumalanga, KwaZulu-Natal, and Limpopo, production is mainly focused on grapefruit and Valencia oranges (Mather 2003).

Approximately 77% of the fruit produced is destined for exports, with 18% of the fruit being processed and 5% supplied to the local market (CGA Industry Statistics 2022). The major consumers of South Africa's citrus are Europe (34%) followed by the Middle East (18%), South East Asia (14%), the United Kingdom (10%), the Russian Federation (8%), North America (8%) and Asia (7%) (CGA Annual Report 2022). Africa and other smaller islands only account for about 1% of the total exports (CGA Industry Statistics 2022). Exports generate most of the revenue in citrus farming, to which citrus pests can pose a challenge. International markets have regulations to protect themselves against the potential incursion of phytosanitary pests, including those associated with citrus (Moore & Manrakhan 2022). These regulations aim to make sure that citrus farmers produce high-quality fruit, free of any pests and diseases, in an environmentally friendly manner (using fewer chemicals); therefore, it is necessary to find alternative ways to achieve this (Goble et al. 2011, Malan et al. 2018).

1.1.3 Important citrus pests

Citrus pests pose a challenge to citrus production and trade on a global scale. In South Africa, over 100 species of insect and mite pests have been reported to be associated with citrus production; however, not all are considered major pests, with some only reaching pest status when conditions are favourable (Peña et al. 2002, Moore & Manrakhan 2022). The most

important citrus pests are hemipterans (e.g., scales, aphids), and another group of important citrus pests are lepidopteran pests (e.g., fruit boring and piercing moths, leaf miners, and leafrollers) (Peña et al. 2002). Other pests such as flies, mites, and thrips can also cause damage (Peña et al. 2002).

Pests can be categorised as cosmetic pests, production pests, phytosanitary pests and disease vectors, based on the type of damage they cause and how severe the damage is (Peña et al. 2002, Moore & Manrakhan 2022). The category of most importance for an export crop is pests that constrain global trade due to their phytosanitary status and consequent quarantine regulations (Moore & Manrakhan 2022). The most important pests of citrus in South Africa include the Natal fruit fly, *Ceratitis rosa* (Karsch) (Diptera: Tephritidae), Mediterranean fruit fly, *C. capitata* (Wiedemann) (Diptera: Tephritidae), California red scale, *Aonidiella aurantia* (Hemiptera: Diaspididae), citrus mealybug, *Planococcus citri* (Hemiptera: Peseudococcidae), citrus thrips, *Scirtothrips aurantii* (Thysanoptera: Thripidae), African citrus triozid, *Trioza erytreae* Del Guercio (Hemiptera: Triozidae) and the false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (Grout & Moore 2015, Aidoo et al. 2021, Moore & Manrakhan 2022), which is the topic of this thesis. Pests can cause direct and indirect losses for the industry. Direct loss occurs, for example, when FCM lay eggs on the fruit, and once the eggs hatch, the larvae bore into the fruit, causing the fruit to rot from the inside and drop prematurely from the trees (Goble et al. 2011). Indirect loss occurs from the phytosanitary restrictions put in place by importing countries, to prevent the introduction and establishment of these pests in destination countries (Moore 2021).

1.1.4 Control of citrus pests

In the early days of the citrus industry, the control of citrus pests relied heavily on the use of chemical pesticides. Most of these were deemed to be safe for humans because of their short residual action (Bedford et al. 1998). These early insecticides included resin wash, lime sulphur, sulphur dust, nicotine sulphate, miscible petroleum oils, oil emulsions, cryolite and tartar emetic plus sugar (Bedford et al. 1998). Oil sprays had to be applied with extra caution due to concern for their effect on the yield and quality of citrus crops (Bedford et al. 1998). From about 1948 onwards, more emphasis was placed on the use of chemical control, with the aforementioned compounds being largely replaced by the organophosphate, parathion

(Bedford et al. 1998). Even though the use of parathion was effective for controlling pests for some time, it was detrimental to beneficial insects and led to the outbreak of new (secondary) pests (Bedford et al. 1998). Even insects that were not previously considered pests suddenly acquired the status of major pests, due to the overuse of parathion (Bedford et al. 1998). In the 1970s, citrus thrips became more prevalent, and parathion was considered no longer effective, particularly against its primary target, red scale, *Aonidiella aurantii* (Hemiptera: Diaspididae). This drove the movement to use new insecticides such as temephos, dimethoate and monocrotophos, albeit also organophosphates (Bedford et al. 1998). Even the new insecticides had their own problems as they led to outbreaks of pests such as red scale and the development of insect resistance against these pesticides (Bedford et al. 1998). In about 1975, red scale became resistant to parathion and forced growers to move towards the use of integrated pest management (IPM) programmes (Bedford et al. 1998). In 1988, the first synthetic pyrethroids were registered for the control of citrus thrips. They were very effective for some time until concerns about red scale outbreaks arose (Bedford et al. 1998). This was despite the use of oil sprays and insect growth regulators (IGRs) to prevent the outbreaks; therefore, growers had to turn to suitable IPM programmes to try and suppress the pests (Bedford et al. 1998). The factors that promoted IPM were the responsibility of growers to the environment, increasing resistance of pests to pesticides and the requirements of international markets with respect to chemical residue regulations (Smith 2023). IPM programmes have been very successful in the South African citrus industry. This is because IPM is cost-effective and incorporates a number of control techniques to suppress pest populations in an ecologically sound manner (Smith 2023). This approach integrates biological control (e.g., parasitoids), cultural practices (e.g., orchard sanitation), behavioural control (e.g., mating disruption), genetic (e.g., sterile insect technique (SIT)) and chemical control (Smith 2023).

This study will focus on FCM because of its strict control measures and phytosanitary regulations especially on fruit destined for the European market. If one moth is found in an export consignment, it results in the whole consignment being rejected as there is zero tolerance for the pest in this market (Goble et al. 2011, Moore & Manrakhan 2022).

1.2 FALSE CODLING MOTH

1.2.1 Classification and Taxonomy

FCM belongs to the order Lepidoptera and the family Tortricidae. In South Africa, FCM was first discovered and described in the KwaZulu-Natal Province by Fuller in 1901 (de Jager 2013). It was named the 'Natal codling moth' and called *Carpocapsa* sp. (Stotter 2009, de Jager 2013). In 1932, FCM was described as *Argyroploce leucotreta* (Eucosmidae: Olethreutidae) by Meyrick (1912) and then later in 1958, it was transferred to the genus *Cryptophlebia* by Clarke (Stotter 2009, Gilligan et al. 2011, de Jager 2013). Komai (1999) then moved *leucotreta* and some of its relatives from *Cryptophlebia* and placed them in the genus *Thaumatotibia*, which was previously a synonym of *Cryptophlebia* (Stotter 2009, Gilligan et al. 2011).

1.2.2 Host Range and Distribution

FCM is endemic to Africa and is distributed throughout sub-Saharan Africa, where it is mostly localised to warm tropical and sub-tropical regions (Gilligan et al. 2011). This moth has been reported to exist in approximately 40 African countries and some islands, including Madagascar, St. Helena, Mauritius, Cape Verde, and Reunion (Gilligan et al. 2011). FCM has established in Israel, but its distribution and host range are still fairly limited (Wysoki 1986, Gilligan et al. 2011). It has also been intercepted in the United States multiple times, but these have been solitary interceptions, and it has failed to establish a population (Gilligan et al. 2011).

FCM is a highly polyphagous pest, recorded on 106 plant species belonging to many different families. The pest status of FCM may differ in certain crops and different regions (Gilligan et al. 2011, Grout & Moore 2015, Carstens & Moore 2020). The larvae of this pest feed primarily on fruit. In Africa, FCM is known to attack citrus (*Citrus* spp.) and is also a pest of avocado, *Persea americana* (Mill) (Laurales: Lauraceae) (Grové et al. 1999, 2010, Gilligan et al. 2011). However, it has also been recorded to cause damage to macadamia, *Macadamia integrifolia* (Maiden & Betche) (Proteales: Proteaceae), and many other horticultural crops.

1.2.3 Life Cycle and Life History

The life cycle of FCM varies between five weeks to three months, depending on environmental factors such as temperature and humidity (Daiber 1980). The females lay eggs on the rind of

the fruit and after a few days, neonate larvae hatch. The larvae then bore into the fruit, which creates an entry point for secondary infections. The development of all five instars occurs inside the fruit and once the fifth instar is ready to pupate, it drops to the ground and pupates in the top layer of the soil, leaf litter and bark crevices. The moths then emerge after a couple of days. There are about six overlapping generations in a year (Daiber 1980, Moore 2002). What sets FCM apart from certain other lepidopteran pests is that it has no diapause and is active throughout the year.

A FCM female oviposits about 100-400 eggs in its lifetime. The eggs are small (< 1 mm diameter), have a flat oval shape and are initially cream-coloured, turning red and brown as they develop (Figure 1.1a) (Daiber 1979a). The duration of the egg stage is climate dependent, between 9-12 days and 6-8 days in winter and summer, respectively (Daiber 1979b). The larva develops through five instars (Daiber 1979b). The early instars have a pale appearance, while the fifth instar has a brownish-pink appearance (Figure 1.1b) (Moore 2002). The larval stage is completed between 25-35 days in the summer and can take between 35-67 days in winter. The larval instars complete their development within the fruit. After the last instar, the larva then drops to the ground to pupate (Moore 2002). The pupae appear dark brown (Figure 1.1c) (Daiber 1979c). This stage takes between 12-24 days in summer and between 29-40 days in winter (Daiber 1979c). The last stage is the adult stage; this stage lives for between 2-3 weeks (Daiber 1980). FCM adults are small, brown to greyish in colour with mottled forewings and grey hind wings (Moore 2002). The females usually eclose a day earlier than males and are bigger than their male counterparts (de Jager 2013). FCM males are distinguished from females by a tuft of hair on their anus and hind wings (Figure 1.2e), which females lack (Figure 1.2d) (Daiber 1980). The males also possess a tuft of elongated scales on their hind tibia (Daiber 1980).

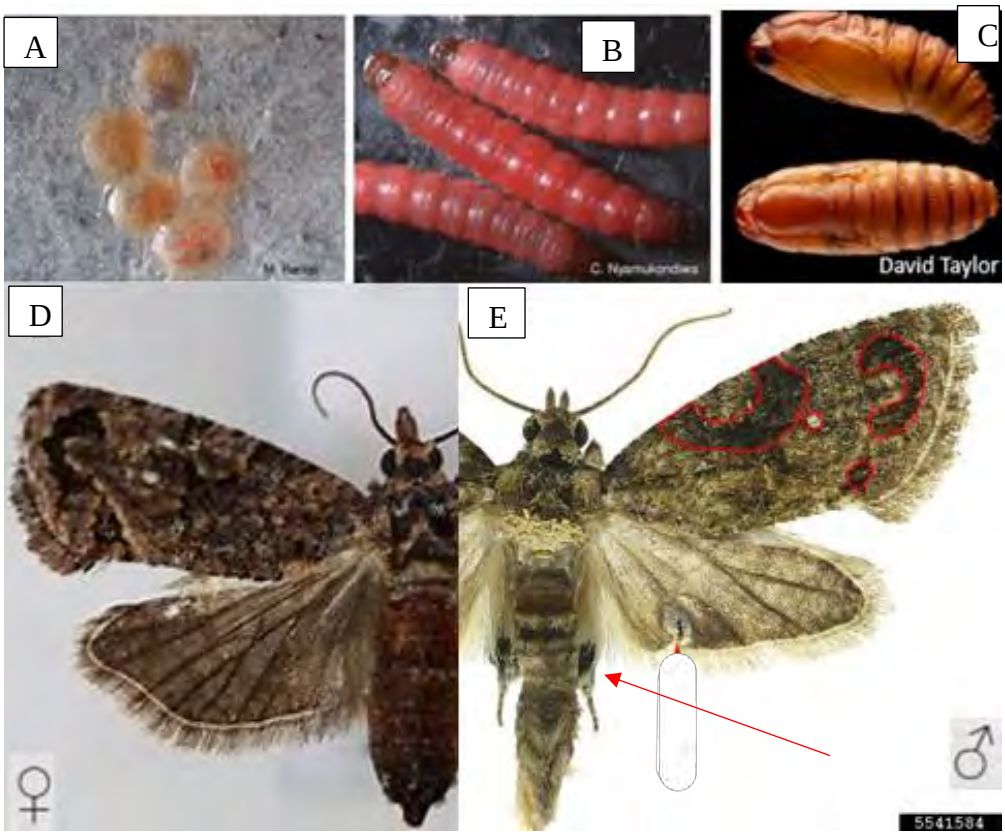

Figure 1.1: (a) FCM eggs. (b) FCM fifth instar, (c) FCM pupae, (d) FCM adult female without tufts on hindleg and abdomen and (e) FCM adult male with tufts on hindleg (indicated by the red arrow) and abdomen.

1.2.4 Economic impact

FCM has quarantine pest status for several export markets (Moore & Manrakhan 2022). If found in a consignment destined for exports it could cause the whole shipment to be rejected; this is a major concern for citrus growers and the industry as a whole (Adom et al. 2021, Moore & Manrakhan 2022). FCM is a major pest of citrus, and it has been reported that although losses are usually less than 2%, they have in the past been as high as 50% in the field, but such losses are now rare as this pest is very well controlled (Moore 2002, Moore et al. 2017). This is because larvae feed on the fruit, damaging and rendering them useless for trade, as fruit usually drop when infested, reducing yield (Moore 2002). This pest is of great economic importance as it is classified as a quarantine pest in European and Mediterranean Plant Protection Organization (EPPO) by the European Commission for Plant Health Regulation and other markets of importance, such as the People's Republic of China, South Korea and the

U.S.A (Moore et al. 2015, Adom et al. 2021). This causes major concern for the agricultural sector, since the majority of the income is generated from exports (Adom et al. 2021). Therefore, due to the importance of FCM, it is crucial that we continue to look for alternative methods for controlling this pest should the existing techniques stop working. Most citrus cultivars are susceptible to attack by FCM, with the exclusion of lemons and limes (Moore 2002, Moore et al. 2015).

1.2.5 Control Strategies

Due to FCM being a quarantine pest, countries to which South Africa exports its fruit have no tolerance for this pest. Most such markets require that the fruit destined for export be disinfected using a cold treatment (Hofmeyr et al. 2015, Hattingh et al. 2020). This entails keeping temperatures below -0.3°C for 22 days (Hattingh et al. 2020). In the field, growers have historically relied on various insecticides to suppress FCM populations, using pyrethroids in the early 1980s and later moving to the use of insect growth regulators (IGRs) (Hofmeyr et al. 2015). Over time, due to overuse of these chemicals, FCM populations developed resistance, particularly to IGRs, making the insecticides less effective. Regulations on chemical residues have also become stricter (Stotter 2009, Hofmeyr et al. 2015). It is also not possible to target FCM larvae that are already within a fruit with insecticides (Stotter 2009). As a result, citrus growers employ an integrated approach that includes a range of management tools (Stotter 2009). Integrated pest management (IPM) is an approach that involves the use of complimentary ecologically friendly techniques in conjunction with chemicals to control FCM (Malan et al. 2018). The aim of this approach is to keep pest populations below a certain predetermined economic threshold (ET) level (Malan et al. 2018). The IPM plan includes pre-harvest and post-harvest techniques. Pre-harvest FCM control involves methods such as monitoring, cultural control (orchard sanitation), genetic control (sterile insect technique (SIT)), biological control (use of naturally occurring enemies), chemical control, and behavioural control (mating disruption) (Malan et al. 2018, Moore 2021, Smith 2023). Behavioural control and monitoring may involve the use of repellent (not yet used in FCM control) and attractant semiochemicals (El-Shafie & Faleiro 2017). Semiochemicals are biological compounds used by living organisms to send specific chemical messages that modify behaviour and physiology (Smart et al. 2014, El-Shafie & Faleiro 2017). The individual that is releasing the semiochemical is called the ‘emitter’, and the individual that receives and

responds to the chemical message is called the ‘receiver’ (Smart et al. 2014). Insects use semiochemicals to locate food and hosts, escape enemies and overcome natural defence systems of their host (El-Shafie & Faleiro 2017).

1.3 SEMIOCHEMICALS

1.3.1 Classification

Semiochemicals are organic compounds that insects and plants use to send specific chemical messages, these messages modify the behaviour or physiology of other species (El-Shafie & Faleiro 2017). Semiochemicals are divided into two groups, pheromones and allelochemicals, according to their effect or function (El-Shafie & Faleiro 2017). The same molecule could act as a pheromone for a species of insect and be an allelochemical (kairomone or allomone) for another (El-Shafie & Faleiro 2017). Pheromones include alarm, sex and aggregation signals, and are used for communication between individuals of the same species (intraspecific) (Smart et al. 2014, El-Shafie & Faleiro 2017). Pheromones are further divided into primer pheromones which have long-term physical changes and releaser pheromones which bring about short-term or immediate changes in behaviour (El-Shafie & Faleiro 2017). Allelochemicals are used to facilitate communication between different species (interspecific) and consist of kairomones, allomones and, synomones, which are signals that benefit the receiver, emitter and both the emitter and the receiver, respectively (Smart et al. 2014). Apneumones are also considered allelochemicals. These compounds are produced by non-living material that benefit the receiver but are harmful to a different living species associated with the non-living emitter (Smart et al. 2014, El-Shafie & Faleiro 2017).

1.3.2 Identification of semiochemicals

Identification of semiochemicals which insects can detect and react to is very important in understanding the role of olfaction in altering insect behaviour (El-Shafie & Faleiro 2017). Identifying semiochemicals involves extraction or headspace collection, identification of active compounds, the determination of the chemical makeup of the found compound and the elucidation of the insect’s behaviour to the found compound (El-Shafie & Faleiro 2017). In cases where organs that produce useful compounds are known, the compound is extracted and then identified (El-Shafie & Faleiro 2017), however, where undesired compounds are

extracted, the identification of the desired compounds is complicated (El-Shafie & Faleiro 2017).

1.3.3 Role of semiochemicals in pest management

Semiochemicals are used as part of an IPM plan to suppress pest populations. In IPM, it is important that strong plumes of the correct blend of pheromones are used to create above-threshold plume strands for proper downwind application of the pheromone source (El-Shafie & Faleiro 2017). Therefore, pheromone dispensers that mimic natural release by insect pests are usually used to achieve efficacy together with optimisation of trap density, design and positioning of the trap (El-Shafie & Faleiro 2017). Semiochemicals have been used in pest management for many years and many agricultural pests have been or are successfully managed using semiochemicals (pheromones), including Asian citrus psyllid, *Diaphorina citri*, tomato leaf miner *Phthorimaea absoluta*, carob moth, *Ectomyelois ceratoniae* and more (El-Shafie & Faleiro 2017). Semiochemicals are preferred to synthetic chemicals, because they are considered to be environmentally safe and are very species-specific, rendering them harmless to non-target species (El-Shafie & Faleiro 2017). Semiochemical based techniques are negatively density dependent and include attract and kill, mating disruption, mass trapping and repellents.

