Dispersal of sterile false codling moth,

Thaumatotibia leucotreta (Meyrick)

(Lepidoptera: Tortricidae), for a Sterile Insect Technique programme on citrus.

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For the degree of:

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Abstract

The false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is an important pest of citrus in South Africa and challenging to manage due to its inconspicuous nature. An effective method currently being employed for the area-wide suppression of the FCM is the Sterile Insect Technique (SIT) and the effective dispersal of sterile moths is very important for success with SIT. This study was conducted in the Addo area of the Sundays River Valley (Eastern Cape) where the programme is commercially used. In this study, sterile male moths were released in different orchards on a citrus farm, and in nearby veld at different times of the year, and their dispersal was monitored through the use of pheromone traps. Various climatic factors were monitored. This provided insight into the local dispersal of sterile male FCM adults in response to abiotic cues (particularly climatic factors). The movement of the FCM in four citrus cultivars, namely lemons, navel and Valencia oranges and mandarins and in the nearby veld (open field), was determined at six different stages of the year.

Results clearly indicated that sterile FCM movement is concentrated within citrus orchards, as very few moths were trapped beyond 30 m from the release point, particularly in navel and Valencia orchards. Of the climatic factors measured, minimum and maximum temperatures had the most significant influence on FCM dispersal, and based on the results, various recommendations are made for the releases of sterile FCM in an area-wide SIT management programmes on citrus. A better understanding of the dispersal capabilities of the FCM in an agricultural system, under different conditions and at different times of the year, is invaluable not only in improving release strategies in an SIT programme but in planning future control strategies against the FCM.

Declaration

I, Gideon Daniel Wagenaar (student number 20435031), hereby declare that this thesis for a student's qualification to be awarded is my own work and that it has not previously been submitted for assessment or completion of any postgraduate qualification at another university or for another qualification.

Gideon Daniel Wagenaar   Date: 31/03/2015

Chapter I GENERAL INTRODUCTION

1.1 Introduction

Effective control is important due to associated economic losses and the pest's phytosanitary status for many export markets. The false codling moth (FCM), *Thaumatotibia leucotreta (*Meyrick) (Lepidoptera: Tortricidae), is a pest of citrus in South Africa (Newton, 1998). Several different modes of control are registered and used commercially, targeting different life stages (Moore & Hattingh, 2012). One of these is the Sterile Insect Technique (SIT), which was commercialised in the Western Cape in 2007 and in the Eastern Cape in 2011, and now being applied over several thousand hectares (Nepgen, 2014). The principle of SIT for FCM is to flood citrus orchards, weekly with large numbers of radiation-induces sterile moths at a target ratio of 10 sterile moths to one wild male moth (Stotter, 2009).

This area-wide means of controlling the FCM has been thoroughly researched, and has resulted in a reduction in FCM infestation of up to 94% (Hofmeyr & Hofmeyr, 2006). However, one aspect of the application of an SIT programme for FCM that has warranted further research is the dispersal capacity and behaviour of the released sterile moths. A report on such a study follows.

The FCM is the most important phytosanitary pest restricting the export of South African citrus into many of South Africa's overseas markets, including the UK, USA, China, Iran and Japan. A record 113 million cartons of citrus were exported from southern Africa in 2013 (CGA 2013). South Africa is the second largest exporter of citrus in the world (CGA 2013).

1.2 Distribution of the false codling moth

The insect is endemic and indigenous to Africa, generally south of the Sahara and mostly in tropical and subtropical areas (Schwartz, 1981).Although the FCM occurs in all citrus producing areas of southern Africa, pest pressure varies dramatically in the different regions and is generally less abundant in the far northern areas (Moore & Kirkman, 2011a). The moth is known to occur in citrus in South Africa, Mozambique, Zimbabwe, Swaziland (Hepburn, 1947; Stofberg, 1954) and Malawi (Sweeney, 1962).

1.3 Host plants of the false codling moth

The FCM has invaded cultivated crops from its wide range of indigenous host plants (Gunn, 1921; Stofberg, 1939, 1954; Pearson, 1958; Schwartz, 1981). Schwartz (1981) reviewed some 21 cultivated and 14 indigenous wild host plants in southern Africa alone. In cultivated crops it is particularly severe on citrus (fig. 1), but also attacks many other deciduous, subtropical and tropical fruits (Daiber, 1980). A recent survey in the Western Cape revealed few alternative hosts (Honiball, 2004). This is not the case in the Eastern Cape (Kirkman & Moore, 2007), as FCM infestation reaches its peak relatively early in the growing season before the fruit matures (Moore *et al.*, 2005), indicating a build-up on other hosts before ripe citrus fruit are available in meaningful quantities.

Navel oranges yielded three times as many moths as Valencia fruit in laboratory trials (Georgala, 1968). Grapefruit and mandarins are less susceptible, and in lemons and limes larval development is rarely, if ever, completed (Gunn, 1921; Newton, 1998; Moore & Kirkman, 2011a). The FCM is also known as a pest of acorns, walnuts, olives, tea seeds, and almonds and infests cotton in most equatorial areas (Newton, 1998).

Fig. 1 Navel orange infested with the false codling moth

This may be because of their greater acidity and excessive juice. Unlike Zimbabwe and East Africa, there is no record of the FCM attacking cotton in South Africa. Pearson (1958) suggested that under South African conditions the moth preferentially confines itself to ripening citrus fruit during late summer and winter when cotton bolls might be susceptible.

1.4 Life history of the false codling moth

In South Africa the FCM has about six generations per year (Bloem *et al*., 2003) and the main adult activity peaks occur in summer and autumn (Hofmeyr & Calitz, 1991). The generational peaks are in December, January, March, May, September and November in the Eastern Cape (Moore pers. comm). The life cycle of the FCM includes egg, larval, pupal and adult stages. The complete life cycle ranges from 30 days (under optimal conditions) to 174 days (under least optimal conditions) (Venette *et al.*, 2003). Within an uninterrupted supply of plant hosts, adult FCM remain active throughout the year and there is no diapause (Diaber, 1980).

1.4.1 Egg. The egg is flat (Fig 2) oval and translucent with a shiny reticulate sculpture. It measures approximately 1 mm in diameter and is frequently inconspicuously in a depression of the rind (Daiber, 1979a). In laboratory cultures eggs are laid on any clean flat surface. At an optimum temperature of 25°C females can lay three to eight eggs per fruit and up to 800 over their life span. If there are numerous females many eggs can accumulate on the fruit. However, only a few survive due to larval competition for food and cannibalism (Newton & Crause, 1990). The egg is susceptible to parasitism by trichogrammatid parasitoids (see 1.6.4) for about half of its typical life span of six to 12 days (Hepburn, 1947; Georgala, 1969; Daiber, 1979a; Schwartz, 1981). Hatching occurs at all times of the day.

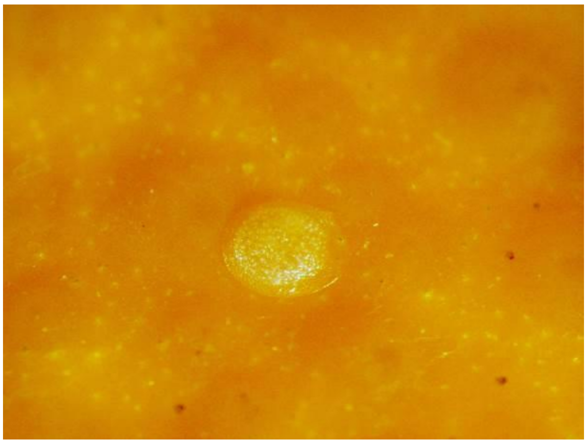


Fig 2 False codling moth egg on a fruit surface (Peter Stephen, Citrus Research International)

1.4.2 Larva. The first instar is extremely delicate and frequently suffers high mortality. Low humidity causes egg and first instar mortality in laboratory cultures, while low winter temperatures are lethal to those stages in the field (Catling & Aschenborn, 1978; Daiber, 1980). There are five larval instars (Stofberg 1954; Daiber 1979b). Younger larvae feed near the surface while older larvae bore towards the centre. Temperature and poor food quality can slow down the rate of larval development. The young larva is often cannibalistic towards eggs, and the larva completes its development in a single fruit (Catling & Aschenborn, 1978). If the host has a hard rind, such as an acorn, entrance is made at the base or attachment to the cup where softer tissue exists. When the host has a soft rind, such as citrus or peaches, the larvae will burrow into the rind almost anywhere. Larvae prefer the navel end (of a navel orange) – or an injured area or cut in the rind. In some hosts, such as avocado, the entrance is marked by the formation of a raised blemish on the rind (Diaber 1979b; Newton & Crause 1990).

The larval period lasts 12 to 33 days in warm weather and 36 to 67 days in cool weather. By the time the larva is ready to leave the fruit, the fruit might already have dropped (Daiber, 1979c; Newton, 1998).

Fig 3 A false codling moth larva in an orange

1.4.3 Pupa. After the pre-pupal stage, the insect pupates in loose soil beneath surface debris or in cracks in the soil. It constructs a silken cocoon incorporating trash and soil particles. Pupae are cream coloured and soft, maturing to a hardened dark brown (Gunn 1921; Newton, 1998). The completed cocoon closely resembles the soil and is difficult to find. The cocoon invariably lies on the soil surface (Stofberg, 1954). The pre-pupal and pupal stages occur within the cocoon. The pre-pupal stage is light beige in colour (Newton, 1998) while the pupal stage is dark brown (Stofberg, 1954). The pupal stage is completed within 21 to 80 days in the field, depending on the time of year (Daiber, 1979c).

1.4.4 Adult. The adult moth is small and dark brown to grey. Males are smaller than females and can be distinguished by densely packed, elongated scales on the hind tibia, an anal tuft of scales, and a scent organ near the anal angle of each hind-wing (Gunn, 1921; Hepburn, 1947; Stofberg, 1954; Georgala, 1969; Daiber, 1980; Newton, 1998). Females mate shortly after their emergence from pupae, within two to three days (Stofberg, 1954), and commence laying eggs.

Fig 4 Adult sterile false codling moth

1.5 Economic importance of the false codling moth

The South Africa citrus industry is large with South Africa ranked as the second largest exporter in the world after Spain (CGA Key Industry Statistics, 2013). In 2013 South Africa exported approximately 113 million cartons (each weighing 15 kg) of citrus varieties to foreign countries (CGA Key Industry Statistics, 2013). Annual losses of more than R100 million ten years ago to the southern African citrus industry are attributed to the FCM (Moore, 2004a). These losses are mostly caused by a reduction in yield at orchard level, caused by fruit dropping off the trees and post-harvest decay due to undetected infested fruit that are packed and exported. All citrus cultivars are susceptible to attack with the exception of lemons and limes.

Navel oranges, which contribute significantly to the total citrus production in South Africa, are particularly susceptible to FCM attack (Georgala, 1968). Within the cultivar greater numbers of eggs are laid on certain selections than on others (Love et al, 2014). Infested fruit drop from trees as early as November, when fruit are no more than 15 to 20 mm in diameter (Stofberg, 1954; Newton, 1998). In extreme cases of infestation FCM can cause reductions of up to 80% (Hofmeyr, 2003). Newton *et al.* (1986) reported that in surveys in the Rustenburg and Nelspruit areas of Mpumalanga Province area 20% to 30% of total fruit drop was due to the FCM, while up to 90% of fruit drop on farms in the Citrusdal area, Western Cape Province, was due to FCM infestation. Newton (1988a) noted losses of 10% to 20% in certain navel orange cultivars on selected farms in the Citrusdal area between 1982 and 1985. Control of the FCM is therefore extremely important.

Some foreign markets regard the FCM as a phytosanitary pest and will reject an entire consignment if its presence in fruit is recorded (Moore, 2002a; Kirkman, 2007). The increased risk and potential threat of FCM established outside southern Africa recently resulted in a zero tolerance policy for FCM enforced at pack houses that send fruit to sensitive markets. The USA in particular is concerned with the establishment there of the FCM due to a similar climate to that of South Africa. If the moth were to establish itself in the USA, substantial economic losses would be experienced (Stibick, 2008). Certain markets, for example, the USA, require a cold sterilization process on fruit destined for these markets.

1.6 Control of the false codling moth

1.6.1 Population monitoring

Population monitoring systems allow a forecast of the FCM populations present in citrus orchards and allow growers to make important decisions for appropriate and necessary control interventions

(Schwartz, 1972). Inspecting for FCM eggs on citrus fruit is difficult as they are very small and transparent. The only effective means of monitoring FCM population levels is a pheromone-based trapping system (Hofmeyr, 2003). The trap consists of a sex pheromone dispenser that attracts male moths and a polybutene-based adhesive for ensnaring the moths. These components are contained in a beige PVC pipe or a yellow delta trap.

This kind of monitoring system has been regarded as essential for the development of a practical programme for any means of effective control of the FCM (Schwartz, 1972). For the first couple of years of monitoring with these traps, it is important to observe infestation, fruit drop and damage in relation to the trap counts and to capture the data of each orchard. This historic data can assist growers to decide when to apply control measures (Hofmeyr, 2003). These threshold values (of 10 adult male moths per trap per week) do not apply anymore due to the phytosanitary status of the FCM and as a result corrective measures should be applied regardless of the population levels in traps and fruit inspection points (Moore, 2011). A peak in trap catches can be used for accurate timing of a corrective application by assuming that a peak in egg hatch would occur two weeks later (Moore, 2011).

1.6.2 Orchard sanitation

Orchard sanitation is the regular removal and destruction of all fallen and hanging fruit which are infested, damaged or decaying, and remains the single most important FCM control measure, with other measures being complimentary to sanitation (Moore & Kirkman, 2008). Research has shown that it is possible to remove an average of up to 75% of FCM larvae from an orchard by conducting weekly orchard sanitation from December to June (Moore & Kirkman, 2008). Until the 1980s the only method of controlling the FCM in citrus orchards was sanitation (Moore, 2002).

The purpose of this sanitation procedure is threefold (Du Toit, 1998). Firstly, for control of FCM, secondly for control of fruit fly and thirdly, for removal of fungal spores from the orchard, which are capable of causing primary infection or secondary decay of fruit.

Fig 5 An example of poor orchard sanitation

Schwartz (1974) stated orchard sanitation should begin immediately after harvesting of an orchard is completed. All fruit on the ground and left hanging in trees must be removed, thus eliminating any possible means of FCM completing its life cycle over winter. This will help restrict the overall size of the FCM population during the following season. However, numerous field trails with the FCM throughout the country indicated that fruit infestation most often peaks during early December, so it is essential that orchard sanitation starts no later than early December (Moore et al, 2004). This will enable the removal of invested fruit ensuing from the normal November/December peak in FCM population to restrict the overall size of the FCM population during the following season.