1.3.3.1 Attract and Kill

This technique entails the use of semiochemicals or attractants to lure the targeted insect pest to a source that contains a killing agent (El-Shafie & Faleiro 2017). The killing agent can either be an insecticide, pathogen or sterilant. This technique is aimed at reducing the fitness and fecundity of the target insect, or killing it, and it is used against FCM (El-Shafie & Faleiro 2017, Moore 2021). As stated above, this technique is density-dependent, and is, therefore, more effective against low to moderate population numbers, as it reduces mating success. In South Africa, there is currently one product that is commercially available for the attract and kill method against FCM, with the name Last Call FCM (Insect Science, Tzaneen, South Africa), and targets male moths (Moore 2021). This product is made up of a combination of

synthesised female pheromones and pyrethroid, in the form of a gel (Moore 2021). Alone it is usually not adequately effective, so it should be used in an IPM programme.

1.3.3.2 Mating disruption

The aim of this method is to modify insect behaviour to reduce its population size. This is achieved by flooding the area where the target insect is present, with synthetic sex pheromones that stop males from finding females and mating with them (El-Shafie & Faleiro 2017). Mating disruption does not completely prevent successful mating in pests but rather reduces and delays it. For example, a delay in males locating females with which to mate, can reduce their fecundity by up to 50% as females have a critical period in which to mate and reproduce (El-Shafie & Faleiro 2017). Mating disruption can either be competitive or non-competitive (desensitisation). Competitive mating disruption is the mechanism by which males respond to synthetic pheromone plumes produced by semiochemical dispensers rather than ones that are released by actual females. The efficacy of this mechanism decreases with increasing insect populations (density-dependent) (El-Shafie & Faleiro 2017, Moore 2021). Desensitisation occurs due to overexposure of insects to pheromones or semiochemicals in an environment i.e. the male olfactory receptor system gets habituated (El-Shafie & Faleiro 2017, Moore 2021). In South Africa mating disruption was first introduced in the 1990s (Moore 2021). At this time, the products were based on a pheromone extracted from the oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), and the level of efficacy against FCM was not sufficient for it to be commercialised (Moore 2021). In other trials that were conducted using Isomate (Shin-Etsu, Japan) (based on the FCM pheromone), in accordance with other semiochemical based techniques, mating disruption was more effective when pest population density was low (about 55% reduction in infestation in areas with high pest abundance and 75% in areas with low pest abundance) between December and the end of April (Moore 2021). When initiated in October rather than November, a 51% to 80% reduction in infestation was recorded, respectively (Moore 2021). Currently, the products available for mating disruption of FCM are: Isomate FCM (Shin-Etsu, Japan), Splat-FCM (ISCA Technologies, Riverside, CA, USA), Checkmate FCM-F (Suttera LLC., Bend, OR, USA), and X-Mate (BASF, Ludwigshafen, Germany) (Moore 2021).

1.3.3.3 Mass Trapping

This technique involves deploying high densities of pheromone traps to eliminate a high number of insects and thus reduce larval damage (El-Shafie & Faleiro 2017). This technique is effective in cases where male-emitted and female-emitted pheromones are released and attract females and males into traps (El-Sayed et al. 2006, El-Shafie & Faleiro 2017). This is often used for insects such as weevils and beetles. The trapping of females and males directly reduces egg-laying and mating (El-Sayed et al. 2006, El-Shafie & Faleiro 2017). No such technology is currently used for FCM.

1.3.3.4 Repellents

The term repellent comes from the Latin verb *repellere*, which means to reject (Deletre et al. 2016). A substance only qualifies as a repellent if it causes an organism to move away from an odour source or prevents the pest from locating and recognising its host (Nerio et al. 2010, Diaz 2016, Deletre et al. 2016). Insect repellents can be derived from three sources, namely plants, insects and synthetic compounds. Plants use physical or chemical defence mechanisms, they have organs or structures that they use to keep their enemies away, such as trichomes in *Urtica,* and via the emission of volatile organic compounds (Howe & Jander 2008, Mithöfer & Boland 2012, Deletre et al. 2016). A study by Khan et al. (1997) showed that stem borer infestations were higher in mono-cropped maize fields than those which were intercropped with Sudan grass, *Sorghum sudanense* (Stapf) (Poales: Poaceae), due to the attraction and trapping of the stem borer by the grass (Khan et al. 1997, Midega et al. 2015). Molasses grass, *Melinis minutiflora* (Beauv) (Poales: Poaceae), was found to be an oviposition deterrent and used as a repellent of stem borers in push-pull strategies after it was confirmed that it emitted semiochemicals (ocimene and nonatriene) that pushed these insects away (Khan et al. 1997, 2000, Midega et al. 2015). Studies show that molasses grass reduces stem borer infestation by up to 80% when intercropped with maize (Khan et al. 2000). These grasses also provided a habitat for a variety of parasitoids of *Chilo partellus* (C. Swinhoe) (Lepidoptera: Crambidae) and *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), further reducing stem borer infestations in maize fields (Khan et al. 1997, 2000, Midega et al. 2015). Insects produce repellents in the form of semiochemical excretions, such as allomones and alarm pheromones (Deletre et al. 2016, Kirk et al. 2021). Thrips from the family Phlacothripidae produce a variety of species-

specific droplets (allomones) that act as repellents or deterrents and have antifungal properties. For example, when little fire ants, *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae) come into contact with droplets from *Gynaikothrips ficorum* (Marchal) (Thysanoptera: Phlaeothripidae), they are repelled and can be attacked by worker ants (Kirk et al. 2021). Another example is that of aphids, when they are threatened by disturbances and predation, they secrete droplets of a defence or alarm pheromone, the function of which is to repel conspecifics and alert them of danger (Verheggen et al. 2010). All of these semiochemicals can be exploited in pest management as repellents of these species. Synthetic compounds are manufactured by humans rather than nature (Deletre et al. 2016). They are manufactured to serve specific purposes such as repellence, as antifeedants or irritants. Yoon et al. (2014), using 24% DEET (N,N-Diethyl-meta-toluamide), a common active ingredient that is used in many insect repellents, demonstrated that DEET significantly repelled Asian tiger mosquitoes, *Aedes albopictus* (Skuse) (Diptera: Culicidae) and had a repellence of over 90% (Fradin 1998, Dogan et al. 1999, Logan et al. 2010, Yoon et al. 2014). Five different types of repellents can be identified, based on the change in behaviour that they induce in the target organism: (1) true repellents, (2) odour masking, (3) contact irritancy, (4) deterrence, and (5) visual masking (Chinthaka et al. 2023).

In medical entomology, a substance that induces an organism's movement away from its preferred host is called an expellent or a true repellent (Deletre et al. 2013, 2016, Kirk et al. 2021). These are used to manage insect pests by creating a barrier preventing the pest from entering a space occupied by their preferred host (Nerio et al. 2010, Deletre et al. 2016). Therefore, true repellents reduce the chance of an encounter between a pest and its host. True repellents can be identified by testing them using bioassays that prevent contact between the insect, and changes in the environment that affect insect behaviour (stimulus) (Deletre et al. 2016). Testing true repellency occurs in the absence of the host as the presence of the host may have a masking effect and disturb the behaviour of the insect (Deletre et al. 2016). Repellence is mostly studied using a tubular olfactometer system, as has been done for pests of veterinary importance and medical entomology (Deletre et al. 2016). Coriander, *Coriandrum sativum* L. (Apiaceae) essential oil, is an example of a true repellent of *A. albopictus* adults, which vector chikungunya virus and dengue virus (Benelli et al. 2013, 2014). Coriander was as effective as DEET when tested at the same dosages and at lower dosages (Benelli et al. 2013, 2014). This essential oil repelled more than 50% of mosquitoes for at least 75 minutes, with 100%

repellency observed after 60 minutes when the highest concentration was used (Benelli et al. 2013).

Odour masking is when attraction by the host is inhibited by a certain odour or compound. This reduces the attractiveness of the host and interferes with the ability of the insect to locate the host using olfactory cues (Deletre et al. 2016). Odour masking agents change the host chemical, therefore making it less attractive and disturbing the seeking ability of the pest (Deletre et al. 2016). The Silverleaf whitefly, *Bemisia tabaci* (Gennadius) biotype B (Hemiptera: Aleyrodidae) is a polyphagous pest of tomato, *Solanum lycopersicum* L. (Solanaceae); this pest also vectors viruses to the plant and may result in up to 100% crop losses (Togni et al. 2010). A study by Togni et al. (2010), showed that when *C. sativum* is intercropped with tomato, the attractiveness of tomato to the females of the pest is reduced significantly. The negative stimulus from coriander, which is not a host of this pest, makes it difficult for the insect pest to locate its host (Togni et al. 2010). More adults and nymphs of *B. tabaci* were found on tomato plants that were monocropped than those that were intercropped with coriander, suggesting that coriander masked the attractiveness of tomato volatiles (Togni et al. 2010). During this study, repellency by coriander on *B. tabaci* was not observed. Therefore, in pest management, odour masking agents are used to hide preferred hosts from their pests. Masking effects should be evaluated by using a bioassay that prevents contact between the insect and the stimulus and with the host volatile (Deletre et al. 2016).

When an insect pest makes a movement away from the stimulus after contact occurred, this phenomenon is termed an irritant (Grieco et al. 2005, Deletre et al. 2016). This is applied in order to break physical contact between insects and hosts (Deletre et al. 2016). It reduces the chance of the insect feeding on its host, prevents oviposition and prevents pathogen transmission (Deletre et al. 2016). The effectiveness of an irritant is tested in an arena where contact between the insect pest and the tested product is allowed, the natural host is not included (Grieco et al. 2005, Deletre et al. 2016). The compound irritancy is usually determined in trials by measuring the time an insect takes in contact with treated material before moving away (Deletre et al. 2016). For example, *Aedes aegypti* mosquitoes were shown to have contact irritancy response to SS220 ((1S,2'S)-2-methylpiperidinyl-3-cyclohexene-1-carboxamide), a synthetic repellent developed by the U.S. Department of Agriculture (USDA) (Grieco et al. 2005).

Compounds that disturb or prevent feeding behaviour are called deterrents or antifeedants (Griffiths et al. 1991, Koul 2008, Deletre et al. 2016). These can cause the change in behaviour either after having fed on the host or in the moment of contact with the host (Koul 2008, Deletre et al. 2016). Feeding on the host is inhibited, but the pest does not necessarily move away from its host. Deterrents impede continued feeding by insect pests on hosts (Deletre et al. 2016). This can be tested in the presence of both the test compound and the host. Fruit extract of the Argentinian Chinaberry tree, *Melia azedarach* L. (Meliaceae), has been shown to act as an antifeedant to various pest species, the most sensitive ones being coleopterans, such as *Epilachna paenulata* Germ. (Coleoptera: Coccinellidae), which is a pest of squash and melons. It can inhibit up to 75% of feeding activity (Carpinella et al. 2003).

Some compounds or objects prevent the insect pest from visually locating its host. This is called visual masking (Deletre et al. 2016). The aim of visual masking in pest management is to disrupt the recognition of the host by its pest (Deletre et al. 2016). This is done using a visual stimulant together with a trap or insecticide to kill the pest (Deletre et al. 2016). Visual response of insects involves colour vision (Gibson & Torr 1999). In a study conducted using tsetse flies, *Glossina* spp., it was discovered that these biting flies are visually attracted to the colour phthalogen blue and prefer landing on black, therefore traps are phthalogen blue on the outside to attract the flies away from their preferred host and black on the inside for landing (Gibson & Torr 1999).

In the context of this study, repellents are botanicals, derived as secondary metabolites from plants. They are derived from plant parts such as flowers, cloves, rhizomes, leaves, barks, stems, roots, seeds and fruits (Lengai et al. 2020). Botanicals are an alternative to synthetic insecticides for the management of pests and are an attractive option as such because they represent a large reservoir of chemical structures with pesticidal activity, do not harm non-target species, and are less phytotoxic (Guleria & Tiku 2009, Lengai et al. 2020). Plant secondary metabolites include phenolics, terpenes, alkaloids, lignans and glycosides, which play an important role in plant defence systems against herbivores and phytophagous insects. These molecules can be developed to serve as new pest control agents (Guleria & Tiku 2009). Development of a botanical pesticide requires knowledge of which pests a plant is resistant to and the defence compounds produced (plant-insect interactions) (Guleria & Tiku 2009, Ikeura et al. 2013). Ikeura et al. (2013), successfully showed how neem seed kernel oil cake significantly repelled cabbage moth (*Mamestra brassicae* L.) larvae from feeding on

komatsuna (*Brassica rapa* var. *peruviridis*) and spinach (*Spinacia oleracea* L.) in laboratory and field trials (Ikeura et al. 2013). The feeding percentage of the larvae on neem seed kernel oil cake treatments was 52%, 70% and 88% one day, two days and seven days post-exposure, respectively. This was lower than that afforded by the chemical, which recorded feeding percentages of 67%, 97% and 100% one, two and seven days post-exposure, respectively. (Ikeura et al. 2013).

Botanical use in agriculture dates back more than 150 years before the major classes of synthetic chemicals existed in Europe and North America and about two thousand years ago in China, Egypt, India and Greece (Isman 2006). Since their discovery, synthetic chemicals have become more prominently used in agricultural pest management, due to their efficacy, and farmers moved away from using botanicals (Isman 2006). Because of the overuse of synthetic chemicals many problems developed, such as pest resistance, poisoning of applicators, farmworkers and even consumers (Isman 2006, Guleria & Tiku 2009, Khater 2015, Jhala et al. 2019). Synthetic chemicals also accumulate in the environment as they are not easily biodegradable, causing groundwater and soil pollution (Lengai et al. 2020). Therefore, some of these were deemed unsafe for the environment and humans; they also killed non-target species (endangering biodiversity), which was problematic as some of these species were crucial for the functioning of the ecosystem (Isman 2006, Khater 2015, Lengai et al. 2020). Due to these unforeseen issues, governments responded by putting regulations in place and restricting the use of synthetic chemicals (Isman 2006). Alternative pest management products with reduced health and environmental impacts were, therefore, sought (Isman 2006). Some of these were insecticides derived from plants such as Pyrethrum (*Tanacetum cinerariifolium*), neem (*Azadirachta indica*), and essential oils, which have been developed into insecticidal products and are increasingly used in IPM. (Lengai et al. 2020).

However, repellents are rarely used on a practical level, due to the constant availability of cheaper effective alternatives, sustainability, standardisation, inadequate formulations for delivery and challenges, such as regulations on registration (Isman 2006, El-Shafie & Faleiro 2017). They can either be used alone or in combination with an attractant as part of a push-pull strategy (El-Shafie & Faleiro 2017, Souto et al. 2021). This requires an understanding of the chemical ecology of the insect pest (El-Shafie & Faleiro 2017, Souto et al. 2021).

This study focused more on potential repellents and their use against agricultural pests, particularly FCM. An example of a repellent is verbenone, which is now commercially

available for the control of the mountain pine beetle (*Dendoctonus ponderosae*) (El-Shafie & Faleiro 2017). The use of repellents against agricultural pests is still limited. Repellents have been exploited more in medical and veterinary entomology. Saini *et al*. (2017) investigated the potential for the use of chemical odours from animals that are not fed on by tsetse flies, *Glossina* spp. (Diptera: Glossinidae), as potential repellents (Saini et al. 2017, Olaide et al. 2019). They used the waterbuck (*Kobus ellipsisprymnus defassa*) Ruppel (Bovidae), which is known to live in tsetse habitats, but is not infected by nagana, transmitted by tsetse flies, even though they belong to the same family group as cattle, which are usually affected (Saini et al. 2017). The active compounds of this repellent were geranylacetone, pentanoic acid, guaiacol and δ-octalactone. These were applied to cattle by use of neck collars, worn by the cattle. The results showed that the waterbuck repellent reduced nagana (trypanosome) infections in cattle by over 80% (Saini et al. 2017). Therefore, the success of the use of repellents in medical and veterinary entomology shows that there is potential for the exploitation of repellents for the control of agricultural plant pests.

Studies conducted on potential repellents of FCM are limited. Dambuza (2022) tested oviposition repellence of plants, essential oils and some pesticides registered for FCM control. An extensive literature review was done to identify all the plants and essential oils that have been reported to have a repellent effect against lepidopteran pests. Thirty-three products were found to have a repellent effect. These were then further tested and only eight significantly showed potential repellence to FCM oviposition. Of the eight products, two were essential oils (lavender & peppermint), two were plant crude extracts (garlic & marigold), three were synthetic chemicals (Delegate®, Coragen® & Warlock®) and mango fruit. The study was conducted using choice and no-choice trials in darkness in a controlled environment room. During choice trials, gravid FCM females were exposed to potential repellent-treated and untreated oranges. In no-choice trials, FCM gravid females were exposed only to potential repellent-treated oranges. In both trials, the number of eggs oviposited on the fruit was counted. In the no-choice trials conducted by Dambuza (2022), the compounds showed the following percentage reduction oviposition per six fruit relative to the control: garlic- 64.7%, marigold- 64.3%, lavender- 63.1%, peppermint- 63.8%, Delegate- 58.8%, Coragen- 65%, Warlock- 46.8% and mango- 37.6%. In choice trials, these compounds also reduced the number of eggs laid by FCM on the fruit significantly. This means that there is a potential to improve FCM control using repellents, especially plant-based based environmentally safe repellents. Since these products were already tested and evaluated in the laboratory using real fruit, this

presented an opportunity to further investigate the repellence of these compounds to determine if they are true repellents or odour maskants and to continue to better understand the potential role of these compounds against FCM.

1.4 STUDY AIMS

Therefore, this research thesis aimed to: (1) determine if the identified potential repellents are true repellents or are odour masking. In the previous study, true repellence trials were not done, so it is therefore not yet clear if the identified compounds are true repellents or simply just odour masking. (2) Determine the effects of the various compounds on the oviposition of FCM using real (in the presence of VOCs) and artificial fruit; (3) Evaluate potential attractants of FCM for potential use in a push-pull system for FCM control.

Chapter 2: Evaluating the deterrent nature of botanical compounds against FCM

2.1 INTRODUCTION

Botanical pesticides are biologically active mixtures, extracts and compounds from plant material (Pavela 2016). Plants produce secondary metabolites such as phenolics, terpenes, and alkaloids to defend themselves against herbivores (Pavela 2016, Fragkouli et al. 2023). The use of botanicals as repellents exploits these plant defence mechanisms. For example, the use of neem oil, *Azadirachta indica* Juss. (Meliaceae) and pyrethrum, *Chrysanthemum cinerariifolium* (Trevir.) Vis. (Asteraceae), which dates back to ancient civilizations, in the management of insect pests is very well-documented (Pavela 2016, Isman 2020). Botanicals are seen as good alternatives to synthetic pesticides for the management of pests and as part of IPM systems (Grzywacz et al. 2014, Pavela 2016, Insung et al. 2017, Dolma & Reddy 2022). These botanicals can function as either insecticides, bactericides, fungicides, herbicides, nematicides or repellents (Fragkouli et al. 2023). A repellent can be broadly defined as a substance that prevents the herbivore's ability to track, locate and recognise its host and causes movement of the pest away from its host (Deletre et al. 2016). As discussed in the preceding chapter, five types of repellents are known: true repellents, odour maskants, contact irritants, deterrents, and visual maskants. True repellents create a barrier preventing herbivores from flying into, landing on their hosts and cause oriented movement away from the odour source (Nerio et al. 2010), and are of interest in this chapter.