Larvae most often leave fruit soon after they have fallen. Therefore, for the best results, sanitation must be conducted at least at weekly intervals (Moore & Fourie, 1999; Kirkman *et al*., 2008). Mature larvae sometimes leave the fruit while they are still on the tree. It is therefore strongly recommended that obviously infested fruit on the tree must be removed during sanitation (Schwartz, 1974).

Fig 6 An example of an orchard where good sanitation has been conducted

The false codling moth is an extremely challenging pest to control. Eggs are laid continually during the fruiting period of citrus and on hatching the larva bores into the fruit within a few hours. As a result it is imperative that producers put a concerted effort into sanitation. In a well-replicated experiment in the Eastern Cape Province, Ullyett and Bishop (1939) found that the total loss of fruit after November was reduced from 6.1% in un-sanitised trees to 3.3% with sanitation once a week.

From April until completion of the harvest, orchard sanitation should be done on a weekly basis and where possible more often. All fruit removed from the orchards should be burnt in an incinerator, buried under a 30 cm layer of soil or crushed with a fruit crusher (Fig 7) (Hepburn, 1947; Georgala, 1969; Newton, 1998). Caution should be taken not to dig a pit that cannot be filled and covered immediately, as larvae from the first lot of fruit will have time to escape and developed further. Fruit can also be placed in old petrol drums half-filled with water, sealed with lids and left for six days (Stofberg, 1954).

Fig 7 Fruit collected from orchard sanitation crushed by a fruit crusher

1.6.3 Microbial control: granulovirus

Three virus products based on the *Cryptophlebia leucotreta* granulovirus (CrleGV) are registered in South Africa for the control of the FCM on citrus and have been successfully incorporated into Integrated Pest Management (IPM) programmes (Moore, 2002). These products are Cryptogran, Cryptex (Moore, 2002) and Gratham (Moore, pers. comm.). When applied correctly FCM control has been recorded for up to 17 weeks with a single application, with an average of 70% reduction in infestation over that time (Moore *et al.*, 2004). Up to 87% reduction in FCM infestation has been recorded in field trails on navel oranges with a single application of Cryptogran (Kirkman *et al*., 2008). Molasses has been shown to significantly and consistently improve the efficacy of virus products for the control of the FCM (Moore *et al*., 2004). This is likely to be the result of the feeding attractant and sticker effects of molassses (Hilliar & Hill, 2013). The sugar component of molasses may cause neonate larvae to feed more actively on the surface of the fruit before attempting penetration into the fruit (Moore *et al*., 2004). This will result in the ingestion of a lethal dose of virus before penetration behaviour begins (Hilliar & Hill, 2013). Cryptex is now registered to be applied without molasses (Hilliar & Hill, 2013).

1.6.4 Biological control

Biological control forms an important part of the natural control of the FCM in many areas. Ullyett & Bishop (1939) listed 25 known natural enemies of the FCM of which 12 species are known to occur in South Africa. Many biological control options have been tried against the FCM in citrus orchards. These include parasitoids, pathogens (e.g. CrleGV referred to in the previous section), and predators. Four egg parasitoids and two larval parasitoids have been identified as having potential as biological control agents (Moore & Fourie, 1999). Compared to larval and pupal parasitoids, egg parasitoids should be considered the most promising, because if applied correctly and if they are effective, they will control the FCM before damage occurs, in the same way as insecticides do. However, larval and pupal parasitoids only control the pest after the damage has been done (Newton, 1998; Moore & Fourie, 1999). Nevertheless, conditions in some regions are more conducive to the proliferation of this natural enemy, namely egg parasitoids (*Trichogrammatoidea cryptophlebiae*), than in other regions.

The commercial rearing and releasing of natural enemies for suppression of the FCM was considered as early as 1939 (Ripley *et al.*, 1939). Probably the most effective predators are ants, which have been shown to dramatically reduce planted false codling moth pupae in research trials (Bownes *et al*., 2014). This is a strong justification for not poisoning ants on an orchard floor but rather just eliminating them from entering trees, where they can disrupt natural enemies of other pests.
Release of the egg parasitiod *T. cryptophlebiae* should be initiated as early as October, and should be released repeatedly while the fruit susceptible. Four releases of 25 000 per hectare are usually adequate, except in the Western Cape where a fifth release per hectare is required (Moore *et al.*, 2004). Undisrupted by injudicious spraying, between 80% and 100% egg parasitism of the FCM has often been recorded, resulting in up to a 67% reduction in infestation in navel oranges from December to harvest (about May) to the total elimination of the FCM by harvest time (Moore & Fourie, 1999; Moore & Richards, 2000, 2001 & 2002). Every effort possible should be made to avoid disruptive sprays thripicides have the worst affect during the season.

1.6.5 Chemical control methods

No insecticides were registered for use against the FCM on citrus until the early 1980s. However, the first chemical trials for FCM control were conducted by Gunn in about 1926, but the results were not satisfactory (Hepburn & Bishop, 1954). Twenty-one years later in 1947 DDT was found to reduce fruit infestation by about two thirds or more (Hepburn, 1947, 1949a). Gammexane, fixed nicotine (Myburg, 1948) and parathion (Thiophos) (Hepburn, 1949a) were also found to reduce infestation by the FCM, but not to the same extent as DDT. In later field trails two synthetic pyrethroids, cypermethrin and deltamethrin, applied two to three months before the harvest, reduced fruit drop due to the FCM by an average of 90% (Hofmeyr, 1983b). In some regions a reduction in efficacy of pyrethroids due to resistance development was subsequently reported (Hofmeyr & Hofmeyr, 2005). Through the years the main focus on the application of insecticides has been to achieve the highest kill of the target pest possible (Debach, 1974). This philosophy, however, comes with its own problems, namely, many of these products have not been entirely compatible with integrated pest management programmes and have been detrimental to natural enemies, causing secondary pest repercussions.

Nomolt SC (teflubenzuron), a benzoylurea insecticide, is also registered for the control of the FCM on citrus. The efficacy of Nomolt has been questioned recently due to multiple cases of resistance (Moore, 2002), to the pyrethroids and in its use. Alsystin (triflumuron) is also registered for chemical control of the FCM in citrus. However, the FCM has developed resistance to this product in the Western Cape Province (Hofmeyr & Pringle, 1998) and possibly in the Mpumalanga Province (Moore, 2000). Alsystin is also known to be detrimental to the egg parasitoid, *Trichogrammatoidea cryptophlebiae* (Hattingh & Tate, 1997).

Two other pyrethroids, Meothrin and Cypermethrin, are registered for commercial FCM control. They are potentially toxic to a wide range of natural enemies and their effectiveness is variable (Hofmeyr, 2003; Moore *et al*., 2004b).

In 2011 two new chemical insecticides were registered for use against the FCM, namely Delegate and Coragen. These two products appear to have comparable efficacy, usually reducing FCM infestation by between 50% and 60% if applied correctly (Moore & Hattingh, 2012).