The use of repellents is common in medical and veterinary entomology and is currently a growing field in agricultural entomology, given increasing regulations on the use of synthetic chemicals in pest management programmes (Pimentel 2005). Therefore, there is an increasing need for more environmentally safe control methods (Pimentel 2005). Compared to toxicants and other insecticides, which usually aim to reduce pest populations, repellents work by changing the pest behaviour through olfactory, gustatory and irritant cues, thereby protecting the crop (Isman 2006). For example, Landolt et al. (1999) conducted a study testing several essential oils as repellents of the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), an important pest of deciduous fruit, especially apples. Landolt et al. (1999) showed that eight

essential oils significantly repelled codling moth neonate larvae as they turned away once reaching the treated area on a glass rod treated with a potential repellent. The notable repellents were rue, *Ruta graveolens* L., garlic, *Allium sativum* L. and tansy, *Tanacetum vulgare* L. (Landolt et al. 1999). In the case of an odour maskant, the pest is simply prevented from recognising the odour and locating the host by reducing the attractiveness of the host (Togni et al. 2010). For example, Thiery & Visser (1986), demonstrated that blending non-host plant odours with potato plant, *Solanum tuberosum* L., volatiles prevented orientation response by Colorado potato beetle *Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae) (Thiery & Visser 1986). Therefore, for a compound to be labelled as a true repellent there must be an orientation by the pest away from the odour source.

Dambuza (2022) explored botanicals as deterrents of FCM. He identified eight products, two of which were plant crude extracts (marigold, and garlic), two essential oils (peppermint and lavender), three synthetic chemicals (Coragen®, Delegate®, and Warlock®) and mango fruit that significantly prevented or reduced oviposition by FCM. This chapter focused on further evaluating these compounds said to have a deterrent effect on FCM identified by Dambuza (2022) to determine if these compounds are true repellents or odour masking, as they were not tested for true repellence. Dambuza (2022) referred to these compounds as deterrents only because his experimental setup was not designed to test for true repellence. Real fruit, a natural host of the pest, was used to conduct those experiments. As a result, this study aimed to explore the true repellence of these compounds using artificial fruit. In this study, a mango crude extract was used in place of whole fruit. This is because extracts are more concentrated compared to fruit and are easier to formulate and apply.

2.2 METHODS AND MATERIALS

2.2.1 Insect Rearing

Following the methods by Love et al. (2014), egg sheets and pupae were obtained from a mixed culture (a hybrid population from interbreeding FCM from the Western Cape, Eastern Cape, and Mpumalanga provinces of South Africa) established by Moore (2002). The culture is held at the Zoology and Entomology Department, Rhodes University. The pupae were reared to

adulthood, where the temperature was set to 26°C ± 2°C, 50-60% relative humidity, over a 12/12 h light/dark cycle. The culture is maintained on the diet which was formulated by Moore et al. (2014) and consists of maizemeal, wheat germ, milk powder, brewer's yeast, nipagin, and sorbic acid. The eggs were surface sterilised and placed in jars containing an autoclaved diet that were stoppered with cotton wool. When ready to pupate, the larvae moved into the cotton wool. Individual pupae were removed carefully from the cotton wool and sexed by counting the number of segments on the abdomen. Four segments were indicative of a female: five a male (Peterson 1965). Pupae were placed individually in 30 ml glass vials and allowed to eclose (Figure 2.1) (Love et al. 2014, Mgocheki & Addison 2016). Within 24 h of eclosing, a virgin female and male were paired together in the same vial, which was stoppered with water-moistened cotton wool. The pair were allowed to mate overnight at room temperature in a controlled environment (CE) room with relatively low humidity (RH) of between 20% and 45% before being used in a trial the following day (Love et al. 2014). Only females that oviposited in the vials were used for the repellence trials.

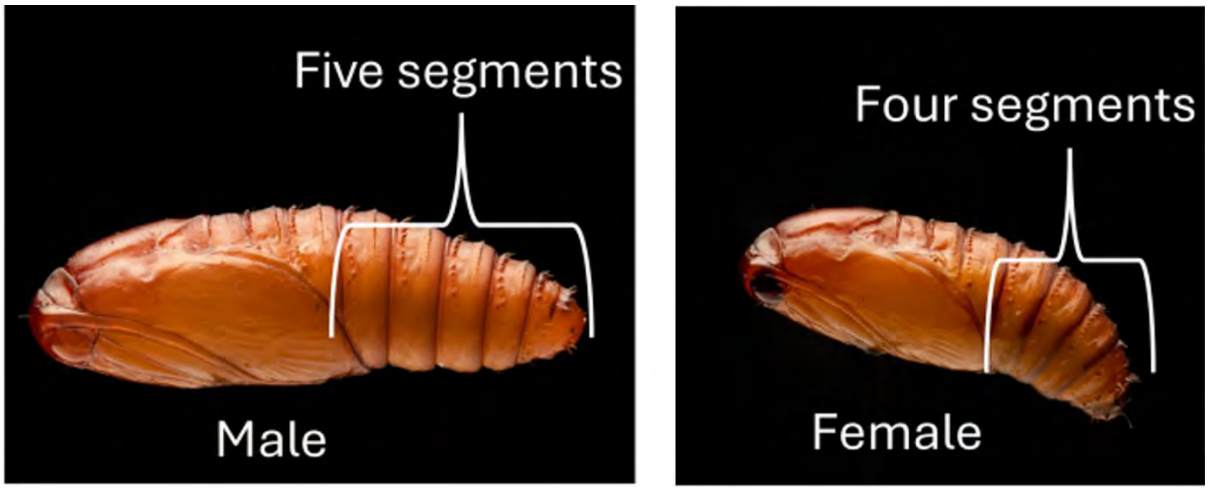

Figure 2.1: Differences in the number of FCM abdominal segments between male (left) and female pupae (right) (Photo credit: David Taylor).

2.2.2 Identification and collection of potential repellents

Eight compounds were evaluated and identified to have a repellent effect on FCM oviposition by Dambuza (2022), including two essential oils, lavender oil (*Lavandula angustifolia*) and peppermint oil (*Mentha piperita*), three crude plant extracts, garlic (*Allium sativum*), mango (*Mangifera indica*) and marigold (*Tagetes patula*), and three registered chemical pesticide

products, Delegate® (Corteva Agriscience, South Africa), Coragen® (FMC, South Africa), and Warlock® (ADAMA, South Africa). The plants were purchased from Sunnyside Garden Centre in Makhanda South Africa; pure essential oils were purchased from eOil.co.za (Johannesburg, South Africa) in accordance with Dambuza (2022); samples of insecticides were acquired from Citrus Research International (CRI) in Gqeberha South Africa; mango peels were acquired from Unifrutti in Hoedspruit, South Africa; garlic was purchased from Farmbox Fruit and Veg store in Makhanda.

2.2.3 Preparation of solutions/suspensions

2.2.3.1 Plant extract suspensions

Plant extract suspensions were prepared using ultrasound-assisted extraction (UAE) at a standard concentration (10 mg/ml) following the methodology described by Dambuza (2022), according to Landolt et al. (1999) and methodology described by Wong Paz et al. (2015). Fresh plant material (leaves, flowers, peels and bulbs) was dried overnight in an oven at 85℃ for garlic and mango, marigold leaves were dried at the same temperature for 8 hours. and then ground to a powder using a coffee grinder (Kambrook) for about five minutes, weighed on a balance machine and mixed with 500 ml of 100% methanol (solvent) in a conical flask. The flask was sealed with foil paper and placed in an ultrasonic bath or ultrasonic probe system for 3 h at room temperature (25℃) (Wong Paz et al. 2015). Following this, the mixture was then filtered using suction filtration with a Buchner funnel containing filter paper attached to the flask. The filtered product was weighed on a balance machine, and the solvent evaporated in a fume hood. The resulting crude extract was weighed and dissolved in distilled water in a beaker to achieve the desired concentration of 10 mg/ml (Elsayed 2020).

2.2.3.2 EO solutions

EO solutions were prepared at a standard concentration of 10 mg/ml. The oil was transferred into a plastic vial and weighed on a balance machine (3 g). The oil was then diluted in 300 ml 90% ethanol in a plastic beaker to achieve the desired concentration (Kostić et al. 2008). Ethanol is used to solubilise EO into water-based sprays so they can be diluted without separating, forming a homogenous mixture (Moore 2017).

2.2.3.3 Insecticide suspensions

All commercial products were used at rates registered for use against FCM on citrus in South Africa. Coragen® registered rate is 17.5 ml/100 L water, Delegate®, registered rate is 20 g/ 100 L water, and Warlock® registered rate is 100 ml/100 L water. Ratios were simplified to use 1 ml of the sample, which was then dissolved in water to achieve the recommended concentration. This equates to 1 ml/ 5.714 L of water, 1 g/ 5 L water and 1 ml/ 1 L water for Coragen®, Delegate® and Warlock®, respectively

2.2.4 True repellence or odour masking trials

These were conducted in controlled environment (CE) rooms under nocturnal conditions, with temperatures being kept at 25°C (± 1.5°C) and low relative humidity (26%) using artificial oranges (DEJUCA) (orange floaters, 115 mm diameter, purchased online from takealot.com) rinsed with distilled water before use (Figure 2.2). Nocturnal conditions were used because FCM adults are mostly active at night (Stofberg 1954). Oviposition behaviour by FCM females in response to these compounds was evaluated in both no-choice and choice trials. In addition to oviposition in choice trials, the insects' choice for treated or non-treated fruit was also recorded.

2.2.5 No-choice trials

In no-choice trials, six artificial oranges were carefully placed an equal distance from each other in a mesh insect cage (60×30×35 cm) with seven gravid FCM females. The positive choice (untreated artificial oranges) was excluded; therefore, six treated artificial oranges (dipped in the same potential repellent) were placed in the cage with FCM females. A control treatment was included (untreated artificial oranges). Oviposition was measured every hour for four hours, by counting the number of eggs oviposited on the artificial oranges (Love et al. 2014). The above experiments were replicated five times for each treatment, with adults arising from new generations.

Figure 2.2: Orange floaters used as artificial oranges (AOs) (Photo credit: David Taylor).

2.2.6 Choice trials

A Y-tube setup (Figure 2.3) was designed and built using 20 L rectangular tubs (unbranded), purchased from the Crazy Store in Makhanda. Two plastic bottle-size holes were cut symmetrically on the long side of the tubs. The tubs were then joined by a joining tube (2 L bottle) cut open on both ends to fit in the tubs and secured in place using silicone and Sellotape (Figure 2.3: B and C). The joining tube was cut in the middle and joined with an extended entry tube (2 L plastic bottle) (Figure 2.3 C). Small holes were made in the lids of the containers to allow for air.

Three untreated artificial oranges served as the control and three treated artificial oranges (dipped in the potential repellent solution) were placed at an equal distance from each other in the separate chambers. Fourteen gravid FCM females were released simultaneously in the entry tube. The number of eggs laid on the artificial oranges, the number of females that remained in the entry tubes (neutral), and the number of females in the control and treatment chambers were counted after 24 h.

The above-mentioned experiments were repeated five times for each treatment, with adults arising from a new generation.

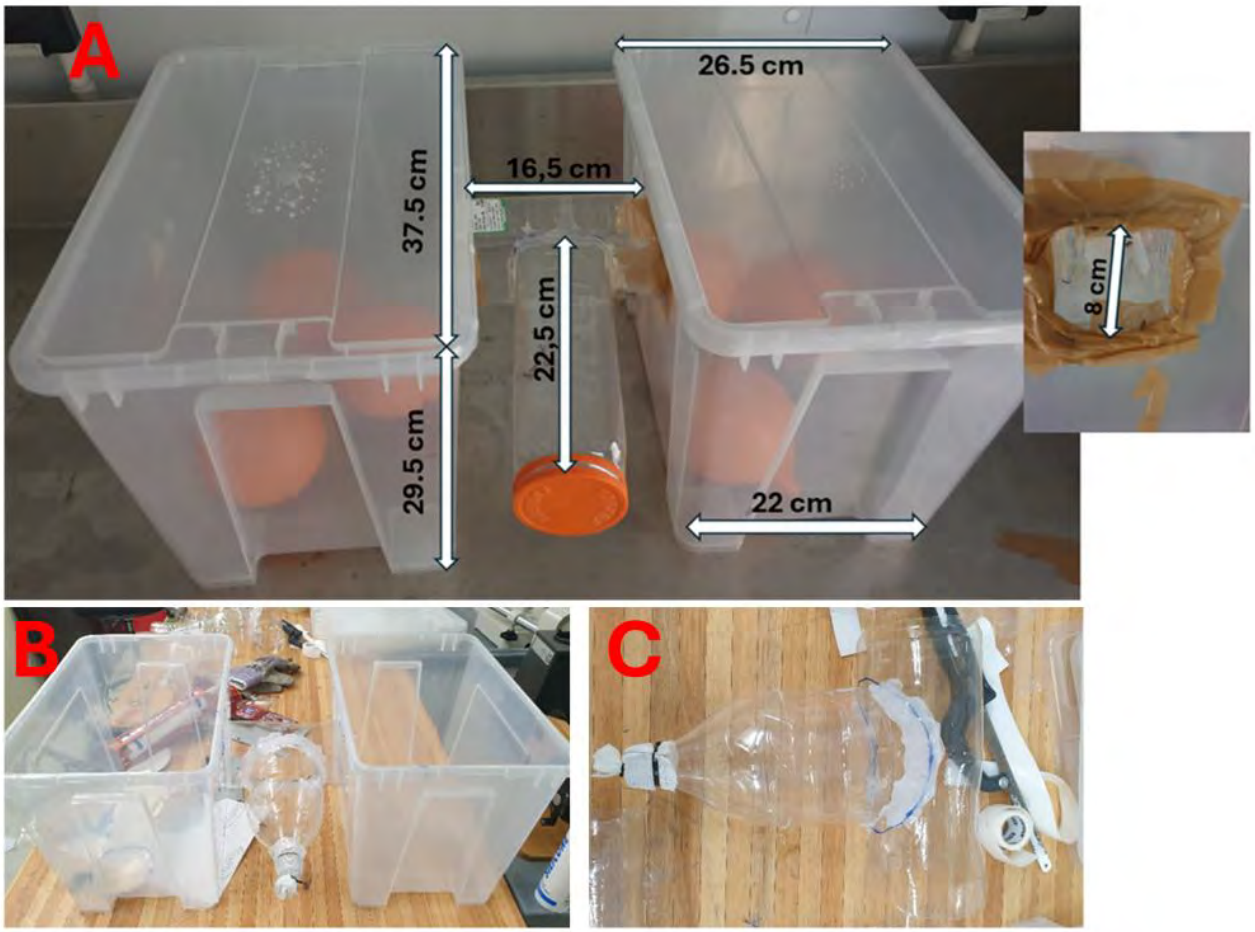

Figure 2.3: Dimensions and variations of the olfactometer used to run choice trials. A: Dimensions of the Y- tube olfactometer setup, B: Y- tube olfactometer setup with joints held together by silicone and C: Y- tube olfactometer tubes.

2.2.7 Statistical analysis

In no-choice trials, to investigate whether different compounds are true repellents of FCM (reduce total oviposition on artificial fruit treated with different compounds), a generalised linear model (GLM) was used. The number of eggs oviposited was modelled as a function of the repellent compound applied. The compound variable was specified as a categorical fixed effect variable with 9 levels (Control/Garlic/Marigold/Mango/Lavender/Peppermint/ Coragen©/Delegate©/Warlock©). The control represents untreated oranges. The GLM was specified assuming a negative binomial error distribution and a log link function. Hypothesis testing was performed using a likelihood ratio test (LRT) ($P < 0.05$). Post-hoc comparisons

were performed using pairwise comparisons of estimated marginal means (Tukey's adjustment for multiple comparisons), using the 'Control' as the reference group.

In choice trials, to investigate whether different compounds are true repellents of FCM, a (GLM) was used. Gravid FCM female choice and number of eggs oviposited per female were modelled as functions of the repellent compound applied. The compound variable was specified as a categorical fixed effect variable with 9 levels (Control/Garlic/Marigold/Mango/Lavender/Peppermint/Coragen®/Delegate®/Warlock®). The GLM was specified assuming either a negative binomial error distribution or a Poisson probability distribution and a log link function. Hypothesis testing was performed using a likelihood ratio test (LRT) or a Wald chi-square test ($P < 0.05$). Pairwise comparisons of estimated marginal means were performed using Tukey's adjustment for multiple comparisons (post-hoc), using the 'control' as the reference group. Each compound had its own model.

All modelling was performed using the '*glmmTMB*' R package (Brooks et al. 2017) and all data were analysed using R version 4.4.0 (R Core Team, 2024).

2.3 RESULTS

2.3.1 No-choice trials

In no-choice trials, there was a significant difference in total number of eggs oviposited between the compounds ($\chi^2 = 124.08$, df = 8, $P < 0.001$). Post-hoc comparisons indicated that all tested compounds resulted in females laying at least 83% fewer eggs on the treated artificial fruit relative to the untreated control (pairwise comparison of estimated marginal means, Figure 2.4, $P < 0.05$). Among the plant crude extracts, garlic demonstrated the highest reduction in oviposition (99.82%), followed by marigold (99.63%) and mango (96.52%) (Figure 2.4). Essential oils also showed substantial reductions in egg deposition with lavender oil reducing oviposition by 86.58% and peppermint oil by 84.19% (Figure 2.4). Similarly, the commercial synthetic chemicals reduced egg deposition with Coragen® achieving a 95.89% reduction, followed by Warlock® (83.84%) and Delegate® (83.29%) (Figure 2.4).

2.3.2 Choice trials

2.3.2.1 Female choice

For the essential oils (Figure 2.5a), both peppermint and lavender recorded significantly fewer females (73.33% and 57.50%, respectively) in the treatment tubs relative to the control (peppermint: χ^2 = 20. 35, df = 1, P < 0. 001; lavender: χ^2 = 9. 55, df = 1, P = 0. 002) (Figure 2.5a). The number of females that did not make a choice (no response) was fewer compared to females observed in the control but not treated fruit for both peppermint and lavender (P < 0. 001; P = 0. 919; P < 0. 001 and P = 0. 730, respectively). Among the plant crude extracts (Figure 2.5b), garlic and marigold demonstrated no significant difference (χ^2 = 3. 29, df = 1, P = 0. 069; χ^2 = 1.48, df = 1, P = 0. 224, respectively). However, a reduction (garlic: 35.71%; marigold: 25.64%) in the number of females in the tub with treated fruit was still observed. Mango demonstrated a significant difference (χ^2 = 16. 19, df = 1, P < 0. 001) with 65.31% more females observed in the tub with treated fruit compared to the control. Across all the crude extracts, the number of FCM females that did not make a choice (no response) was fewer compared to the females observed in the control and treated fruit (garlic: P = 0. 002; P = 0.007; marigold: P < 0. 001; P < 0. 001; mango: P= 0.016; P < 0. 001). Among the chemical insecticides (Figure 2.5c), Coragen© demonstrated a significant difference (χ^2 = 10. 87, df = 1, P < 0. 001), with 57.78% more females observed in the tub with treated fruit. Delegate© demonstrated a significant difference (χ^2 = 16. 30, df = 1, P < 0. 001), as there were 68.18% fewer females observed in the tub with treated fruit, and Warlock® demonstrated no significant difference (χ^2 = 1. 25, df = 1, P = 0. 264), however a reduction of 24.32% in the number of females in the tub with treated fruit was observed. Among the chemical insecticides, the number of females that did not respond (no response) was fewer compared to the females observed in the control and treated fruit for Coragen® and Warlock® (Coragen: P = 0. 031; P < 0. 001, and Warlock: P < 0. 001; P = 0. 001, respectively). The number of non-responding females was smaller compared to the females observed in the control (P < 0. 001), and not statistically different compared to females observed on treated fruit (P = 0. 918) for Delegate©.