1.6.6 Attract-and-kill

This product consists of a synthetic pheromone and a pyrethroid (permethrin) in a gel formulation and is applied by hand using a pre-calibrated dispenser that delivers 50 ɥl drops. Male moths are attracted to the pheromone and are killed soon after making contact with the pyrethroid active ingredient. The only attract-and-kill product registered for controlling the FCM on citrus in southern Africa is Last Call FCM (Moore & Hattingh, 2012). Field trials indicate that its efficacy when used together with mating disruption is superior to that of attract-and-kill against the FCM (Hofmeyr & Hofmeyr, 2002). However, this was tested in a situation of fairly high FCM pressure and by all accounts its efficacy is better in low-pressure FCM regions (Moore & Hattingh, 2012).

1.6.7 Mating disruption

During 1999 the first mating disruption product for FCM control (developed by BASF) was registered for use on citrus. A few years later Isomate was registered (Moore & Hattingh 2012). Another mating disruption product, Checkmate FCM-F, which is a spray-applied capsule suspension, was not as effective as Isomate (Moore & Kirkman, 2010, 2011a). Mating disruption and attract-and-kill are still being used for the control of FCM but to a lesser extent, but both are being used as part of an IPM package and not as a stand-alone treatments.

1.6.8 Sterile Insect Technique

Sterile Insect Technique (SIT), a method of releasing sterile insects into a wild population in an effort to control them, was independently pioneered by three researchers in the early twentieth century. Serebrovskii's genetic studies on *Drosophila melanogaster* (Meigen) (Diptera: Drosophilae) at the Moscow State University in the 1930s and 1940s, supported the principles of Mendelian genetics for the advance of Soviet agriculture by the use of chromosomal translocations to cause inherited partial sterility for pest population suppression (Robinson, 2002). In Canada, SIT proved to be successful in the control of the codling moth, *Cydia pomonella* (Bloem & Bloem, 2000). This technique has also been used to eradicate and suppress American screwworm, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae), in the United States and Mexico. SIT has also been used against tropical fruit flies (Diptera: Tephritidae) in many countries, tsetse flies (*Glossina* spp) in Zanzibar, horn fly *Haematobia irritans* (Linnaeus) (Diptera: Muscidae), on cattle in Texas, and very successfully against pink bollworm on cotton in California (Pedigo & Rice, 2006). This is achieved through reproductive mate attrition. Generally the male insect is exposed to radiation and thereby rendered sexually sterile. With this method, sterile males are released at an over-flooding ratio to the wild males, to ensure that the probability of a female mating with a sterile male is higher than her mating with a wild male (Myburgh, 1963; Hofmeyr *et al*., 2004). Mating with a sterile male prevents the female from reproducing (Kirkman, 2007). The transfer of sterile sperm during mating by the sterile males aids in reducing FCM populations within the orchards.

Varying levels of sterility can be induced in insects for release in an SIT programme. Absolute sterility might not always be required and is in fact undesirable for some species in which increasing amounts of radiation will seriously compromise their competitiveness in the field. Lepidoptera require high doses of ionizing irradiation to be fully sterile and can to a certain extent be regarded as radio-resistant (Lachance & Graham, 1984). If they are exposed to sub-sterilizing doses and either inbred or outcrossed with fertile counterparts, their offspring (F1 generation) shows a higher level of sterility than their parents (inherited sterility), and in addition the level of sterility in F1 females is lower than in F1 males.

Spatial modelling work on the SIT (Plant *et al.*, 1984; Wolf *et al.*, 1971; Lewis & Driessche, 1993; Marsula & Wissel, 1994; Barclay, 1992) has confirmed that the consideration of spatial effects is important in the development of effective SIT programmes. Numerous experimental studies have shown that most male moths fly upwind in response to detecting a conspecific female sex pheromone (Carde, 1984). An evenly distributed target population, sparsely dispersed over a wide area, will increase the efficacy of an SIT programme.

For the codling moth the combined release of sterile insects and egg parasitoids was first suggested by Nagy (1973). Experiments by Bloem *et al*. (1998) demonstrated inside field cages that an additive suppressive effect can be realised when sterile moths are released at a 10:1 over-flooding ratio (sterile:wild) together with *Trichogramma platneri* Nagarkatti when compared to containing wild moths that received sterile moths or parasitoids only. In the case of FCM, it was shown to be that an over-flooding ratio of 10:1 (sterile:wild) is maintained in orchards under Sit for it to be successful (Hofmeyr & Hofmeyr, 2004). After mating with a treated male a female FCM will lay largely infertile eggs, thereby reducing the population (Schwartz, 1975). Laboratory experiments by Hofmeyr *et al*. (2004) showed promise in the use of SIT in the control of the FCM in the Western Cape Province of South Africa. In a 35 ha field trial, with SIT Hofmeyr & Hofmeyr (2004) reported a 94.4% reduction in FCM infestation.

Two subsequent trials with SIT conducted in the Eastern Cape and Limpopo achieved more than 80% reduction in FCM infestation (Hofmeyr & Hofmeyr, 2010; Moore 2011b). This technique was commercialised by Xsit (Pty) Ltd in 2007 and is now being applied over more than 4500 ha of citrus in the Western Cape Province and more than 3400 ha in the Sundays River Valley in the Eastern Cape Province with good success (Nepgen, 2014).

Due to the phytosanitary status and resistance by FCM to the commonly used pesticides in all citrus producing areas of South Africa, the potential for SIT has expanded enormously. Other factors, such as insecticidal resistance, the negative effect of insecticides on the environment and consumers opposed to chemical residues on fruit, can be seen as contributing to this potential. The success of an SIT programme is dependent on efficient application of the technology to achieve its objectives in a timeous manner, and also the on dispersal ability of the sterile FCM being released. Another aspect that needs to be studied is the influence or dictation of the weather on the dispersal of sterile FCM.

SIT is not a stand-alone technology, but should be integrated with other pest management technologies, such as bait application, virus sprays and sanitation in an area wide programme (Bloem *et al.*, 2005).

1.7 Dispersal of moths in general

As there is a continued and growing interest in the use of sterile insect technique (SIT) as a tactic for the suppression or eradication of key Lepidoptera pests, such as the FCM and the codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) (Bloem *et al.*, 2005; Carpenter *et al.*, 2005; Simmons *et al.*, 2010; Vreysen *et al*., 2010), the establishment of simple and inexpensive bioassays that can detect differences in the quality of reared, sterilized and released insects, and monitor field performance, is essential.

Animals search for appropriate sources of food, water, mates and oviposition sites for growth and reproduction (Bell, 1991). However, such searching behaviour has costs that animals must balance with potential benefits gained from the resource. These costs include energy expended on movement itself, time taken away from other activities and risk of predation while searching (Bell, 1991). The attraction of male moths to female pheromones is a well-established model for long-distance sexual communication (Mafra-Neto & Carde, 1994; Vetter & Baker, 1984; Vickers et al, 1991; Willis & Arbas, 1991). During pheromone-mediated upwind flight, male moths are considered to be scrambling for females and thus bear major costs of finding a mate (i.e. energy and risk) (Greenfield, 1981; Thornhill & Alcock, 1983). Scramble competition occurs when a finite resource that is shared between competitors, such as a sexually receptive female, is reduced with increasing population density. Fitness (of sterile males) is critical for early arrival (at the females) for mating. Successful location of a calling female depends critically on flight performance. Because muscle efficiency is strongly temperature dependent over a wide range of ambient temperatures, flight muscles of endothermic moths need to be heated before a moth can engage in upwind locomotion (Dorsett, 1962; Heinrich, 2007; Heinrich & Mommsen, 1985; Krogh & Zeuthen, 1941).