2.3.2.2 Oviposition

In choice trials, comparing the number of eggs oviposited on control and treated fruit per compound, lavender demonstrated no significant difference (χ^2 = 0.08, df = 1, P = 0.779) (Figure 2.6a). However, a reduction (25.34%) in the number of eggs per female on treated fruit was still observed. Peppermint demonstrated a significant difference (χ^2 = 25.04, df = 1, P < 0.001), with no oviposition observed on treated fruit (100% reduction). For the plant crude extracts, both garlic and mango demonstrated no significant difference (χ^2 = 0.02, df = 1, P = 0.918; χ^2 = 2.35, df = 1, P = 0.125, respectively) relative to the control. However, a reduction (garlic:10.83%) and an increase (mango: 65.56%) in the number of eggs per female was observed. For marigold, more eggs per female (100%) were recorded on control fruit relative to the treated fruit (χ^2 = 7.183, df = 1, P = 0. 007) (Figure 2.6b). Coragen®, Delegate® and Warlock® demonstrated no significant difference (χ^2 = 0.003, df = 1, P = 0. 953; χ^2 = 3. 57, df = 1, P = 0. 059; χ^2 = 0. 44, df = 1, P = 0. 505, respectively) (Figure 2.6c). However, a reduction (3.76%, 75.14% and 41.01%, respectively) in the number of eggs oviposited per female was observed. Oviposition per female in some treatments was very low, therefore some results were not conclusive.

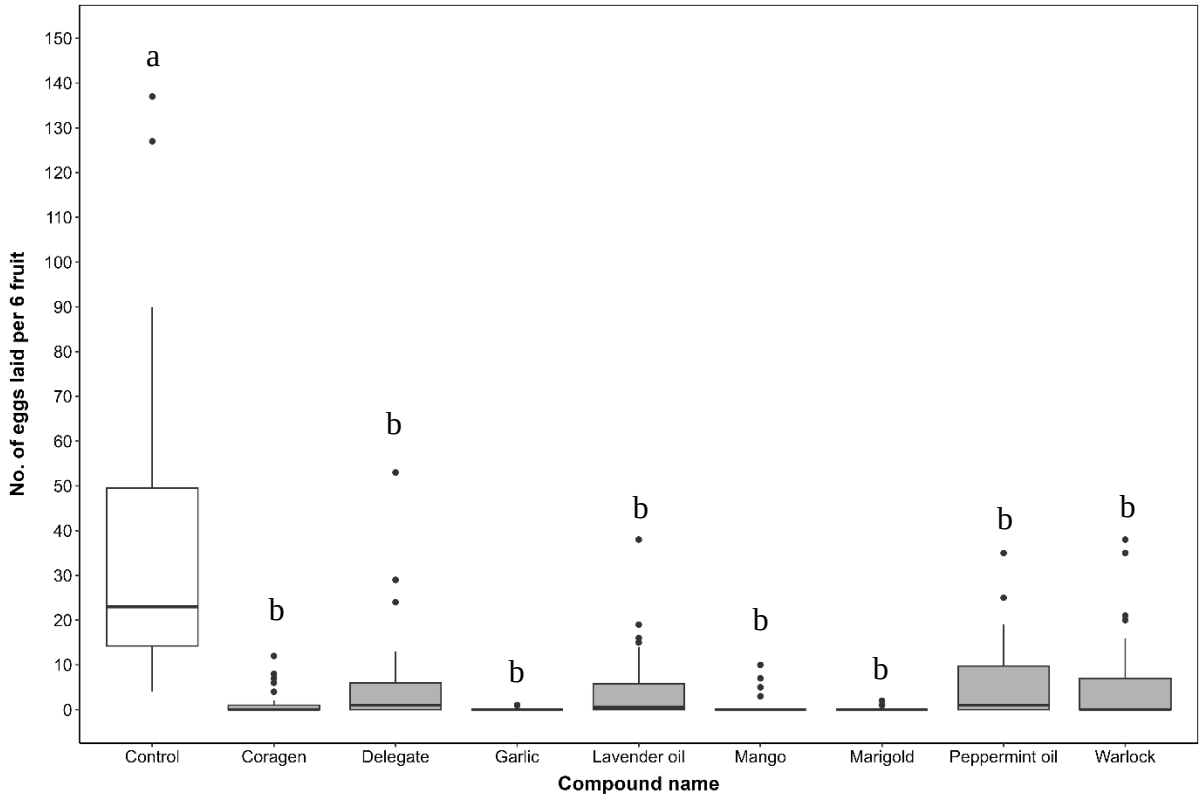

Figure 2.4: Total number of eggs oviposited within 4 h by FCM on artificial oranges, in no-choice trials, treated with plant suspensions (garlic, mango and marigold) (10 mg/ml), essential oils (lavender and peppermint) (10 mg/ml), commercially available chemical insecticides (Coragen®, Delegate® and Warlock®) (registered rates) and a control. Different letters indicate statistical differences between the different compounds relative to the control (Pairwise comparison of estimated marginal means, $P < 0.05$). The box-and-whisker displays the distribution of the data: the boxes represent the interquartile range (IQR) containing the middle 50% of the data, with the horizontal line within each box indicating the median (50th percentile). The whiskers extend to the smallest and largest values within 1.5 times the IQR from the first and third quartiles, respectively, while points outside this range are plotted as potential outliers.

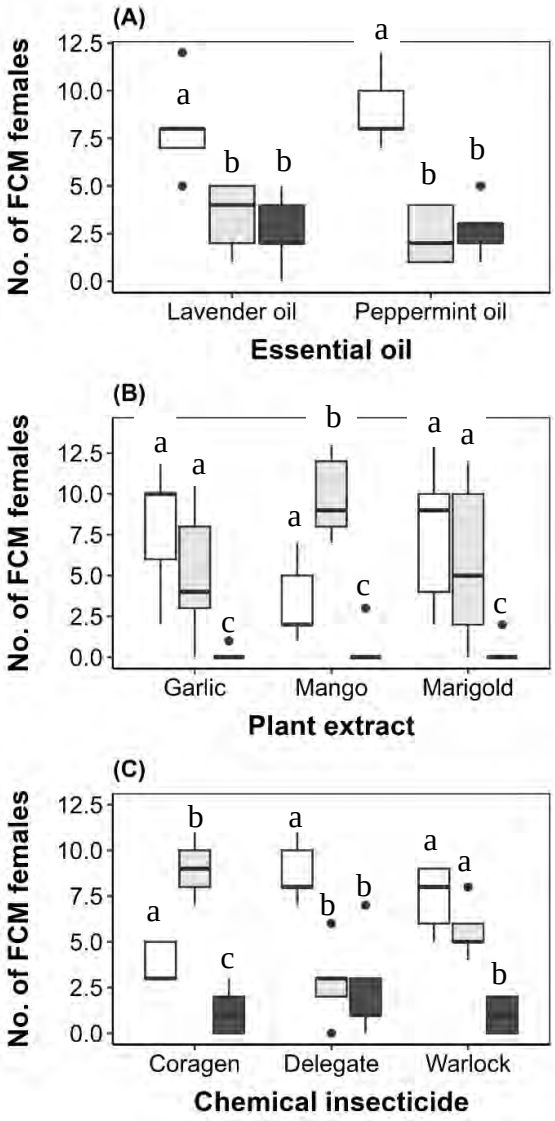

Figure 2.5: Total number of females in tubs with control (white) and treated (light grey) fruit and number of females that did not respond (no response) (dark grey), in choice trials. Fruit was treated with essential oil solutions (lavender and peppermint) (10 mg/ml) (A), plant suspensions (garlic, mango and marigold) (10 mg/ml) (B), commercially available chemical insecticides (Coragen®, Delegate® and Warlock®) (C) (registered rates). Different letters indicate statistical differences between the fruit treated with the different compounds relative to the control (Pairwise comparison of estimated marginal means, $P < 0.05$/Wald's test). The box-and-whisker displays the distribution of the data: the boxes represent the interquartile range (IQR) containing the middle 50% of the data, with the horizontal line within each box indicating the median (50th percentile). The whiskers extend to the smallest and largest values within 1.5 times the IQR from the first and third quartiles, respectively, while points outside this range are plotted as potential outliers.

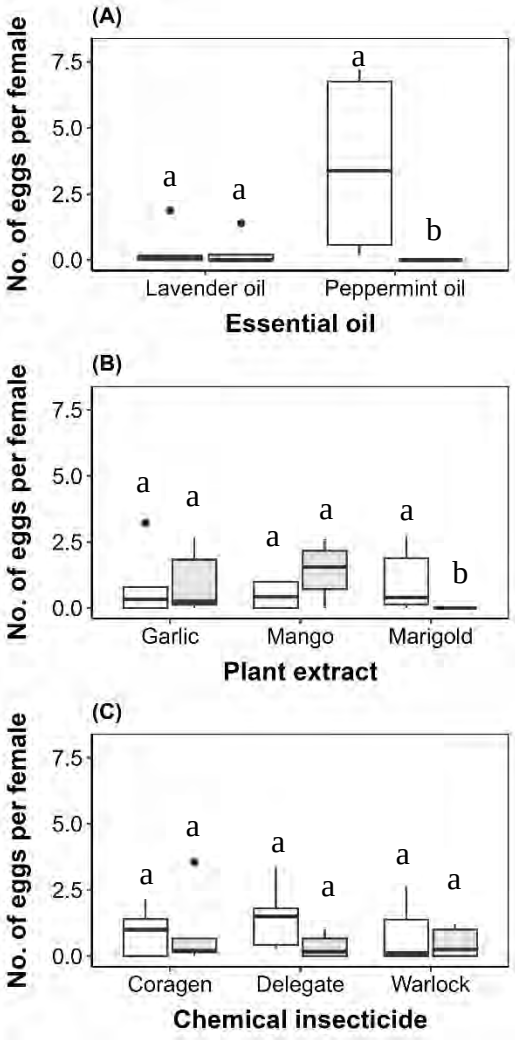

Figure 2.6: Total number of eggs oviposited per female on artificial oranges, in choice trials, control (white) and treated (light grey) fruit. Treated fruit were treated with essential oil solutions (lavender and peppermint) (10mg/ml) (A), plant suspensions (garlic, mango and marigold) (10 mg/ml) (B) and commercially available chemical insecticides (Coragen®, Delegate® and Warlock®) (C) (registered rates) versus untreated fruit/control. Different letters indicate statistical differences between the different compounds relative to the control (Pairwise comparisons of estimated marginal means, $P < 0.05$/Wald's test). The box-and-whisker displays the distribution of the data: the boxes represent the interquartile range (IQR) containing the middle 50% of the data, with the horizontal line within each box indicating the median (50th percentile). The whiskers extend to the smallest and largest values within 1.5 times the IQR from the first and third quartiles, respectively, while points outside this range are plotted as potential outliers.

2.4 DISCUSSION

Other than an unpublished study by Dambuza (2022), no other study has documented the potential use of botanicals as repellents for FCM. This study aimed to further evaluate botanical deterrents that were identified by Dambuza (2022) and establish whether they may be considered odour-maskants or repellents of FCM. This is important in pest management, as it provides clarity on their modes of action and informs practical application. Repellents drive pests away from the odour source by altering their behaviour whereas odour maskants indirectly disrupt the pest's ability to locate its host (Isman 2006, Deletre et al. 2016). Understanding these differences is important in designing IPM systems that combine chemical, behavioural and ecological tactics (Isman 2006). For example, repellents might be more suitable for push-pull systems, whereas odour maskants might be more suitable for use with attractants to disrupt pest-host location dynamics. Accurate classification of deterrents would also allow for testing of their compatibility with natural enemies therefore minimising environmental impacts, while improving sustainability and efficiency of pest control (Iamba & Yoba 2019).

The studies that have investigated the potential of botanicals as agricultural pest repellents mostly look at choice assays in the presence of the host and do not investigate the nature of the so-called repellents (Jankowska et al. 2009, Iamba & Homband 2020). Therefore, even though these compounds are referred to as repellents, they could simply be odour maskants. Not many no-choice trials have been conducted for botanical repellents. However, these are important as they are representative of more realistic field conditions where pests do not usually have a variety of choices (Sangha et al. 2017). Including no-choice trials informs on how deterrents perform when the pest is not given choices, whereas choice trials indicate if the pest has a preference when exposed to treated and untreated fruit, therefore informing us about the nature of its mode of action (repellent or maskant).

Deletre et al. (2016) stated that for a compound to be called a true repellent, it should be tested in the absence of the natural host. This is done to prevent the host from disturbing the insect or having a masking effect, as a result, we conducted true repellence assays using artificial oranges.

In this study, we identified three repellents: lavender oil, peppermint oil and the chemical insecticide, Delegate®. These compounds are identified as repellents because, in olfactometer assays, FCM adult females consistently moved away from the odour source, preferring the

untreated control fruit over the treated alternatives. The same was observed for oviposition as FCM preferred ovipositing on control fruit over treated fruit. Garlic, marigold and Warlock acted as odour maskants, FCM females were not repelled by their odours, but there was very low oviposition on treated fruit compared to the control. These compounds deterred oviposition very well in no-choice trials but did not repel females in choice-trials, with a lot of oviposition observed. These results were unexpected as both compounds have been reported to deter oviposition by FCM (Grové et al. 2012, Fullard & Hill 2013). It is unclear what might have caused this; we can speculate that this might be due to our methodology. With more replication, this may be clarified.

Lavender oil is primarily from the flowers of Lavandula angustifolia, it has a complex composition, containing volatile organic compounds such as terpenes which are responsible for its insect-repellent properties. It significantly repelled FCM females and reduced oviposition in both trials. Whilst there was little oviposition on control and treated fruit, the observed number of oviposited eggs per female on treated fruit was still 25.34% less compared to the control. This supports the findings by Dambuza (2022) and is congruent with other research. For example, lavender oil was reported to significantly repel oviposition by the box tree moth (BTM), *Cydalima perspectalis* Walker. (Lepidoptera: Crambidae) on treated boxwood plants compared to the control (Szelényi et al. 2020). Landolt et al. (1999) reported that lavender oil significantly repelled neonate larvae of codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae). Lavender oil also significantly repelled oviposition by the Mediterranean fruit fly *Ceratitis capitata* Wiedemann reducing it by up to 94% (Ouarhach et al. 2022). These show evidence of lavender oil as a deterrent against a variety of insects, which is consistent with our finding that lavender oil is a true repellent for FCM females.

Peppermint oil is one of the most used essential oils in insect pest management. Our study demonstrated that peppermint is the strongest botanical repellent of FCM among the deterrents that were evaluated. These results are consistent with a study by Dambuza (2022), in which peppermint was a strong oviposition-deterrent against FCM. Peppermint oil has also been reported to repel a variety of insect pests such as the tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), peach fruit fly, *Bactrocera zonata* (Diptera: Tephritidae) and common mosquito, *Culex pipiens* (Diptera: Culicidae) (Tajdar et al. 2020, Lo Pinto et al. 2022, Farag et al. 2024). Therefore, if it continues to maintain its repellence in semi-field trials it can be tested in field trials, with potential for registration of a product for commercial use.

Chemical insecticides have been reported to impact the reproductive potential of FCM (Fullard & Hill 2013). Delegate® is a chemical insecticide that attacks the nervous system of target insect pests, through contact and ingestion (Delegate® 250 WG). Fullard and Hill (2013) also reported that FCM adults oviposited significantly less on fruit treated with Delegate at the registered standard concentration in detached fruit bioassays. In our study Delegate was the only chemical insecticide that acted as a repellent. Therefore, because Delegate is applied to infect and kill insect pests like FCM through contact and ingestion, its repellence of FCM may prevent FCM from coming into contact with it and being killed. A consequence could be that although a degree of protection of the fruit is provided, the FCM population may not be as effectively suppressed as would be the case without the repellent effect.

Garlic, marigold and Warlock acted more as odour maskants. This was not expected for garlic, as it is often reported as a repellent for some insect pests. Mobki et al. (2014) reported that garlic extract repelled red flour beetle, *Tribolium castaneum* (Herbst) larvae and adults at reasonably low concentrations. In this study, garlic did not repel FCM females, but although the number of FCM adults that moved towards treated fruit in olfactometer assays was not significantly lower than the control, oviposition and number of eggs per female on garlic treated fruit was still much lower relative to the control. Therefore, it was concluded that garlic is acting more as an odour maskant. Garlic macerate oil from garlic bulbs reduced oviposition by *Tuta absoluta* Meyrick (Lepidoptera; Gelechiidae) on tomato leaves in laboratory and greenhouse trials, with repellency increasing with dosage (Lo Pinto et al. 2022). Garlic essential oil has also been reported to be an oviposition repellent of velvetbean caterpillar, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), a pest of soya beans (Ribeiro et al. 2015). Therefore, this can be developed as an odour maskant to apply in orchards. Currently, there are no registered odour-masking botanicals against FCM. There is, therefore, potential for more research exploring odour maskants. Marigold was the strongest odour maskant. Whilst FCM distributed evenly between the control and treated fruit chambers FCM still oviposited more on control fruit. Jankowska et al. (2009) reported that when cabbage was intercropped with pot marigold, *Calendula officinalis* L. and French marigold, *Tagetes patula nana* L., the number of cabbage aphid, *Brevicoryne brassicae* L. and flea beetles (*Phyllotreta*) was significantly lower. Intercropping with marigold plants also reduced oviposition by some lepidopterans, namely, the small white butterfly, *Pieris rapae* L., large white butterfly, *P. brassicae* L., cabbage moth, *Mamestra brassicae* L. and diamondback moth, *Plutella xylostella* L. (Jankowska et al. 2009, Iamba & Homband 2020). Calumpang et al. (2015) reported that

there were fewer landings by the eggplant fruit and shoot borer (EFSB), *Leucinodes orbonalis* Guenee (Lepidoptera: Crambidae) on the host plant when marigold was present. Marigold also significantly reduced damage by EFSB on eggplants and eggplant shoots and significantly repelled EFSB females (Calumpang et al. 2015). Instead of acting as a repellent, marigold is possibly blocking the odour that is attractive to the insect pest (Deletre et al. 2016). Therefore, if effective in semi-field trials, marigold can be further tested as an FCM maskant by spraying of suspensions.

Warlock did not repel FCM females, but oviposition was very low on all the Warlock-treated fruit; FCM females oviposited 41.01% less eggs on treated fruit. Therefore, this compound was classified as an odour maskant as it did not repel adults, but oviposition on treated fruit was reduced. This might be due to FCM sensing the active constituents of this insecticide, discouraging their use of treated fruit as suitable oviposition sites. Published literature on how Warlock impacts the reproductive potential of its target pest, and their behaviour once applied is limited. However, the main constituent of Warlock, emamectin benzoate has been reported to reduce percentage of larval hatch of eggs and mating frequency in corn earworm adults, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (López et al. 2010). In contrast to this, it has been reported that emamectin benzoate effects on different life stages of DBM can vary based on sub-lethal concentration applied, when treated with LC_{20} dose of the chemical, the average single female oviposition number increased by 30.9 eggs (Liu et al. 2022). This shows that further research is required to evaluate how Warlock impacts reproductive potential and behaviour of FCM.