Most wild male codling moths are short distance dispersers (Schumacher *et al.*, 1997), often remaining within 1 km of the release site. This form of dispersal is probably typical of sterile codling moths as well. While individual sterile male codling moth have been observed to fly great distances (up to 8 km in one study) (Mani & Wildbolz, 1977), most males are recaptured near release locations. Data in a study by Thistlewood et al (2004) indicated that very few moths were recaptured beyond 800m from any release site, and none more than 3 km away.

Numerous experimental studies have shown that most male moths fly upwind in response to detecting a conspecific female sex pheromone (Carde, 1984). Mating occurs when a male successfully follows a female pheromone plume to its source. The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is known to be able to migrate over long distances (Mackenzie, 1958; Lorimert, 1981; Chu, 1986). However, little is known about its dispersal ranges within active host crops or local dispersal ranges.

The dispersal of released irradiated codling moths was studied using mark-release-recapture tests (Wesling & Knight, 1994; Bloem *et al*., 1998). Reports were made on the progress in Tunisia during the last two years with mass-rearing of the carob moth and assessing the performance of irradiated substerile males in the field. These field assessments were done over a period of three years (Mediouni, 2005).The data showed that the substerilising dose of 400 Gy did not affect the ability of males to disperse under field conditions.

1.8. Dispersal of the false codling moth

Being nocturnal, the FCM is a night flyer (Diaber, 1978) and not known to fly during the day (Stofberg, 1954). Pheromone trapping in transects across the agricultural landscape has shown that FCM males are concentrated within citrus orchards or very close to them. However, some FCM male individuals were trapped at distances of up to about 1.5 km from the nearest citrus orchard or known host plants (Stotter, 2009). This raises questions about the FCM’s dispersal ability. While relatively little is known about the dispersal capabilities of the FCM, significant genetic variation exists between populations in South Africa. Very little is known about female FCM dispersal, particularly after mating (Stotter, 2009). The dispersal of insects over a given area as well as the dispersion ability of individuals plays a significant role in SIT. Controlling a population of insects capable of moving vast distances, such as medfly, normally requires isolation of the treatment area to prevent reinvasion (Hendrichs *et al.,* 2002).

The FCM has been previously described as a poorly dispersing species (Newton, 1998). This assumed poor dispersal ability may be responsible for the occurrence of genetically distinct populations, which may be separated from each other by less than one kilometre (Timm, 2005). It is clear that FCM populations are higher both within citrus orchards and surrounding vegetation when there are mature citrus fruit available for infestation, while population sizes, and movement of males, are significantly lower during winter when there is no mature fruit on trees (Stotter, 2009).

FCM males have been found to respond to FCM females up to one kilometre away (Omer, 1939; Timm, 2005). Schwartz (1981) found that females dispersed up to 35 m after mating to lay their eggs. It may be possible that males disperse further than females while searching for a mate. Timm (2005) suggested that FCM individuals may vary genetically in their capacity to disperse over long distances, with dispersal possibly being limited within agricultural systems where host plants occur in high density. This will further be aided in a perennial crop such as citrus, where various cultivars ripening and being harvested at different times are being produced in the same area.

In the Citrusdal region of the Western Cape it was found that the FCM is concentrated within or very close to citrus orchards (Stotter, 2009). Exceptions to this occur where alternative host plants are situated close to orchards. Rather, it is clear that the FCM does not move into orchards from surrounding indigenous vegetation (Stotter, 2009). Citrus orchards appear to be the reservoir for the FCM to move into surrounding vegetation. It is clear that relatively few FCM male individuals move further than a few hundred metres from citrus orchards. Therefore, FCM control strategies can focus primarily or even solely on the orchard environment (Stotter, 2009). The dispersal ability of the sterile FCM determines the success of an SIT programme and the effective application of the technique and the influence of other factors that might influence the dispersal ability of the release sterile moths such as the weather.

Chapter II OBJECTIVE OF THE STUDY

2.1 Objective of the study

The objective of the study was to understand the dispersal biology and behaviour of sterile male false codling moths within an SIT programme and thus to help in planning control strategies. The researcher sought to understand how far, in which directions and how successfully released moths dispersed, and to identify the factors dictating and influencing their dispersal. This information is important to assist with all the control strategies influenced in any way by moth dispersal and the decision-making affected by these findings. However, most importantly, these findings may help to improve sterile moth release strategies, such as when to and when not to release, the spacing between release transects and whether there should ever be any alteration in the numbers of moths released per hectare or per area.

Chapter III MATERIALS AND METHODS

3.1 Trial sites

The trials were conducted at the Addo Research Station (33°34’14’’S, 25°42’36’’E) belonging to the Institute for Tropical and Subtropical Crops (ITSC) of the Agricultural Research Council. It is situated near Addo in the Sundays River Valley in the Eastern Cape Province of South Africa.

Table 1 Details of trial sites (orchards) used in the study

Cultivar	Year planted	Orchard number	Tree spacing between rows	Tree spacing within rows	Tree height and density
Navels (Washington)	1998	C8	2 m	2 m	3 m high and dense
Valencia (Midknight)	2000	E3	6 m	3 m	2 m high and sparse
(Lemon Limoneira)	2002	F9	6 m	1.5 m	2.5 m high and sparse
Soft citrus (Nules Clementine)	2000	J6	6 m	3 m	2.5 m high and dense

The fifth site (veld), were open veld, mostly with low non FCM host bushes next to the farm.

3.2 Trial layout

The layout of the trial was identical for each of the five sites (four orchards and an open veld). The experimental design was a random complete block with a central release point. Traps were set out in the four main directions (North, East, South and West) and at four distances (30, 60, 100, 150 m) from the release point (Figure 8). Traps were hung on poles where there was no suitable tree in the correct position in the orchards and in the open veld (Fig 14).

	A	B	C	D	E	F	G	H	I	J	K	
1	o	o	o	o	o	150 m	o	o	o	o	o	1
2	o	o	o	o	o	o	o	o	o	o	o	2
3	o	o	o	o	o	o	o	o	o	o	o	3
4	o	o	o	o	o	o	o	o	o	o	o	4
5	o	o	o	o	o	o	o	o	o	o	o	5
6	o	o	o	o	o	100 m	o	o	o	o	o	6
7	o	o	o	o	o	o	o	o	o	o	o	7
8	o	o	o	o	o	o	o	o	o	o	o	8
9	o	o	o	o	o	o	o	o	o	o	o	9
10	o	o	o	o	o	60 m	o	o	o	x	o	10
11	o	o	o	o	o	o	o	o	o	o	o	11
12	o	o	o	o	o	o	o	o	o	o	o	12
13	o	o	o	o	o	30 m	o	o	o	o	o	13
14	o	o	o	o	o	o	o	o	o	o	o	14
15	o	o	o	o	o	o	o	o	o	o	o	15
16	150 m	100 m	o	60 m	30 m	o	30 m	60 m	o	100 m	150 m	16
17	o	o	o	o	o	o	o	o	o	o	o	17
18	o	o	o	o	o	o	o	o	o	o	o	18
19	o	o	o	o	o	30 m	o	o	o	o	o	19
20	o	o	o	o	o	o	o	o	o	o	o	20
21	o	o	o	o	o	o	o	o	o	o	o	21
22	o	o	o	o	o	60 m	o	o	o	o	o	22
23	o	o	o	o	o	o	o	o	o	o	o	23
24	o	o	o	o	o	o	o	o	o	o	o	24
25	o	o	o	o	o	o	o	o	o	o	o	25
26	o	o	o	o	o	100 m	o	o	o	o	o	26
27	o	o	o	o	o	o	o	o	o	o	o	27
28	o	o	o	o	o	o	o	o	o	o	o	28
29	o	o	o	o	o	o	o	o	o	o	o	29
30	o	o	o	o	o	150 m	o	o	o	o	o	30
	A	B	C	D	E	F	G	H	I	J	K	

Moth release point

Traps in northerly direction

Traps in easterly direction

Traps in westerly direction

Traps in southerly direction

Fig 8 Trial layout for all orchard sites.