The results for mango and Coragen were inconclusive. This is because in the no-choice trials, these two compounds deterred oviposition by FCM. This is consistent with a study by Dambuza (2022). Grove et al. (2012) reported that mango in South Africa (orchards in Limpopo and Mpumalanga) is not a natural host for FCM as a result of antixenosis. Even though FCM can oviposit on mango under artificial conditions, it is not a preferred host (Grové et al. 2012). Therefore, more tests need to be conducted for mango. Coragen has been reported to have an oviposition deterrence effect on FCM (Dambuza 2022) Therefore, more testing for Coragen is also required as the large number of eggs of oviposited on treated fruit cannot be explained. Therefore, more replicates are needed to get more conclusive results for these two compounds.

Whilst it is important to establish the deterrent nature of these compounds, in reality, they are not going to be applied on artificial fruit. Therefore, it is important to establish whether their

degree of repellence is retained in the presence of real fruit given that FCM cue into fruit volatiles for oviposition This could have implications on the of these compounds as deterrents. These findings could also add another tool to reduce FCM populations in the field that would have the effect of reducing the chances of interceptions during exports.

Chapter 3: Comparing oviposition by FCM on real and artificial fruit treated with the same compound

3.1 INTRODUCTION

FCM shows oviposition preferences that are influenced by various factors (Newton 1989). This is largely because the selection of a suitable oviposition site by phytophagous insects is very important for the development of their offspring (Renwick 1989). Orientation, landing and recognition of a plant host by insects are often guided by the various volatile organic compounds (VOCs) emitted by their host plant (Renwick 1989, Webster et al. 2010). Newton (1989) demonstrated that FCM oviposited more eggs on premature ripe and injured Navel and Valencia oranges than on healthy ripe fruit. Mkiga et al. (2019) also demonstrated that FCM preferred laying eggs on oranges than on other suitable hosts, such as eggplant. Other tortricid pests, for example, the codling moth (CM) also show oviposition preferences, with the leaves of apple trees being chosen as their primary site for oviposition (Blomefield et al. 1997), likely owing to the presence of a naturally occurring compound, α-Farnesene, found in apple leaves. This compound has been linked to significantly increased oviposition by CM in laboratory studies (Wearing & Hutchins 1973). Other volatiles such as Ethyl (E, Z)-2,4-decadienoate (Et-E,Z-DD) have been shown to improve oviposition by CM in laboratory studies but have proved ineffective when tested in orchards (Knight & Light 2004). This shows that oviposition by CM and other insect pests, including FCM, is largely stimulated by chemical cues.

In pest management, highly effective botanical repellents in the presence of the actual host would be preferred over ones whose repellence is reduced in the presence of the actual host due to the release of attractive host VOCs. The role of repellents is to create a barrier between the natural host and their pest to prevent the pest from being able to locate and recognise its host (Deletre et al. 2016). Deletre et al. (2016) stated that to test for true repellence of a compound the natural host must be absent. But for a repellent to be approved and commercialised it must be effective in the presence of the natural host. In evaluating an *in vitro* blood-feeding method (i.e., in the absence of a natural host), Rutledge and Gupta (2004) demonstrated that the results obtained with the *in vitro* method were not necessarily accurate or the same as the results obtained using humans, the natural host. As a result, they concluded

that the *in vitro* method needed some improvements (Rutledge & Gupta 2004). For improved results, Klun et al. (2005) developed a new *in vitro* system by improving the Klun & Debboun (K&D) test module, coupling it with a membrane-blood reservoir. The new *in vitro* method showed similar repellence results compared to their performance during *in vivo* trials. Even though results differed slightly, this demonstrated that *in vitro* systems can be adapted and improved to provide results that correlate with *in vivo* systems (Klun et al. 2005).

Therefore, given that discrepancies in realised repellence may differ depending on whether an artificial or natural host is used (Zeilinger et al. 2015), this study investigated the effects of these VOCs released by oranges on the degree of repellence of the potential repellents of FCM identified in the previous chapter.

3.2 METHODS AND MATERIALS

3.2.1 Insect rearing

Insects were reared following the methods described in the previous chapter (Chapter 2, section 2.2.1). Egg sheets and pupae were sourced from a mixed FCM culture established by Moore (2002), combining populations from South Africa's Western Cape, Eastern Cape, and Mpumalanga provinces. The culture was mixed to enhance genetic diversity within the population. The culture, maintained at the Zoology and Entomology Department of Rhodes University, was reared to adulthood under conditions of 26°C ± 2°C, 50–60% relative humidity, and a 12 h light/dark cycle. The diet, formulated by Moore et al. (2014), included maize meal, wheat germ, milk powder, brewer's yeast, nipagin, and sorbic acid.

Eggs were surface sterilised and placed in jars with an autoclaved diet, stoppered with cotton wool. Larvae moved into the cotton wool to pupate, after which pupae were carefully removed, sexed by abdominal segment count (four for females, five for males) (Peterson 1965), and placed individually in 30 ml glass vials for eclosion.

Within 24 h of eclosion, virgin males and females were paired in vials stoppered with water-moistened cotton wool and allowed to mate overnight in a CE room with 20–45% relative humidity. Only females that oviposited in the vials were selected for the trials (Love et al. 2014, Mgocheki & Addison 2016).

3.2.2 Identification and collection of potential repellents

The same botanicals, chemical insecticides and essential oils used in the previous chapter (Chapter 2) were used: two essential oils (lavender and peppermint), three crude plant/fruit extracts (garlic, mango and marigold) and three chemicals (Delegate®, Coragen® and Warlock®). The plants were sourced from Sunnyside Garden Centre in Makhanda, essential oils from eOil.co.za in Johannesburg, insecticide samples from CRI in Gqeberha, mango peels from Unifrutti in Hoedspruit, and garlic from Farmbox Fruit and Veg store in Makhanda.

3.2.3 Preparation of plant suspensions

All the plant suspensions were prepared as described in the previous chapter (Chapter 2, section 2.2.3.1). Plant extract suspensions were prepared using ultrasound-assisted extraction (UAE) at a standard concentration of 10 mg/ml, following the methods of Dambuza (2022), based on Landolt et al. (1999) and Wong Paz et al. (2015). Fresh plant materials, including leaves, flowers, peels, and bulbs, were dried overnight in an oven at 85℃, ground into a powder with a coffee grinder (Kambrook) for approximately five minutes, and weighed. The powdered material was mixed with 500 ml of 100% methanol in a conical flask, sealed with foil, and processed in an ultrasonic bath or probe system for three hours at 25℃ (Wong Paz et al. 2015). The mixture was then filtered using suction filtration with a Buchner funnel and filter paper. The filtrate was weighed, and the solvent was evaporated in a fume hood. The resulting crude extract was weighed again and dissolved in distilled water in a beaker to obtain the final concentration of 10 mg/ml (Elsayed 2020).

3.2.4 Preparation of essential oil solutions

EO solutions were prepared at a standard concentration of 10 mg/ml. The oil was transferred into a plastic vial and weighed on a balance machine (3 g). The oil was then diluted in 300 ml 90% ethanol in a plastic beaker to achieve the desired concentration (Kostić et al. 2008).

Ethanol is used to solubilise EO into water-based sprays so they can be diluted without separating, forming a homogenous mixture (Moore 2017).

3.2.5 Insecticide suspensions

All commercial products were used at rates registered for use against FCM on citrus in South Africa (Coragen®: 17.5 ml/100 L water, Delegate®: 20 g/ 100 L water, and Warlock®:100 ml/100 L water). Ratios were simplified to use 1 ml of the sample, which was then dissolved in water to achieve the recommended concentration as previously described.

3.2.6 Orange VOCs effect on oviposition

These trials were conducted in complete darkness in a CE room as described in the previous chapter (Chapter 2, section 2.2.5). All real fruit (Palmer Navels) used in this study were provided by Citrus Research International following collection from orchards near Addo in the Sundays River Valley in the Eastern Cape Province, South Africa. Upon arrival, all fruit were rinsed thoroughly with distilled water, dried and stored in a cold room at 4°C to preserve fruit health. The artificial oranges used were the same as in the previous chapter. They were washed with pure water and soap followed by a final rinse with distilled water. Oranges, artificial and real, were allowed to dry before use. Oviposition on the oranges was tested in choice trials, with the choice being either real fruit or artificial fruit.

A total of six oranges, three artificial and three real oranges, were placed one by one into a cage (60×30×35 cm) with seven gravid FCM females. All oranges (artificial and real fruit) were dipped in the same potential repellent, plant crude extract (garlic, mango and marigold), essential oil (lavender oil and peppermint oil) or chemical insecticide (Coragen®, Delegate® and Warlock®) at the prepared rates. The fruit were dipped to get uniform coverage of the entire surface and to reduce loss of treatments through runoff. Three untreated artificial oranges and three untreated real oranges were placed into a cage of gravid FCM females equidistant from each other, serving as a control.

The above-mentioned experiment was replicated five times for each treatment.

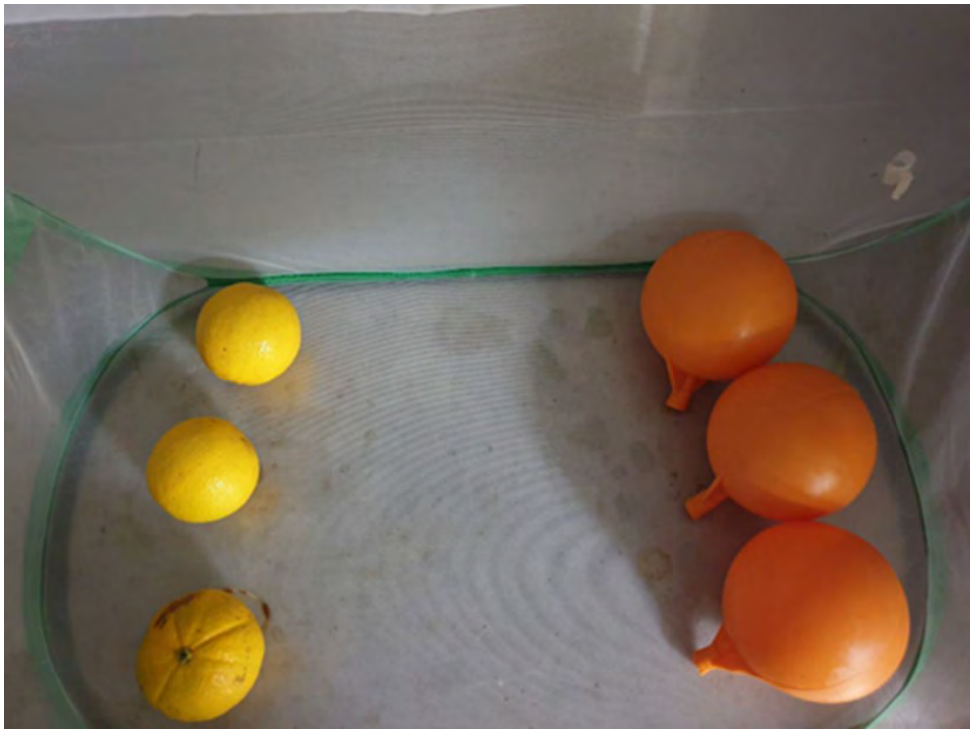

Figure 3.1: Real and artificial fruit treated with the same compound in a netted cage.

3.2.7 Statistical analysis

In orange-type choice trials, to investigate whether FCM had a preference for oviposition between real fruit and artificial fruit treated with each potential repellent, a GLM was used. The number of eggs oviposited was modelled as a function of the repellent compound applied and orange type. The compound variable was specified as a categorical fixed effect variable with 9 levels (Control/ Garlic/ Marigold/ Mango/ Lavender/ Peppermint/ Coragen®/ Delegate®/ Warlock®) and the orange variable was specified with two levels (Real fruit/Artificial fruit). The GLM was specified assuming a negative binomial error distribution or a Poisson probability distribution and a log link function with replicate included as a random effect. Hypothesis testing was performed using a likelihood ratio test (LRT) or a Wald chi-square test ($P < 0.05$). Post-hoc comparisons were performed with a pairwise comparison of estimated marginal means (Tukey's adjustment for multiple comparisons), comparing oviposition counts between real fruit and artificial fruit, with 'Real fruit' as the reference group. Each compound was analysed individually comparing oviposition on artificial and real fruit. All modelling was performed using the '*glmmTMB*' R package (Brooks et al. 2017). All data were analysed using R version 4.4.0 (R Core Team, 2024).

3.3 RESULTS

Comparing the number of eggs oviposited on artificial versus real fruit treated with the same compound, the control (untreated) demonstrated a significant difference (χ^2 = 25. 16, df = 1, *P* < 0. 001). There was a 96.17% reduction in the number of eggs oviposited on artificial fruit compared to real oranges (Figure 3.2A). Among the essential oils (Figure 3.2B), both lavender and peppermint demonstrated a significant difference (χ^2 = 10. 99, df = 1, *P* < 0. 001; χ^2 = 75. 57, df = 1, *P* < 0. 001, respectively). A 90.82% and 91.57% reduction in oviposition on artificial fruit relative to real oranges was observed, respectively. Among the plant crude extracts (Figure 3.2C), garlic demonstrated no significant difference (χ^2 = 0. 11, df = 1, *P* = 0. 739). However, a 20% reduction in the number of eggs oviposited on real oranges compared to artificial fruit was observed. Mango and marigold demonstrated significant differences (χ^2 = 44. 36, df = 1, *P* < 0. 001, χ^2 = 111. 68, df = 1, *P* < 0. 001, respectively), with oviposition only recorded on real oranges. Oviposition was, however, still low. Among the synthetic commercial chemicals (Figure 3.2D), Coragen® and Delegate® demonstrated significant differences (χ^2 = 10. 68, df = 1, *P* = 0. 001, χ^2 = 19. 82, df = 1, *P* < 0. 001). A 96.53% and 46.72% reduction in oviposition on artificial fruit compared to real oranges was observed, respectively. Warlock® demonstrated no significant difference (χ^2 = 0. 71, df = 1, *P* = 0. 399). However, there was 18.42% more oviposition on artificial fruit.

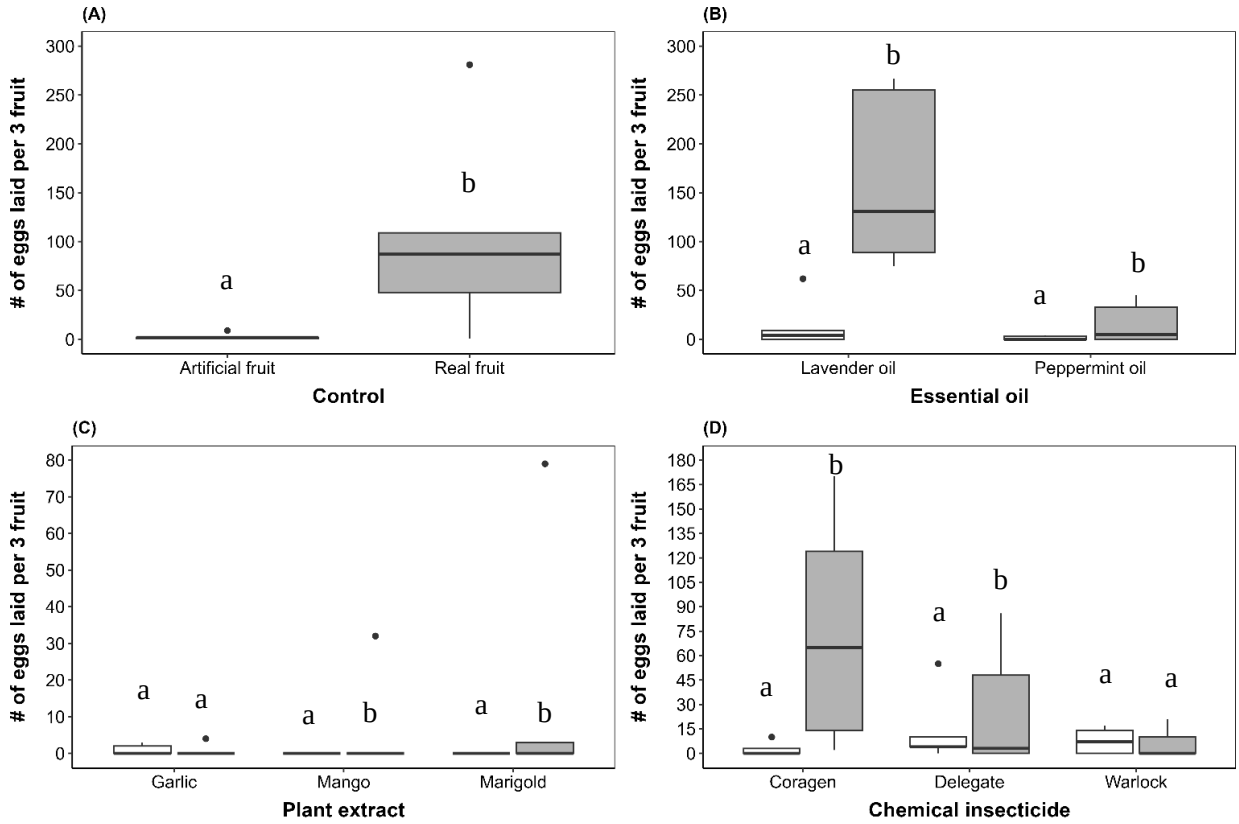

Figure 3.2: Total number of eggs oviposited by FCM on artificial fruit (white) versus real oranges (grey) treated with the same compound. (A) untreated (control) oranges, (B) fruit treated with essential oil solutions (lavender oil and peppermint oil) (10 mg/ml), (C) plant suspensions (garlic, mango and marigold) (10 mg/ml) and (D) commercially available chemical insecticides (Coragen®, Delegate® and Warlock®) (registered rates). Different letters indicate statistical differences in oviposition on artificial and real fruit treated with the same compound ($P < 0.05$). The box-and-whisker displays the distribution of the data: the boxes represent the interquartile range (IQR) containing the middle 50% of the data, with the horizontal line within each box indicating the median (50th percentile). The whiskers extend to the smallest and largest values within 1.5 times the IQR from the first and third quartiles, respectively, while points outside this range are plotted as potential outliers.

3.4 DISCUSSION

Repellent performance in the field can be impacted by many different factors, including temperature, rainfall and the presence of natural host volatiles (Zeilinger et al. 2015, Macchioni et al. 2019). Although establishing the nature of deterrents is important, for them to be

considered effective enough their impact in the presence of the host must be established. This is done to determine if the repellents would be economically viable for field application and to see if they repel pests in the presence of the natural host. Zeilinger et al. (2015) reported that volatiles released by natural hosts can overpower the repellence of compounds. Some studies have shown that volatiles that repel pests when used alone, can act as attractants when combined with other volatiles (Webster et al. 2010). Therefore, this further shows the need to establish repellence of the compounds in the presence of their natural host. In medical entomology, studies are designed so that experiments are more reflective of real-world conditions and pest densities to accurately assess the effectiveness of repellents (Novak & Gerberg 2005). As a result, human subjects are preferred for repellence trials of mosquito repellents over animal and artificial membranes, as using humans generates more representative data (Trongtokit et al. 2008).