The letters on the X-axis indicate the orchard rows and the numbers on the Y-axis the trees within the orchard rows. The letter X in row J indicates where a tree has been removed. The grey part indicates Washington Navels and the white part Bahianinha Navels. A grey line on the white part shows where Washington Navels have been planted between Bahianinha Navels.

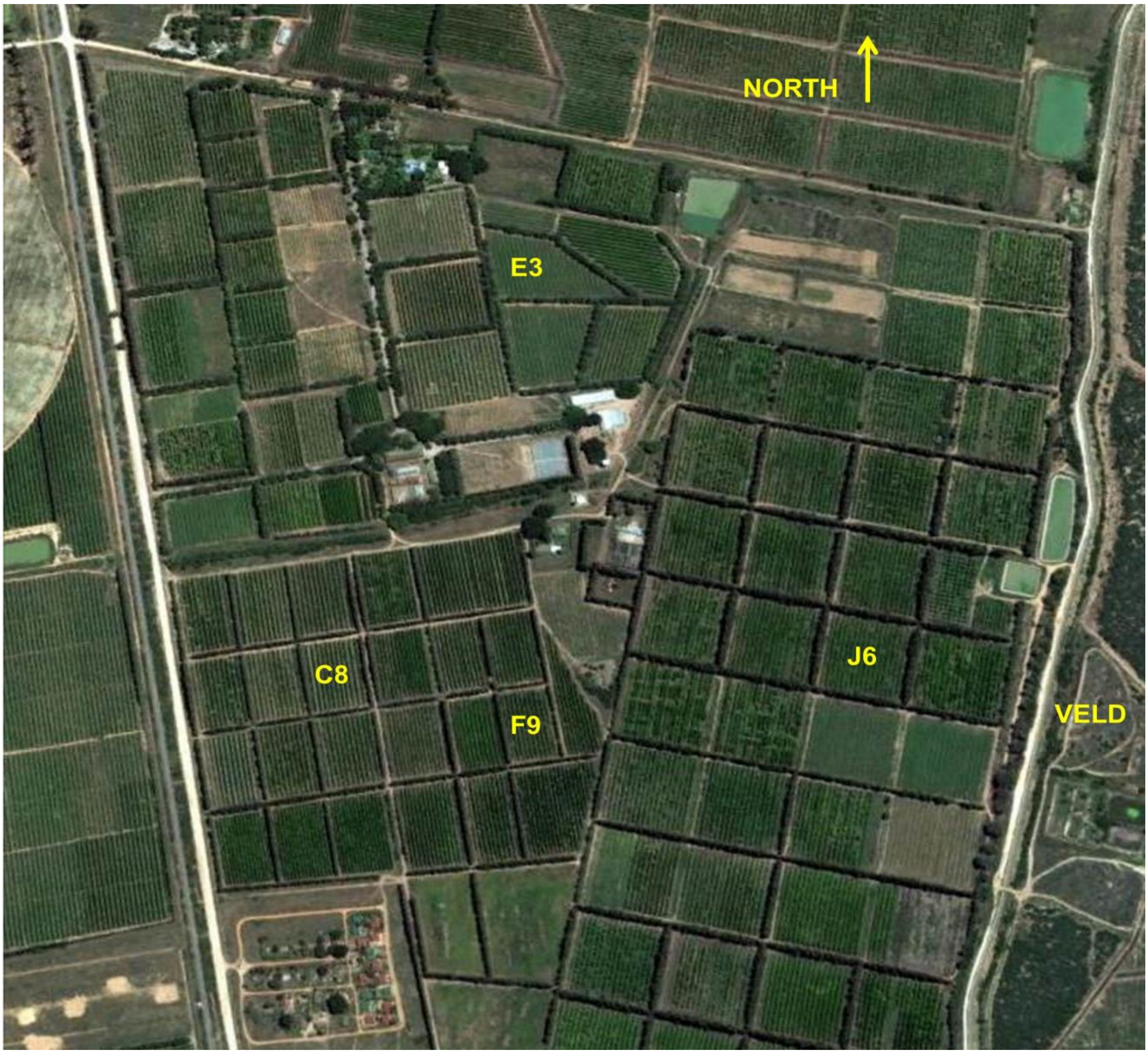


Fig 9 Map of the Agricultural Research farm in Addo (33°34'14''S, 25°42'36''E), indicating the orchards in which the sterile FCM were released (Google Maps). (Refer to table 1 for more detail).

3.3 Weather data

The weather data recorded were minimum and maximum temperatures, wind direction, wind speed, relative humidity and rainfall. This was done on a daily basis for the seven-day period that the yellow delta traps were checked after the release for each of the six release dates. These weather parameters were captured only from after dusk until after midnight, as this is the period that moths are known to be active, as they are nocturnal (Stotter, 2009). Weather data recorded by the weather station on the Addo Research Station farm were used. Weather parameters such as rainfall, minimum and maximum temperatures, relative humidity and wind speed were also correlated to the different wind directions.

3.4 Source of moths

Sterile FCMs were supplied by Xsit (Pty) Ltd which is conducting a commercial FCM SIT programme in the Sundays River Valley. Xsit is jointly owned by River Bioscience (Pty) Ltd and The Technology Innovation Agency (TIA) of the Department of Science and Technology. Xsit's production facility is situated in Citrusdal in the Western Cape (32° 35' 20" S 19° 00' 42" E) and moths are transported to the Eastern Cape with cold-immobilized transport. The moths were released on the same morning of their arrival at Xsit's premises in the Sundays River Valley and were kept in a cooler box with ice cubes until they were released. Releases were done within an hour from the time the moths were received from Xsit. On each occasion of the six releases, Xsit supplied 200 g sterile FCMs for the trial which were divided into 40 g groups of sterile FCMs.

3.5 Preparation of the moths for release

3.5.1. Weighing the moths

In order to determine the number of sterile moths released on any occasion, the average mass of one sterile moth had to be determined. This was done by weighing on each of the 10 occasions to determine an average weight per moth.

3.5.2 Dyeing the moths

It was necessary to determine whether the dye had any detrimental effect on the released moths. Consequently, 100 g (4 000 of sterile moths) were coloured with fluorescent powder dye (rocket red) and another 100 g were left undyed. To dye the moths they were put into a container and powder dye added. The container was then shaken gently until the moths were covered with dye. Yellow delta traps were set out in the four main directions (north, east, south and west) and at four distances (30, 60, 100, 150 m) from the release point (Figure 8). The dyed and undyed moths were then released simultaneously in the central release point. The traps were checked after five days when the numbers of dyed and undyed moths caught in the traps were counted and compared. The trial was conducted twice, once on 8 December 2012 and again on 9 December 2013.

Analysis of variance (Anova) was performed on the number of dyed and undyed FCMs trapped, using GLM (General Linear Models) Procedure of SAS software (Version 9.2; SAS Institute Inc, Cary, USA). Randomised block split plot Anova was done considering release dates as block replicates, types of FCMs (dyed and undyed) as the main plot factor and the traps' direction and distance as subplot factors. The Shapiro-Wilk test was performed to test for normality (Shapiro, 1965). Percentages were subjected to square transformation to improve normality (Snedecor, 1980). Fisher's -least significant difference was calculated at the ≤5% level to compare treatment means (Ott, 1998). A probability level of ≤5% was considered significant for all tests.

3.6 Release of sterile moths in dispersal trials

The sterile moths received from Xsit were placed in a cooler box with ice cubes to keep them cold. For each release point (site) 40 g of moths (determined to be approximately 1625 moths) were then weighed in a petri dish (fig.10) and released (as described in 3.2 – Trial layout). The moths were weighed and released after they were removed from the cooler box and warmed up (allowed to reach ambient temperature) so that they could get active before their release. They were then released manually into the canopy of the release point trees. Most of the sterile released moths settle in the canopy of the release point tree (fig. 11), some of the released moths fell on the ground where they could be preyed on by ants. Releases were done about nine ’o’clock in the mornings. The moths were released on 18 December 2012 and on 29 January, 11 March, 6 May, 16 September and 4 November 2013.

Fig 10 Sterile FCM’s (40 g) being weighed for each release point

Fig. 11 Sterile moths on citrus leaves after being released.

3.7 Trial monitoring

Sixteen traps loaded with Lorelei pheromone lures (River Bioscience, Port Elizabeth, South Africa) were used to catch the released moths at each release point. The traps were set out as described in section 3.2 (Trial layout) (Figure 8). The yellow delta traps were positioned so that the prevailing wind (usually south-easterly) could blow through the trap and carry the pheromone plume into the orchard, as FCM adult males are believed to fly upwind. The monitoring of traps was done for seven consecutive days after each release. On each day all the moths (sterile and wild moths) found in each trap were removed and counted.

Fig. 12 A yellow Delta FCM pheromone trap hanging in a citrus tree

Traps were hung approximately 2 m above ground level and in the canopy of the tree. All tree branches around the trap were removed to ensure free movement of the moths in and out of the traps.

Trap management was conducted to ensure that the traps remained effective. The stickiness of the sticky liners (trap floors) was maintained to ensure that the moth captures were not compromised. Foreign matter on the sticky liner, such as dust and leaves, was removed every day. New sticky liners were used on each release date.

The Lorelei dispenser contains liquid pheromone and is permanently sealed. Its polyethylene tip, responsible for pheromone release, is protected by a transparent, plastic cap, which is removed when in use. To insert the Lorelei pheromone dispenser into the yellow delta trap, a cross was cut just to the side of the roof apex, using a sharp knife (Fig 13a). Each cut was about 2 cm long. The dispenser was then inserted through the centre of the cross (Fig 13b), with the stopper of the dispenser flush against the roof of the trap (fig 13b) (May *et al*., 2010). Lorelei dispensers, was replaced after 5 months.

Fig 13a A cross-cut in the roof of the yellow delta trap into which the Lorelei pheromone dispenser will be inserted.

Fig 13b A Lorelei pheromone dispenser inserted into a yellow delta trap

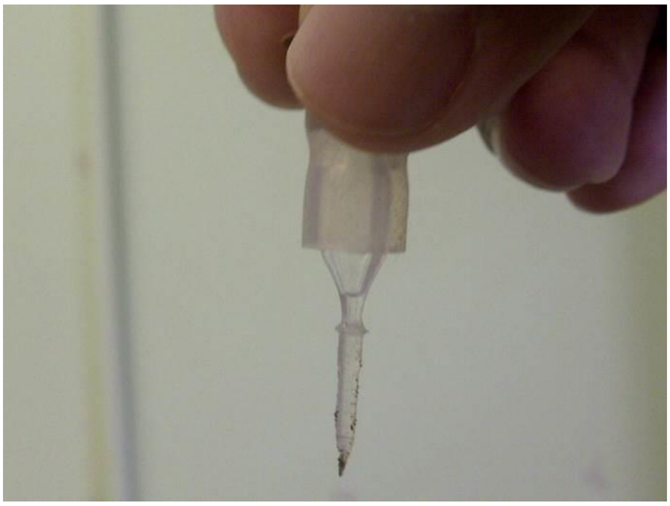


Fig 13c A Lorelei pheromone dispenser with the protective plastic cap removed to reveal the polyethylene tip through which the pheromone is dispensed.

Fig 14a

Fig 14b

Fig 14 Traps hunged on poles where there is not suitable or no tree in the correct position.

3.8 Data analysis

Analysis of variance (ANOVA) was performed on the percentage of moths trapped using a General Linear Model (GLM) procedure with SAS software (Version 9.2; SAS Institute Inc, Cary, USA). A randomised block split plot ANOVA was done considering release dates as block replicates, release points as the main plot factor and trap direction and distance as subplot factors. A Shapiro-Wilk test was performed to test for normality (Shapiro, 1965). Percentages were subjected to logit transformation to improve normality (Snedecor 1980). Fishers LSD tests were calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of ≤ 5% was considered significant for all tests.

Pearson correlations were calculated between trap count data and weather parameters to investigate if there was any linear relationship between moth movement and weather conditions. Scatterplots were used to visualise the relationship between these variables. A partial least squares (PLS) regression was conducted (XLStat, Version 2011, Addinsoft, New York, USA) with total moth counts at each distance as dependent variables and weather parameters as independent variables to determine the joint effect of weather conditions on moth movement.

To determine the effect of wind direction a contingency table was set up for trap direction against wind direction. A Chi-squared test was conducted to determine if the number of moths trapped in a specific direction was independent of wind direction.

Chapter IV RESULTS

4.1 Weather data

Details of the wind direction and speed recorded during the seven-day wind monitoring periods following releases of sterile FCM is given in Table 2.

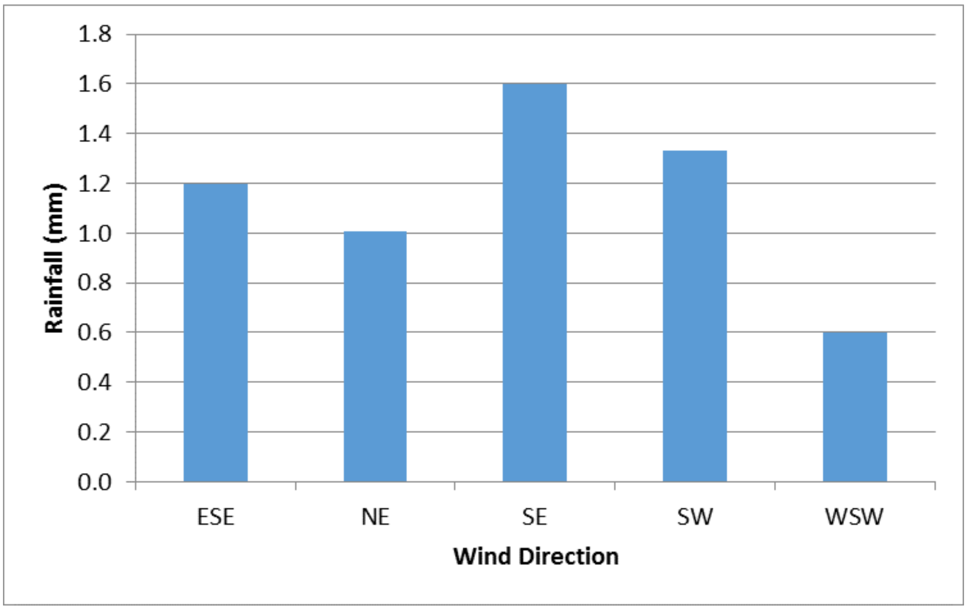


Figure 15 Wind speed recorded for each wind direction

Figure 15 indicates that the wind speed was the highest when the wind blew in a south-easterly direction and the lowest when the wind blew in a west south westerly direction over the trial period.