Here, a control was included to support the prediction that when given a choice, FCM would prefer to oviposit on real fruit compared to artificial fruit due to real fruit releasing volatiles that are attractive to FCM (Renwick 1989, Thompson & Pellmyr 1991, Love et al. 2014). Our results support our prediction as FCM oviposited almost exclusively on real fruit in the control assays. Gratton & Welter (1998) reported that leafminers, *Liriomyza helianthin* (Diptera: Agromyzidae), exhibited a preference for oviposition on their natural host plants and related species over non-host species. Plants outside the subfamilies of the host plant were rarely used for oviposition (Gratton & Welter 1998). Additionally, Love et al. (2014) reported that when exposed to different Navel cultivars FCM oviposited the least on Fischer Navels in the early maturing groups. *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), has been observed to have a preference for ovipositing on tobacco, maize and sunflower in laboratory studies compared to other species (Firempong & Zalucki 1989). Female *C. capitata* have been found to oviposit more on sweet citrus as the oils produced, which are based predominantly on limonene, stimulate oviposition (Ioannou et al. 2012). CM has been reported to oviposit more on medium and late-maturing apple varieties in regions with contrasting climatic conditions (Akroute et al. 2023). The South American leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), has been reported to prefer ovipositing on tomatoes over other crops such as potatoes and eggplants (Subramani et al. 2021). Volatile compounds, such as p-quinone and 2-carene in tomatoes, stimulate oviposition by *T. absoluta*, while 1-fluorododecane in other plants may deter oviposition (Subramani et al. 2021).

Lavender oil did not retain its strong repellent effect in the presence of real fruit, although minimal oviposition was observed on artificial fruit. If we look at the oviposition on real fruit treated with lavender, it is similar to the control. This may be because the presence of natural host volatiles overrides the repellent effect of lavender oil. As a result, this compound cannot be applied in the field or further evaluated in semi-field trials. Whilst it may be possible to counteract the presence of host VOCs by increasing the concentration used and techniques such as encapsulation, the degree to which this would need to occur may not be economically viable for field application (Ayllón-Gutiérrez et al. 2024). Our results are contrary to findings that the chemical linalool, which forms a major constituent of lavender oil, has a deterrent effect on oviposition by citrus pests such as *C. capitata* (Ioannou et al. 2012). Dambuza (2022) also reported that lavender oil significantly deterred oviposition by FCM on real oranges. It is important to note that in the study by Dambuza (2022), FCM had a choice between treated and untreated real oranges, therefore it is possible that this made it a lot easier for the moths to cue host volatiles. It is not clear what might have caused FCM females to oviposit on lavender-treated real fruit in our study, but it has also been reported that the repellent effect of lavender oil can diminish when insects are simultaneously exposed to attractive pheromones (Kheloul et al. 2019). In our study the moths were left for 24 h, therefore perhaps overtime the repellent effect of lavender oil diminishes making it easier for moths to pick up on the oranges. It has been reported that air circulation and temperature can affect the rate at which essential oils evaporate (Lane 2024)., Delegate and Peppermint oil maintained its strong repellence even in the presence of real fruit. Whilst there was more oviposition on real fruit treated with peppermint relative to treated artificial fruit, oviposition was very low. This suggests that the active components of peppermint oil effectively override attractive cues released by real fruit. This was expected, as peppermint oil has been reported to have a repellent effect greater than 80% on brown-winged leafhopper, *Pochazia shantungensis* (Hemiptera: Ricaniidae) in both laboratory conditions in the absence of attractants and in field conditions where it was evaluated in the presence of the pest attractants (Ryu, Tae-Hee et al. 2016). Therefore, this compound is a good candidate for further evaluation in semi-field trials for its efficacy proven efficacy in more natural conditions.

All plant extracts significantly deterred oviposition by FCM, on both artificial and real fruit. Oviposition was generally very low in plant crude extract trials. This suggests that active components may effectively override attractive cues released by the natural host as the results are consistent between artificial fruit and real fruit. When FCM did oviposit, they oviposited

more on real fruit in the mango and marigold treatments. However, the presence of orange volatiles did not reduce the effectiveness of the plant crude extract in deterring oviposition. For garlic and marigold, this supports our findings from the previous chapter that these are odour maskers and prevent FCM from being able to locate, recognise and oviposit on real fruit and is contrary to the report that the presence of host plant attractants can neutralise the deterrent effect of garlic (Denoirjean et al. 2022). Literature on how the presence of host volatiles affects marigold repellence is limited. Positively, in addition to the repellent effects reported for marigold, marigold plants have also been reported as natural enemy attractants. This, therefore, makes it suitable for IPM systems as it may enhance biological control (Song et al. 2017). As suggested in the previous chapter, more tests need to be conducted for mango, as the results in our studies have been inconsistent. Therefore, it is difficult to conclude the nature of its mode of action. Mango was expected to deter oviposition throughout our laboratory assays as it is not a natural host of FCM (Grové et al. 2012). Green immature mangoes (Tommy Atkins and Keitt) have also been reported not to be a host of Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae) in both field and laboratory settings (Peña et al. 2006). Even though fruit flies are not related to FCM, extracts from green immature mango may warrant investigation, as green mangoes could contain volatiles with potential repellent properties that are lost during the ripening process.

Excessive oviposition was observed on real fruit treated with Coragen relative to artificial fruit. This is also because oviposition on Coragen treated fruit was similar to the control. Therefore, the presence of real fruit volatiles overpowers any deterrence that Coragen has; this was expected as Coragen did not maintain strong deterrence in choice trials in the previous chapter. Our results are contrary to findings by Dambuza (2022) and Fullard & Hill (2013), who observed that Coragen significantly reduced oviposition by FCM on real fruit at the standard commercial recommended concentration. Delegate and Warlock maintained a strong oviposition deterrence. Warlock was the strongest chemical deterrent, as there was no difference in oviposition between artificial and real fruit. However, FCM oviposited more on real fruit treated with Delegate relative to artificial fruit, but oviposition was low, meaning that Delegate maintains its repellence. Therefore, Delegate and Warlock can be further evaluated in semi-field trials.

The difference in performance of these compounds in the presence of host volatiles outlines the importance of understanding the interaction between repellents and host volatiles. While testing repellents on artificial objects is valuable, the presence of host volatiles can affect the

outcomes. This study shows the need for testing repellents in the presence of a natural host to validate lab assays done using artificial objects, as effective pest control systems must account for the chemical ecology of the pest-host repellent system.

Since peppermint oil and Delegate maintained strong repellence in the presence of real fruit volatiles. This supports their potential use in practical pest management. They can be evaluated in conjunction with strong attractants in push-pull systems. This would push pests away from treated fruit while pulling them towards traps or decoys, maximising pest control efficacy. Garlic and marigold can be further evaluated as potential odour maskants. Their potential as odour maskants suggests that they reduce the attractiveness of host volatiles (Deletre et al. 2016). Studies need to be done to determine the concentration and application method that will best mask host VOCs without reducing fruit quality and interfering with other control agents for controlling FCM and other pests of citrus in the same system. After all important laboratory studies have been conducted, more realistic field studies must be conducted to test the effectiveness of these compounds under natural conditions, including different fruit varieties and pest densities. Additionally, assessment of the economic feasibility of using these compounds in large-scale operations considering material cost and returns is needed. It is important to note that in this study we did not incorporate a control choice component within the treatments, which would have constituted a multi-choice assay. Therefore, future research should investigate the effects of VOCs using a multi-choice assay approach, this would provide more information on the effectiveness of these compounds.

Chapter 4: Evaluating potential attractants of FCM for push-pull systems

4.1 INTRODUCTION

In the agricultural industry, there is an increased need for pest control methods that are less damaging to the environment, and safe for humans (Bhattacharyya 2017). The push-pull technology is an environmentally friendly pest management approach that uses semiochemicals to manipulate insect behaviour (Cook et al. 2007, Hassanali et al. 2008, Bhattacharyya 2017). Using an alternative method like push-pull systems reduces the amount of pesticides used and can additionally enhance IPM systems, soil fertility and weed control (Hassanali et al. 2008, Bhattacharyya 2017). In this system, semiochemicals are used to repel pests from crops or natural hosts and attract them to trap crops (Figure 4.1) (Bhattacharyya 2017). For the success of push-pull, a good understanding of plant-insect interactions on different plant species and their chemical ecology is needed (Khan et al. 2010). The push-pull system is made up of two components, namely the 'push' and the 'pull' (Figure 4.1). For the "push" component, the natural host is protected either directly by modifying the plant itself or by planting a companion crop between the rows of the main crop (Pickett et al. 2014), which elicits a repellent or deterrent effect on pest behaviour. Ideally, a push crop should also be suitable for the pest's natural enemies, such as parasitoids, attracting more and therefore increasing their population and diversity (Pickett et al. 2014). Trap plants are grown along the perimeter of the main crop, attracting pests away from the main crop towards them, this is the "pull" component (Pickett et al. 2014). Trap plants may also encourage oviposition thereby reducing oviposition on the main crop. They may also reduce the target pest population by incorporating natural pesticides or using plant innate defence systems (Pickett et al. 2014).

Sobhy et al. (2022) showed that silverleaf, *Desmodium uncinatum* (Fabales: Fabaceae) and greenleaf, *Desmodium intortum* (Fabales: Fabaceae), emitted volatiles that repelled oviposition by the target pest, *S. frugiperda,* when intercropped with maize. The same result was achieved when a three-way hybrid Brachiaria Mulato II (*Urochloa ruziziensis* × *Urochloa decumbens* × *Urochloa brizantha*) was used as a push crop. These plant species also emitted compounds that attracted parasitoids of *S. frugiperda.* As a result, *S. frugiperda* infestations were reduced as larval mortality increased. Similarly, a push-pull system has also been shown to be effective in

the control of maize stem borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), a pest of maize (Khan et al. 2011). Again, *Desmodium* spp. is planted between the rows of maize, which pushes the stem borer away from the main crop. A highly attractive trap crop, Napier grass, *Pennisetum purpureum* (Poales: Poaceae) is included at the edge of the field to pull the pest towards it and, therefore, away from the main crop (Khan et al. 2011). As a nitrogen-fixing plant, Napier grass has the added benefit of improving soil fertility and can also be used as fodder for livestock (Khan et al. 2011). These examples demonstrate that push-pull systems are plausible for the control of important lepidopteran agricultural pests.

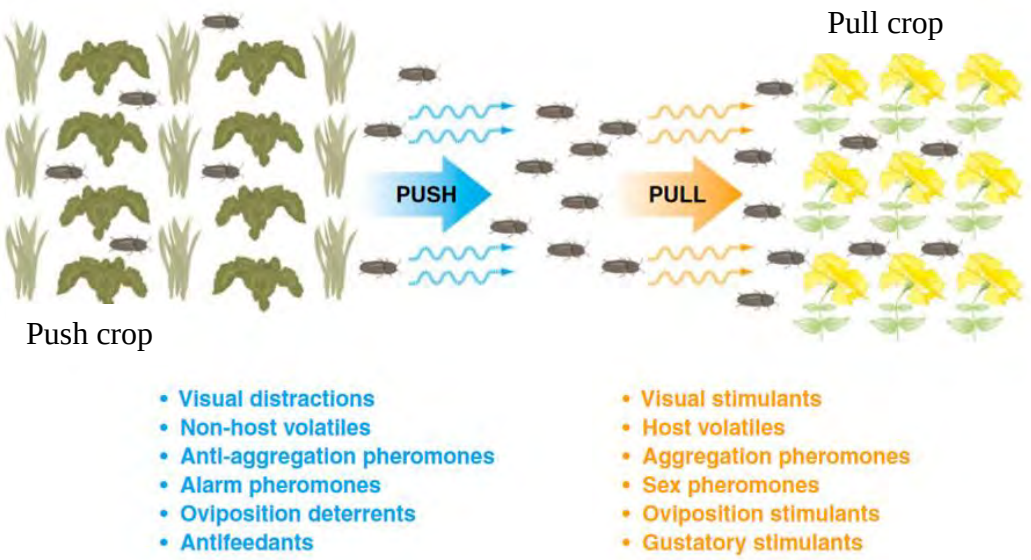

Figure 4.1: A representation of the "push" and "pull" components and generalised mode of action of a push-pull system (Cook et al. 2007).

To date, a push-pull system for FCM control has not been investigated. Volatile organic compounds (VOCs) that have the potential to be repellents were initially investigated by Dambuza (2022) and were further evaluated in this thesis. However, for a complete push-pull system, two components are needed. Therefore, attractants for FCM need to be investigated as they have not been explored before. An attractant is a compound that induces the orientation of a pest towards it and preferably encourages oviposition (Chambers et al. 1974, Gregg et al. 2018, Mkiga et al. 2019). Several attractants of agricultural pests have been reported. For example, Usha Rani & Pratyusha (2014), demonstrated that tobacco cutworm, *Spodoptera litura*, (F., 1775) (Lepidoptera: Noctuidae) oviposited more on two Castor plant, *Ricinus communis* L., phenolics, namely coumaric and syringic acids. They also demonstrated that

larvae of Castor plant semi-looper, *Achaea janata* L. (Lepidoptera: Noctuidae), and Tobacco cutworm orientated more often towards arms treated with these two phenolics in Y-tube trials, compared to an untreated control. López-Guillén et al. (2020) also confirmed the susceptibility of *R. communis* to herbivory by *A. janata* and *S. litura*, further proving that there is a level of attraction. Hu et al. (2022) demonstrated that in the oriental fruit fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), mature males were strongly attracted to clove bud essential oil, especially during the day. Mkiga et al. (2019) demonstrated that FCM had a strong oviposition for sweet pepper and chilli pepper (*Capsicum* spp.). Even though a complete push-pull system is not yet available for FCM, some potential components of it are. It has been demonstrated that traps baited with pheromones were sufficient for monitoring FCM populations in citrus orchards; traps containing pheromones such as (Z)- and (E)-8-dodecyl acetate trapped high numbers of FCM males (Newton & Mastro 1989). Therefore, in the case of a push-pull system, this would be exploited as a pull component, as FCM males are attracted to pheromone-baited traps and would be paired with a push component, a true repellent of FCM. Even though this method focuses on trapping males only, it shows that there is potential for further research and exploitation of attractants, especially ones that are effective against females and preferred for oviposition, as seen in Mkiga et al. (2019), and the development of push-pull systems against FCM. This chapter focused on evaluating the attractiveness of four essential oils (clove bud oil, castor oil, cypress oil, and sage oil) against FCM for potential use in an FCM push-pull system. A push-pull system for FCM would be a tool that could be incorporated in an IPM system. Given that research on the use of botanicals as attractants in agricultural pest management is limited, these compounds were chosen as they have been shown to have some level of attraction to various insect species of agricultural importance, not necessarily Lepidoptera. This study evaluated attractance of the various EOs using a different methodology.

4.2 METHODS AND MATERIALS

4.2.1 Identification of potential attractants

Potential attractants were identified from the literature based on whether they have acted as attractants or a host of various insects before, including Lepidopterans (Table 4.1). Sage oil,

cypress oil and clove bud oil had relatively low ODI indexes in a study conducted by Dambuza (2022). Positive ODI values represented repellent effect while negative values represented attraction, therefore since these oils had low positive values they were identified to have a potential as attractants. Additionally, FCM females oviposited more on oranges treated with these EOs relative to the control, therefore warranting their evaluation as potential attractants (Dambuza 2022).

Pure essential oils were purchased from eOil.co.za (Johannesburg, South Africa).

Table 4.1: Potential attractants of FCM evaluated.

Attractant(s)/Host Plant(s) and Essential Oils	**Pest attracted/feeding on it**	**References**
Castor Oil (*Ricinus communis*)	Castor semi-looper, *Achaea janata* (L.,1758) (Lepidoptera: Noctuidae)	Usha Rani & Pratyusha 2014, López-Guillén et al. 2020
	Tobacco cutworm, *Spodoptera litura*, (F., 1775) (Lepidoptera: Noctuidae)	
	False codling moth, *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae)	Hamburger et al. 2001
Clove Bud Oil (*Eugenia caryophyllata*)	Fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)	Hu et al. 2022
	False codling moth, *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae)	Dambuza (2022)
Cypress Oil (*Cupressus sempervirens*)	False codling moth, *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae)	Dambuza (2022)

Sage Oil (*Salvia officinalis*)	False codling moth, *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae)	Dambuza (2022)

4.2.2 Insect rearing

Insects were reared following the methods described in the previous chapter (Chapter 2, section 2.2.1). Egg sheets and pupae were sourced from a mixed FCM culture established by Moore (2002), combining populations from South Africa's Western Cape, Eastern Cape, and Mpumalanga provinces. The culture, maintained at the Department of Zoology and Entomology at Rhodes University, was reared to adulthood under conditions of 26°C ± 2°C, 50–60% relative humidity, and a 12 h light/dark cycle. The diet, formulated by Moore et al. (2014), included maize meal, wheat germ, milk powder, brewer's yeast, nipagin, and sorbic acid.

Eggs were surface sterilised and placed in jars with an autoclaved diet, stoppered with cotton wool. Larvae moved into the cotton wool to pupate, after which pupae were carefully removed, sexed by abdominal segment count (four for females, five for males) (Figure 2.1) (Peterson 1965), and placed individually in 30 ml glass vials for eclosion.

Within 24 h of eclosion, virgin males and females were paired in vials stoppered with water-moistened cotton wool and allowed to mate overnight in a controlled environment room with 20–45% relative humidity. Only females that oviposited in the vials were selected for the subsequent trials (Love et al. 2014, Mgocheki & Addison 2016).

4.2.3 Preparation of solutions

Essential oil and plant solutions were prepared following the same methods in the previous chapter (Chapter 2). EO solutions were prepared at a standard concentration of 10 mg/ml. The oil was transferred into a plastic vial and weighed on a balance machine (3 g). The oil was then diluted in 300 ml of 90 % ethanol in a plastic beaker to achieve the desired concentration (Kostić et al. 2008). Ethanol is used to solubilise EO into water-based sprays so they can be diluted without separating, forming a homogenous mixture (Moore 2017).

4.2.4 Attraction Choice Trials

Six artificial oranges, three per tub, were placed in a Y-tube olfactometer with 14 gravid females. Three untreated artificial oranges that served as the control and three treated artificial oranges (dipped in the potential attractant solution) (pull factor) were placed at an equal distance from each other in separate chambers. The 14 gravid FCM females were released in the entry tube. The number of eggs laid on the artificial oranges, the number of females that remained in the tubes (neutral), and the number of females in the control and treatment chambers were counted after 24 h.

The above-mentioned experiments were repeated five times for each treatment, with adults arising from a new generation.

4.2.5 Statistical analysis

A GLM was used to investigate whether different compounds are attractants of FCM. Gravid FCM female choice and number of eggs oviposited per female were modelled as functions of the attractant compound applied. The compound variable was specified as a categorical fixed effect variable with five levels (Control/ Castor oil/ Clove bud oil/ Cypress oil/ Sage oil). The GLM was specified assuming either a negative binomial error distribution or a Poisson probability distribution and a log link function. Hypothesis testing was performed using a likelihood ratio test (LRT) or a Wald chi-square test ($P < 0.05$). Wald chi-square test was used to test if effect of the treatments on oviposition by FCM was significant. Pairwise comparisons of estimated marginal means were performed using Tukey's adjustment for multiple comparisons (post-hoc), using the 'control' as the reference group. Each compound was analysed individually.

All modelling was performed using the '*glmmTMB*' R package (Brooks et al. 2017) and all data were analysed using R version 4.4.0 (R Core Team, 2024).

4.3 RESULTS

4.3.1 Female choice

The number of females in the control chamber and treated chamber did not significantly differ in castor oil treatments (χ^2 = 1.795, df = 1, *P* = 0.180) (Figure 4.2). However, there were 30% more females in the chamber with treated fruit. The number of females that did not make a choice (no response) was fewer than females observed on treated fruit but did not differ significantly from the control (*P* = 0.019; *P* = 0. 308, respectively). For clove bud oil, the number of FCM females recorded in the chamber with treated fruit did not differ from the control (χ^2 = 0.862, df = 1, *P* = 0.383) (Figure 4.2). However, there were 22% more females observed in the chamber with treated fruit. The number of females that did not make a choice was fewer than females recorded on treated fruit, but did not differ significantly from the control (*P* = 0.017; *P* = 0.135, respectively). The number of females recorded in the chamber with treated fruit did not differ significantly from the number of females recorded in the chamber with control fruit for cypress oil (χ^2 = 0.229, df = 1, *P* = 0.633) (Figure 4.2). However, there were 11% more females recorded in the chamber with treated fruit. There were no females that did not respond. Lastly, for sage oil, the number of FCM females recorded in the chamber with treated fruit did not differ from the number of females recorded in the chamber with control fruit (χ^2 = 2.702, df = 1, *P* = 0.100) (Figure 4.2). However, 34% more females were recorded in the chamber with treated fruit. The number of females that did not make a choice was fewer than both the number of females in the tub with treated fruit and the control (*P* < 0.001; *P* = 0.008, respectively).

4.3.2 Oviposition

The number of eggs oviposited per female did not differ significantly for all the treatments when compared to the untreated control (castor oil: χ^2 = 0. 461, df = 1, *P* = 0. 497; clove bud oil: χ^2 = 0. 658, df = 1, *P* = 0. 417; cypress oil: χ^2 = 0. 008, df = 1, *P* = 0. 931; sage oil: χ^2 = 0. 361, df = 1, *P* = 0. 548). However, for castor oil, clove bud oil and sage oil, a decrease in the number of eggs oviposited per female on treated fruit relative to control fruit was recorded (60%, 56%, and 68%, respectively). For cypress oil, an increase in the number of eggs oviposited per female on treated fruit relative to control fruit was also recorded but was approximately 10-fold lower than the other compounds tested (6%) (Figure 4.3).

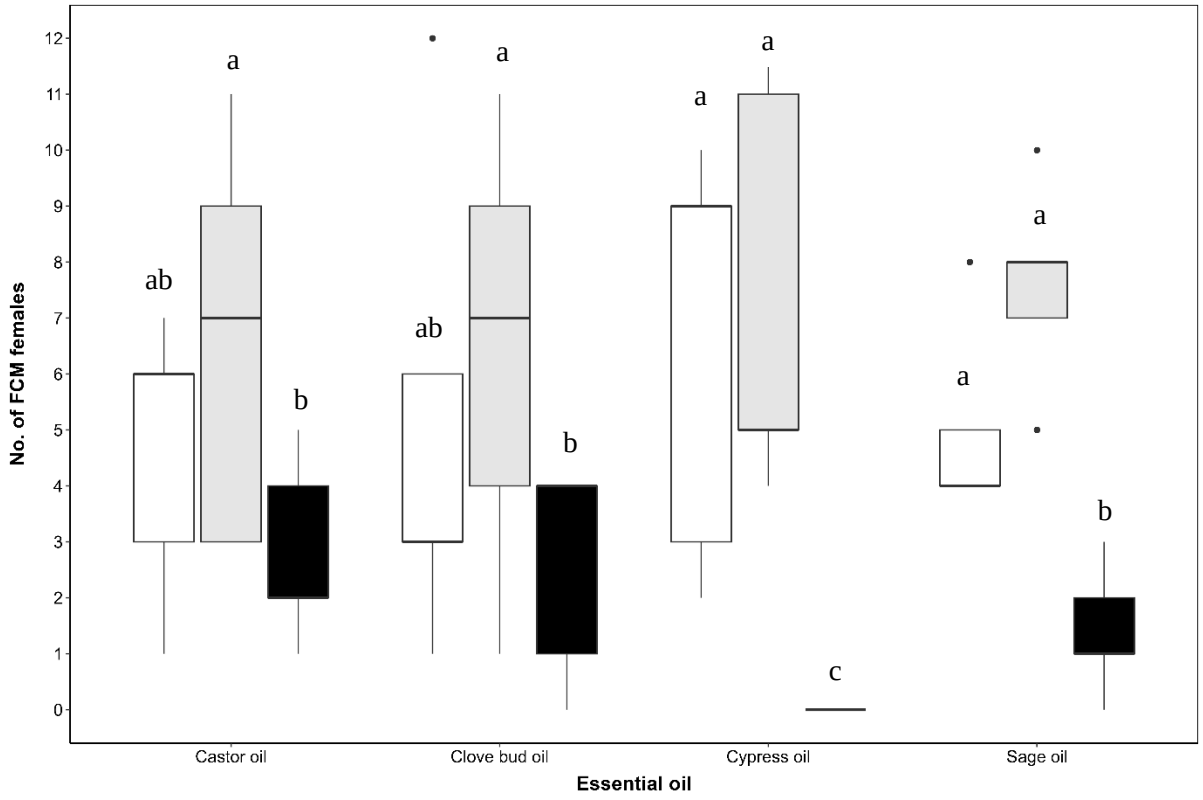

Figure 4.2: Total number of females recorded in the chambers with control (white) and treated (grey) fruit and number of females that did not respond (no response) (black) in attraction trials for each of the four essential oils tested at 10 mg/ml. Different letters indicate significant differences between the fruit treated with the different essential oils relative to the control (pairwise comparison of estimated marginal means, $P < 0.05$/Wald's test). The box-and-whisker displays the distribution of the data: the boxes represent the interquartile range (IQR) containing the middle 50% of the data, with the horizontal line within each box indicating the median (50th percentile). The whiskers extend to the smallest and largest values within 1.5 times the IQR from the first and third quartiles, respectively, while points outside this range are plotted as potential outliers.

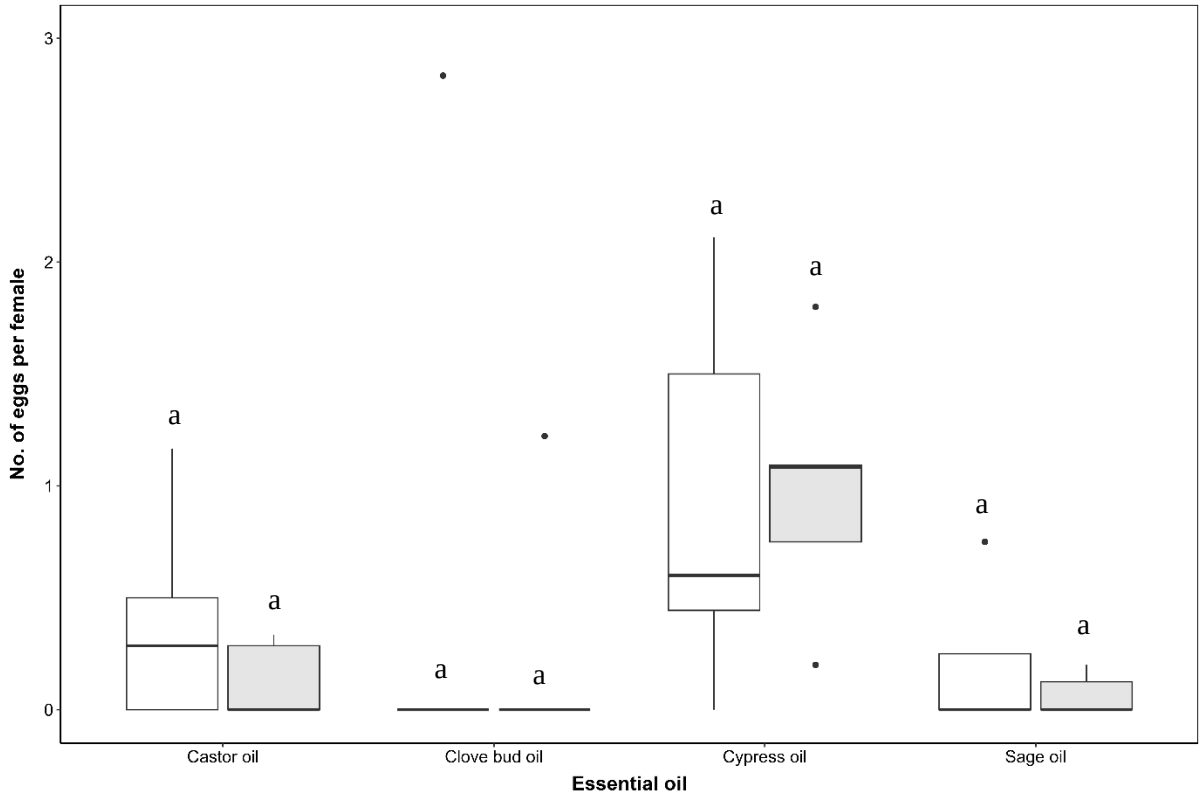

Figure 4.3: Total number of eggs oviposited per female on either artificial control fruit (white) or essential oil treated fruit (10 mg/ml) (grey) in attraction trials. Different letters indicate significant differences between the different essential oils relative to the control (pairwise comparisons of estimated marginal means, $P < 0.05$/Wald's test). The box-and-whisker displays the distribution of the data: the boxes represent the interquartile range (IQR) containing the middle 50% of the data, with the horizontal line within each box indicating the median (50th percentile). The whiskers extend to the smallest and largest values within 1.5 times the IQR from the first and third quartiles, respectively, while points outside this range are plotted as potential outliers.

4.4 DISCUSSION

This study aimed to evaluate certain botanicals as potential attractants for FCM. This is important in pest management as successful attractants can be used in conjunction with repellents in push-pull systems. Attractants lure pests towards the odour source, while repellents push pests away from the odour source (Landolt & Guédot 2008, Deletre et al. 2016). Presently there are no botanicals registered as attractants for FCM. Available attractant traps for FCM use synthetic sex pheromones, that use a combination of (Z)- and (E)-8-dodecenyl

acetate in equal proportions to attract males, with yellow delta-traps being the most effective (Newton & Mastro 1989). However, there have been studies that looked at botanical or plant volatile attractants of lepidopteran pests (Landolt & Guédot 2008, Jakubska-Busse et al. 2023).

These compounds have been shown to have a level of attraction to various insect species, not limited to Lepidopteran pests, given that research on the use of botanicals as repellents or attractants in agricultural pest management is limited, this study evaluated EOs castor oil, clove bud oil, cypress oil and sage oil as potential attractants of FCM females. In this study, we did not evaluate available FCM lures (i.e. pheromones), because we were testing specifically for FCM females, as the goal is to limit oviposition on the fruit. There was no statistically significant attraction of FCM adults to any of the essential oils evaluated. Cypress oil was the weakest of the potential attractants followed by clove bud oil. However, even though castor oil and sage oil showed slightly better attraction, the degree of attraction by both only warranted further evaluation in attraction trials. Oviposition count did not differ significantly between the control and treated artificial fruit for all the potential attractants tested. This might be because egg counts, in general, were too low for results to be conclusive.

Castor, *Ricinus communis*, has been reported as a highly favoured host of FCM in Israel, FCM larvae have been recovered in young fruits and seeds (Hamburger et al. 2001). Hamburger et al. (2001) reported that more catches of FCM were recorded on castor compared to macadamia (*Macadamia terniflora*) nuts and citrus, with male catches reaching 1.4 per trap per day. It was also suspected that the catches that were recorded in citrus orchards might have been because the castor surrounding the orchards was highly infested, with FCM eventually moving into citrus orchards (Hamburger et al. 2001). Additionally, FCM eggs were also recorded on castor bean fruits in Israel between November 2015 and December 2016, this further shows that castor is a suitable host for FCM in Israel (Kaspi et al. 2018). FCM was recorded to establish on castor in South Africa in a study where FCM neonate larvae were placed onto castor fruit and checked for establishment after two weeks (Kirkman & Moore 2007). It is important to note that in Southern Africa, infestation of castor by FCM is extremely rare (CABI 2020).

Due to the previous records, we expected FCM to be attracted to fruit treated with castor oil, however, in this study as reported there was not a strong attraction response by FCM to castor oil. This may be explained by reports that have found castor oil extract to have a repellent effect. For example, hydroethanolic extracts from castor plants have been reported to demonstrate a repellent effect against the agave snout weevil, *Scyphophorus acupunctatus*

(Coleoptera: Curculionidae); both females and males preferred untreated agave tissue relative to tissue treated with *Ricinus communis* extract (Pacheco-Sánchez et al. 2012). Essential oil from the stem of castor plants has been reported to show repellent activity against the red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae), achieving over 80% repellence after one hour of exposure (Salem et al. 2017). Additionally, castor oil solution has been reported to effectively control the eggplant fruit and shoot borer, *Leucinodes orbonalis* Guenée L. (Lepidoptera: Crambidae), by reducing incidents of attack with more applications or higher concentrations generating the best results (Mariame et al. 2023). Therefore, this shows that castor oil in some insects primarily acts as a repellent against insect pests rather than an attractant. However, in this study, castor oil did not repel FCM females. Therefore, more studies need to be conducted on its effect against FCM females with more replicates. Some studies can evaluate behaviour of FCM females in the presence of castor plants and fruit rather than its essential oil, to see if FCM females will respond differently. This is because VOCs often work as a suite not a single compound and some of the elements that induce repellence might not be present in the essential oil. It should also be noted that some of the FCM adults that chose the chamber with castor oil treated artificial fruit were found dead. Whilst inconclusive, castor oil may, therefore, have an insecticidal effect on FCM.

Clove bud oil has been reported to act as an attractant of adult male oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) (Hu et al. 2022). Even though it was more attractive to males than females, this can still be exploited to control the pest (Hu et al. 2022). At 50% concentration, clove bud oil, has also been reported to attract adult males of the Mediterranean fruit fly, *C. capitata* (Moustafa et al. 2012). Therefore, this shows that this essential oil can act as an attractant for pests. Besides the study by Dambuza (2022), where clove bud oil slightly increased oviposition by FCM, it has not yet been shown to have a significant attractant effect against Lepidopteran pests. As a result, it is possible that lepidopteran pests may not respond in the same way as fruit flies. For example, one study reported clove buds, *Syzygium aromaticum* L., to act as a repellent against *T. castaneum*, achieving a 100% repellence at various concentrations (Abo-El-Saad et al. 2011). This further highlighted that various insect pests respond differently to it. In our study, FCM females showed no specific response (no strong attraction) to clove bud oil, as they were distributed evenly between the control and treated fruit. In previous studies by Moustafa et al. (2012) and Abo-El-Saad et al. (2011), clove bud oil did not elicit any response in female fruit flies and only their male counterparts responded and were attracted to it. But it is important to note that FCM and fruit flies are not

closely related therefore responses may differ. Perhaps it would be worthwhile for future studies to look at how FCM males would respond to clove bud oil, as we only looked at female response in this study. Additionally, clove bud oil consists of components like α-copaene, which is very attractive to fruit flies. Therefore, it would be worthwhile evaluating this compound as a potential attractant of FCM (Lull et al. 2023). However, clove bud oil may simply not be attractive to Lepidoptera specifically FCM females in this case. More studies need to be conducted on the effect of clove bud oil on various lepidopteran pests, as information is very limited. Clove bud oil's complicated chemical constituents must be evaluated against different pest and non-pest insects, as clove bud oil can also be a toxic to certain species (Abo-El-Saad et al. 2011). It is important to note that Surinam cherry, *Eugenia uniflora* (Myrtales: Myrtaceae), a plant (fruit) that belongs to the same family as clove bud, has been reported to be a host of FCM (Grové et al. 2019). Therefore, it was a possibility that clove bud could be host or an attractant of FCM if plants are used instead of a crude extract.

Essential oils from sage, *S. officinalis*, have also shown potential in pest management. Information on the use of sage oil as an attractant is very limited. Dambuza (2022), reported that sage oil showed some level of attraction to FCM females along with some of the other potential attractants evaluated. In this study, FCM did not show any significant response to sage oil (weak response or attraction), as there was no significant difference in the number of FCM females in the chamber with treated fruit relative to the control. For it to be further evaluated in push-pull trials, it would have to show a strong level of attraction to FCM females, which it did not. Therefore, warranting further evaluation with more replicates. In contrast to Dambuza (2022), and here, other studies have reported sage oil to act more as a repellent than an attractant and bioinsecticide. It has been reported to cause high mortality, reduce egg deposition and hatchability and cause developmental abnormalities in black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuoidea) (Sharaby & EL-Nujiban 2013). Additionally, it has been reported to act as a repellent and fumigant against many storage pests (Suma & Rajadurai 2023). In field trials, it reduced Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), attraction to baited traps (Youssef et al. 2009). It is important to note that these pests are of a different family and order to FCM, and, therefore, the responses and effects could be different as sage oil did not repel FCM females in this study.

This study aimed to evaluate the potential of selected essential oils as attractants to FCM and, if successful, for them to be tested in push-pull strategies. These were tested under controlled conditions, sage and castor oil may hold some promise none of the essential oils acted as an attractant for FCM. The outcome therefore suggests that volatiles emitted by some of these oils lack chemical cues required to elicit attraction behaviour for oviposition in FCM. Sage oil and castor oil elicited weak attraction(30 and 34% more females on treated fruit) behaviour to FCM females, as a result they should be considered for further evaluation including more replicates or higher concentrations. These findings highlight the complexity of FCM's olfactory preferences. Therefore, there is a need for further exploration of alternative attractant sources for FCM females and research into the behavioural ecology of FCM could provide more information into factors that influence its attraction and oviposition preferences.

While the tested essential oils were not all successful with some showing promise, this study contributes valuable knowledge to the growing body of research that is aimed at developing environmentally friendly and sustainable pest management practices for FCM. Future research should consider a broader spectrum of potential natural and synthetic volatiles of FCM females, as well as the potential of synergistic interactions among chemical compounds. It is important to note that the pull cops evaluated in this study do not belong to the same family as citrus, nor are they closely related. Effective attractants are not necessarily always derived from plant species within the same family as the natural host. Furthermore, citrus is not considered the original host for FCM.

Chapter 5: General discussion

5.1 INTRODUCTION

The South African citrus industry is a major producer and exporter of citrus globally and provides employment for about 100, 000 people (Gianessi & Williams 2012). Due to demand and pressure to increase productivity, growers rely on the continued use of chemical pesticides (Rother et al. 2008). The use of chemical pesticides in agricultural pest management poses risks to human and environmental health (Sharma & Pandey 2021). Additionally, the overuse of pesticides can also result in unnecessary additional costs and the development of resistant pest strains (Gedefaw et al. 2022). Due to these concerns, important export markets such as the European Union (EU) impose strict restrictions on the use of pesticides, including in the form of maximum residue levels (MRLs) on fruit destined for export (Isman 2020, CGA Annual Report 2022). As a result, behavioural control is increasingly important in the South African citrus industry, as it offers an environmentally friendly and sustainable alternative to chemical pesticides that can be added to IPM systems such as botanicals (Morales 2002, Cook et al. 2007).

Botanicals are secondary metabolites derived from plants that act as repellents, attractants, antifeedants, and growth inhibitors (Cook et al. 2007, El-Shafie & Faleiro 2017, Ngegba et al. 2022). The use of botanicals as repellents against FCM has not been widely explored. Besides this study, only Dambuza (2022) has looked at the potential of botanicals and commercial insecticides as repellents for FCM. This study aimed to further this knowledge by establishing the nature of these two essential oils (lavender and peppermint), three plant crude extracts (marigold, garlic, and mango), and three commercially available insecticides (Warlock, Delegate, and Coragen) and identify if they are true repellents or odour maskants. Using artificial fruit, lavender oil, peppermint oil and Delegate were identified as true repellents, whilst garlic, marigold, and Warlock were considered odour maskants (Chapter 2).

The influence of natural host volatiles on the deterrence of these compounds was evaluated in Chapter 3. All the plant crude extracts deterred oviposition by FCM in the presence of natural host VOCs. Within the essential oils only peppermint deterred oviposition by FCM, and two

chemical insecticides (Delegate and Warlock) deterred oviposition by FCM in the presence of VOCs.

Finally, four essential oils were evaluated as potential attractants for FCM for use in a potential push-pull system for FCM control. None of the essential oils were considered attractants. In repellence trials, the mango crude extract significantly reduced oviposition in no-choice trials but served more as an attractant in choice trials and again deterred oviposition in the presence of natural host VOCs. This result means that more trials need to be done to investigate the mode of action of mango extract on FCM, as the result was inconclusive.

This chapter discusses the full results of the study in the context of agricultural pest management, how natural pest volatiles influence the repellence of compounds, the integration of repellents and attractants to form push-pull strategies, discussing the broader impact of the key findings and their implications for pest management, the limitations of the study, and lastly, recommendations for future research.

5.2 REPELLENTS

Insect repellents have been used for a long time in pest management and have evolved with the early repellents in the 1920s, paving the way for modern formulations (Peterson & Coats 2001). DEET (N, N-diethyl-meta-toluamide), first introduced in the 1950s, has remained the most commonly used and effective repellent (Katz et al. 2008). However, due to their low toxicity and similar efficacy to some synthetic chemicals, newer alternatives, like plant-based repellents, such as the oil of lemon eucalyptus and picaridin, became more commonly used (Katz et al. 2008). The need to combat mosquito-borne diseases, responsible for hundreds of thousands of deaths each year, has been a major driving force in the development of repellents (Norris & Coats 2017). Therefore, medical and veterinary pests have long been controlled with repellents (Isman 2006, Isman et al. 2011).

In agricultural pest management, the use of plant-based repellents and insecticides dates back to traditional farming practices (Devi et al. 2016). Traditionally used botanical insecticides include nicotine, neem, rotenone, ryania, sabadilla, and pyrethrum (Arnason 2012, Ikeura et al. 2012). While promoted as an environmentally friendly alternative, the commercial success of botanicals has been limited (Isman 2006), and only a few botanicals have gained popularity in the market namely pyrethrum, neem, and essential oil-based products (Isman 2006, Arnason

2012). Many plant-based substances, especially behaviour-modifying botanicals, have failed to reach commercialisation and achieve worldwide adoption, largely due to regulatory barriers and the availability of cost-effective synthetic chemical alternatives (Isman 2006, 2020). In the context of IPM systems, botanicals are seen as the most suitable for organic food production in developed countries and have great potential in countries that are still developing (Isman 2006, Devi et al. 2016).

Currently, there are no registered botanical repellents for use against FCM. This study identified three true repellents of FCM, including two botanical compounds and one chemical insecticide. This, therefore, shows that there is a need for continued exploration of potential botanical repellents of FCM. Botanical repellents in citrus orchards could be used as standalone treatments to push pests away from orchards or in push-pull systems with attractants. Repellents can also play an important role as part of IPM systems, once they have been deemed to be compatible and not detrimental to non-target organisms.

5.3 INFLUENCE OF VOLATILE ORGANIC COMPOUNDS (VOCs) ON REPELLENTS

Plant volatile organic compounds (VOCs) are the most diverse secondary metabolites that play an important role in mediating communication, defence, and adaptation to environmental stresses like climate change (Abbas et al. 2022). These include compounds like terpenes, aldehydes, alcohols, and esters (Dudareva et al. 2004). These compounds play an important role in facilitating interactions between plants and their environment, such as attracting pollinators and defending against herbivorous insects (Loreto et al. 2014, Abbas et al. 2022). VOCs are synthesised via multiple biochemical pathways and are either released continuously or as a response to stress, such as herbivory (Loreto et al. 2014). Their production is governed by complicated mechanisms, such as transcriptional control, epigenetic modifications, and polyploidy, which all play a role in plant adaptation to their environment and their evolution (Picazo-Aragonés et al. 2020). Advanced technology for VOC analysis, such as headspace collection, EAG (electroantennography) and gas chromatography-mass spectrometry, have improved understanding of VOCs and their biosynthesis and ecological roles (Tholl et al. 2006, El-Shafie & Faleiro 2017). Nonetheless, there are still challenges in the collection and analysis of VOCs, and further research is required to fully harness their potential for pest management, especially when it comes to scaling up their use for industrial applications. VOCs not only play

a role in plant biology but also have a lot of influence on atmospheric chemistry and offer promising applications in agriculture (Loreto et al. 2014).

In agriculture, VOCs play an important role in ensuring sustainable agriculture and the management of pests. They can be used as repellents, attractants, odour maskants, and oviposition deterrents. They can attract or repel pests, and attract natural enemies, as they bolster plant defences against biotic and abiotic stresses (Brilli et al. 2019, Zhou & Jander 2022). Therefore, understanding the chemical interactions of different VOCs within an environment is important for the development of effective pest management systems.

VOCs released by natural hosts, such as fruit and plants play an important role in shaping the degree of efficacy of insect pest repellents. Insect pests use these chemical signals to locate and oviposit on a suitable host (Deletre et al. 2016). Natural host VOCs can interact with repellents, either improving or diminishing their effectiveness (Bruce & Pickett 2011). This study showed that the presence of orange volatiles had an impact on the oviposition preference of FCM. FCM oviposited more on real fruit than artificial fruit, indicating that host plant volatiles were still attractive even in the presence of repellents. While for most of the repellent's oviposition was very low, lavender oil lost its repellent effect in the presence of natural host VOCs, indicating that natural host volatiles managed to overcome lavender oil's volatiles. This emphasises the importance of evaluating the repellency of compounds in both settings, on artificial surfaces and on the natural host. Some of the repellents performed well on artificial surfaces but lost their effectiveness when the natural host VOCs were present which, therefore, disqualifies them from being repellents of that pest. For repellents to be considered for further evaluation and formulation they must perform well in the field and when in the presence of host VOCs. Moreover, some varieties of citrus are more attractive to oviposition by FCM than others. Therefore, there is a high possibility that the efficacy of repellents and odour maskants would vary between different citrus varieties (Love et al. 2014).

5.4 RELEVANCE TO PUSH-PULL SYSTEMS

A push-pull system in pest control is a behaviour manipulating strategy in which natural hosts are made unattractive, and pests are directed towards a trap crop (Cook et al. 2007). This is a semiochemical-based approach that repels pests from the main crop and attracts them to trap crops (Hassanali et al. 2008). Push-pull systems offer a variety of advantages, such as reduced

use of synthetic chemicals, improved natural enemy diversity and effectiveness, and also improved quality of the soil (Hassanali et al. 2008, Bhattacharyya 2017). Development and improvement of these systems require consideration of various cues and spatial ranges that influence the behaviour of the pest during host selection and their sensory modalities (Eigenbrode et al. 2016).

Push-pull systems were originally developed for managing maize stem borers in Africa and have since been adapted for many different pests and crops (Khan et al. 2010). For Lepidoptera, such as maize stem borers, push-pull systems have been implemented with success, reducing pest infestation by up to 80% (Khan et al. 2000, 2010, 2018). Push-pull systems have also been investigated for the control of cotton bollworm, *H. armigera*: neem seed kernel extract was sprayed on cotton, *Gossypium hirsutum* (Malvales: Malvaceae) to push the pest away from the crop and okra (Arka Anamika), *Abelmoschus esculentus* (Malvales: Malvaceae) or pigeon pea (APK 1), *Cajanus cajan* (Fabales: Fabaceae), were planted as a trap crop treated with a nucleopolyhedrovirus (NPV) to infect and kill the pests. This system significantly reduced cotton fruit infestations and damage by cotton bollworms (Duraimurug & Regupathy 2005). This shows the potential of these systems for the management of lepidopteran pests. This last example also indicates that push-pull systems may be compatible with some of the components of IPM systems in citrus; pests can be lured into a trap crop that is treated with a pesticide or virus. This would, therefore, reduce MRLs on fruit as the chemical pesticides would be applied on a trap crop. Push-pull systems have also been successfully investigated for the control of various other pests, such as the Colorado potato beetles, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), pea leaf weevils, *Sitona lineatus* (Coleoptera: Curculionidae), pollen beetle, *Meligethes aeneus* (Coleoptera: Nitidulidae) and Western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae) (Cook et al. 2007). This further shows the potential of push-pull technologies in different systems and for management of various pests in agriculture. When used alone, the components of a push-pull system (attractant and repellent) may have reduced efficacy compared to when paired together, therefore push-pull systems have the potential to improve the efficacy of botanical attractants and repellents (Cook et al. 2007).

Currently, there are no available push-pull systems for the management of citrus pests. Therefore, there is a potential for studies to look at the management of citrus pests using push-pull systems. The citrus industry relies on an IPM system, which involves a variety of techniques, such as biological control, cultural control, SIT and chemical control (Malan et al.

2018). For push-pull systems to be successful in citrus it would have to be compatible with the already existing IPM system, which may be a challenge. The challenge would arise because IPM systems consist of many components including the use of natural enemies, therefore, it is possible that identified repellents and attractants might have negative effects on some of these. Therefore, implementing push-pull systems would be made difficult, and more research would need to be conducted on the extent of potential damage by botanical attractants and repellents on beneficial insects' and if it would be justified to go ahead and implement them.

In this study apparent attractants of FCM to successfully investigate in a potential push-pull system were not identified. However, this study formed a foundation on which future research can build for the exploration of botanicals for use in push-pull systems for management of FCM.

5.5 IMPLICATION IN PEST MANAGEMENT AND BROADER IMPACT

The findings of this study offer valuable information for the development of environmentally friendly and sustainable control strategies for FCM. By identifying repellents and odour maskants of FCM, evaluating the impacts of VOCs on the effectiveness of repellents, and evaluating potential attractants of FCM for use in push-pull systems, this contributed to a growing body of research aimed at reducing reliance on synthetic chemicals and improving IPM systems. The implications of this study go beyond just FCM management and touch on broader pest control strategies and environmental sustainability.

FCM is a major pest of citrus and a phytophagous pest of many crops that can cause notable losses if not managed properly. Current management practices rely on the use of synthetic chemicals, which pose a risk of resistance development by pests, non-target effects, and regulatory constraints. The identification of botanical repellents for FCM offers a promising alternative, using natural compounds to deter pests from ovipositing on fruit and causing damage. Botanicals are biodegradable and pose lower risk to human and environmental health making them an attractive option for IPM programmes (Haritha et al. 2021).

In evaluating the impacts of VOCs on repellents, this study showed that host volatiles can significantly influence the efficacy of repellents. This stresses the importance of considering the complexity of the chemical ecology of pest-host interactions when developing repellents

(Regnault-Roger et al. 2012). This is because for a repellent to be approved for registration, it would have to be best suited for not just the pest but also cause minimal damage to non-target organisms, particularly beneficial arthropods (Regnault-Roger et al. 2012, Lengai et al. 2020). Host volatiles can mask or enhance the repellent effects of various compounds, suggesting that their presence could either compromise or enhance the repellent efficacy of botanicals. Therefore, understanding all these interactions is crucial for the management of FCM using botanicals. This is because FCM relies heavily on the olfactory cues of the host for localisation.

While the study identified effective repellents, the lack of success in identifying strong attractants presents a key challenge. Nevertheless, repellents and odour maskants can be used to enhance the push components of systems and reduce pest pressure on crops. Additionally, using these findings to develop effective push-pull systems for FCM using botanicals is important and will reduce the issue of repellents deteriorating quickly and the chance of pests adapting as exposure to multiple-modal disruptions or multiple stimuli of host-seeking behaviour could reduce FCMs' likelihood of overcoming the repellents. When FCM is exposed to different semiochemicals at the same time (attractants and repellents), they will not have enough time to adapt to botanical repellents as they will be directed away from the repellent treated areas towards the attractant treated areas, therefore increasing the efficacy of repellents.

On a broader scale, this research also has the potential to inspire similar studies for many other pest species. Like FCM, many species of insects rely on olfactory cues to locate their host. Therefore, the approach of evaluating botanical repellents and attractants and investigating the impact of host VOCs can be applied across diverse pest systems. The use of botanicals aligns with the global trend of reducing the use of synthetic chemicals and moving towards more sustainable agriculture. This study also highlighted the importance of integrating chemical ecology into pest management research. The dynamic interactions between pest behaviour, host volatiles, and repellent compounds reveal the complex process of developing control strategies that are effective. As global climate change, global trade, and fast-growing populations increase pest pressure, such nuanced approaches will be important for maintaining agricultural productivity. This study therefore aligns with UN Sustainable Development Goals (SDGs) as it promotes pest management using environmentally friendly techniques, reducing use of chemicals, conserving biodiversity and supporting food security.

5.6 LIMITATIONS AND FUTURE DIRECTIONS

Due to regulations on pesticide use and residue levels on fruit by our export partners, the South African citrus industry is moving towards the use of environmentally friendly control methods for IPM systems (Ndou & Obi 2013, Grové 2022). Therefore, many different avenues have been explored, including the use of botanicals (Coetzee et al. 2022). Botanicals are very diverse and are available in many different forms, including essential oils and crude extracts.

Laboratory studies do not represent real field conditions. Therefore, they do not consider factors that might interact with and affect repellents in the field, such as wind, temperature, and sunlight. The chemical makeup of botanicals is complicated and can vary depending on many factors, such as cultivation practices, cultivar, abiotic factors, and competition within the environment, thus, potentially impacting the effectiveness of these botanicals in field settings (Dougoud et al. 2019). Therefore, results obtained in laboratory studies might not translate into the field. Future research should also consider the effectiveness of the identified repellents and odour maskants in semi-field settings. As stated earlier in this chapter, some varieties of citrus may be more attractive to FCM oviposition than others. Therefore, botanical repellent and attractant efficacy studies would have to be done for all the different citrus cultivars (Love et al. 2014).

This study did not include multiple choice assays with treated and untreated artificial oranges and real fruit. This would have provided a better idea of the effectiveness of repellents when natural host VOCs were present. Multiple-choice assays give a more realistic idea of insects' response to various stimuli; some studies have included multiple-choice assays (Fullard & Hill 2013, Love et al. 2014). Additionally, laboratory assays focus on short-term responses, which are not reflective of long-term patterns of behaviour in the field, nor possible adaptation. In this study many attractants were not evaluated, due to limited literature on botanical attractants of lepidopteran pests. This, therefore, limited the likelihood of success. Furthermore, an increased number of replicates in future studies could provide a better understanding and improve the statistical power of the findings. Additionally evaluating higher concentrations might also yield different or better results.

Improved choice methods for choice assays are required that would account for various behaviours by FCM. An automatic sprayer device, such as the one used in perfumes containing the potential repellent or attractant can be mounted next to the fruit on the Y-tube olfactometer chambers to spray periodically. This would prolong the effect of the botanicals, as repeated

spraying with these low volumes of botanical solution and suspensions would have a longer-lasting effect.

Botanical compound use against citrus pests, such as FCM, is not very well studied. Therefore, more studies need to explore various botanicals against FCM, their efficacy, and how they interact with the environment and other organisms, including beneficial insects. Many botanicals, especially essential oils, have been reported to have concerning insecticidal effects on a variety of non-target beneficial insects (Campolo et al. 2018, Giunti et al. 2022). Therefore, more research is needed to better understand botanicals' long-term non-target effects in the field, given the importance of parasitoids and predators in citrus orchards, not only against FCM, but also other important citrus pests. If successfully efficacious in the field, without phytotoxic effects, future studies could also look at developing these botanicals into dispenser-type systems for application in orchards. When evaluating the impact of natural host VOCs on the effectiveness of repellents, future studies must include untreated artificial and real fruit to look at multiple-choice assays.

In the case of potential botanicals of FCM, a broader spectrum of potential attractants must be evaluated. This is important, as they form integral parts of push-pull systems. Evaluating the synergistic effects of repellents and attractants is very important, as they might work and interact with the pest differently when used together. This control strategy would target female FCM, to prevent them from ovipositing on fruit. Therefore, these botanicals would have to be strong attractants for FCM females.

Pests have been reported to have the ability to develop resistance against synthetic chemicals. This shows the pest's ability to adapt to different chemicals and chemical stimuli (Tiwari et al. 2011, Chen et al. 2020). Therefore, some future studies must look at how continuous exposure of pests to botanicals will affect the efficacy of the botanicals and the long-term effects and potential adaptation of insect pests to repellents and attractants (Crossley et al. 2021).

Some botanicals have been reported to have insecticidal effects (Alkofahi et al. 1989, Isman 1997, Ngegba et al. 2022). Therefore, going forward, research needs to look at IPM compatibility of botanical attractants and repellents. The implication for using these botanical repellents and attractants in orchards where SIT is employed should also be investigated. This would mean investigating all of the constituents of various botanicals, as they could have a negative impact on non-target organisms (Isman 1997). This presents another challenge, as this would be very expensive and takes a long time to do. Consequently, going forward, cheaper

and more efficient methods need to be investigated and potentially employed. Having a standard method to quantify active ingredient(s) in botanicals would help botanicals with regulatory compliance and aid towards ultimate commercialisation. Research in this regard is therefore warranted (Isman 1997).

5.7 CONCLUSION

This thesis has demonstrated true repellence and odour masking by various botanicals and registered synthetic chemicals against FCM in laboratory conditions. These results emphasise the importance of evaluating the nature of the mode of action of botanicals in agricultural pest management. Additionally, this forms a foundation for semi-field studies to be done on the control of FCM, using botanicals of different natures (repellents and maskants). This is a positive result for the management of FCM in the citrus industry because if these can maintain efficacy in the field and be compatible with existing IPM systems and reach registration, the number of chemical insecticides used could be reduced. The South African citrus industry continues to rely on synthetic chemicals for FCM control. Therefore, if more environmentally friendly alternatives were available, chemical residue levels on fruit would be reduced. The use of botanicals in agriculture may even contribute towards improved soil fertility and soil health, ensure the health of farm employees, and improve the diversity of natural enemies and other beneficial insect species like pollinators. The use of botanicals is also advantageous in a manner that repellents and attractants can be used alone or together to form push-pull strategies. Additionally, small-scale growers may be able to crudely produce their own botanicals when the relevant plants are readily available in the environment. The identification of botanical repellents and odour maskants for FCM control offer a promising alternative for strengthening phytosanitary compliance in citrus production. Additionally, incorporation of botanical repellents in IPM systems for FCM may further reduce interception rates during exports and as a result, strengthen market access.

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