Table 2. Absolute and relative frequency of wind direction during the seven-day evaluation periods after all releases of sterile FCM adults in citrus orchards.

Wind direction	**Total number of days wind blew in specific direction**	**% days wind blew in a specific direction over trial period**
ESE	1	2.38
NE	10	23.81
SE	24	57.14
SW	6	14.29
WSW	1	2.38
Total	42	100%

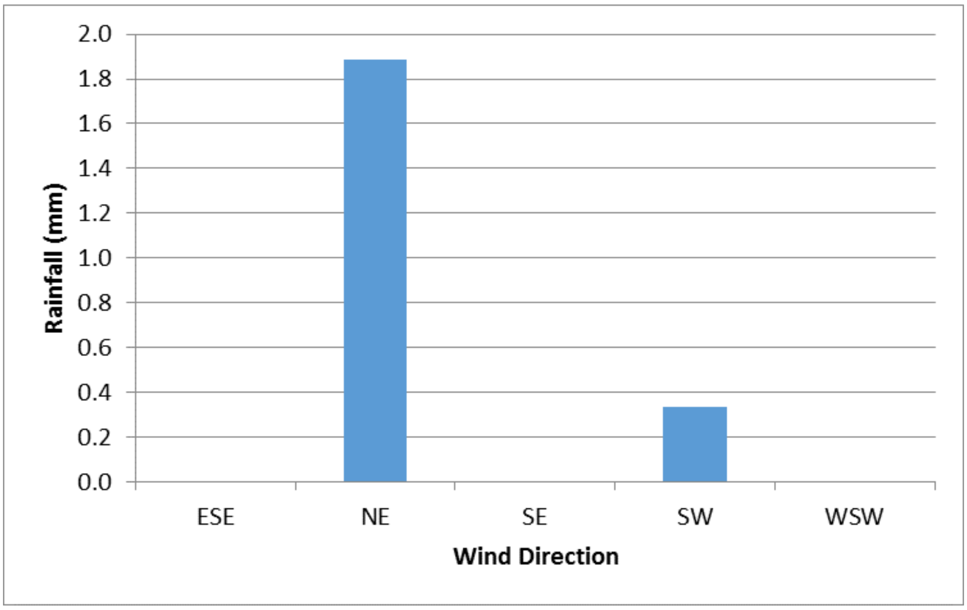


Figure 16 Rainfall recorded per wind direction

Figure 16 clearly indicates that most rain fell of the time when the wind blew in either a north-easterly or south westerly direction. No rain fell over the trial period when the wind blew in the other directions.

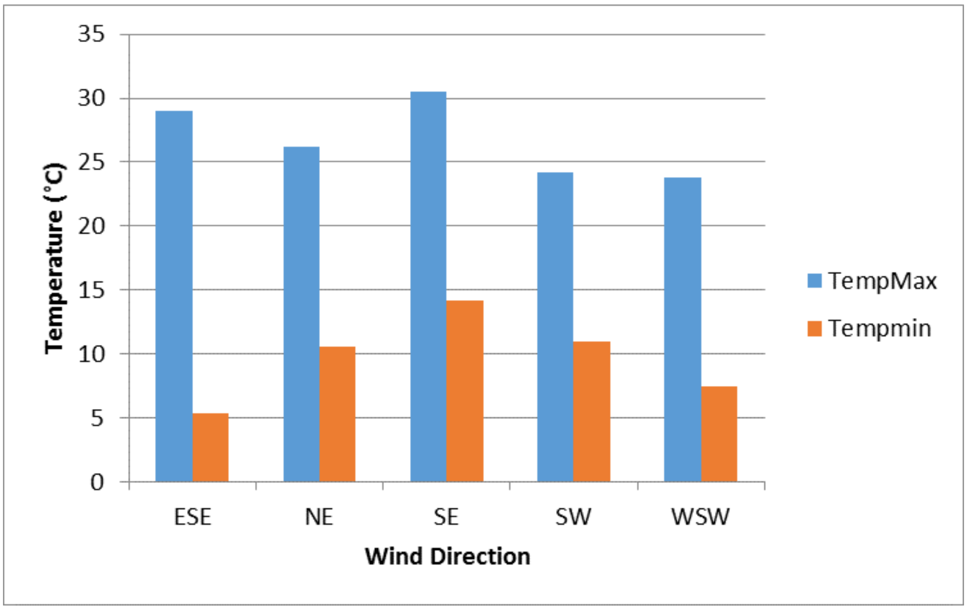


Figure 17 Minimum and maximum temperatures recorded for each wind direction

Figure 17 indicates that the maximum temperature was the highest when the wind blew in a south-easterly and east south easterly direction while the minimum temperature was the lowest when the wind blew east south easterly and west south westerly over the trial period.

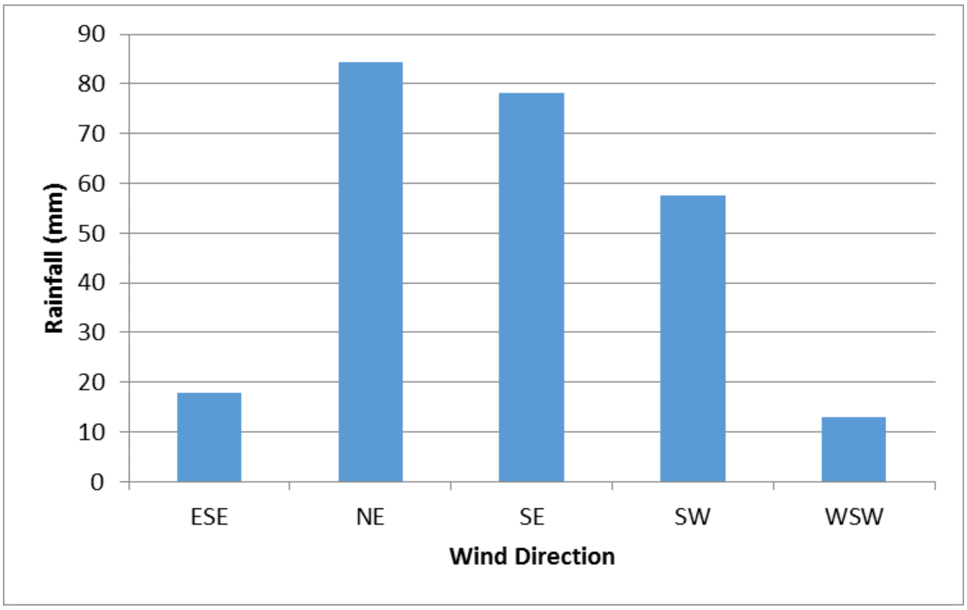


Figure 18 Relative humidity recorded for each wind direction

Figure 18. Lemon indicates that the relative humidity was the highest when the wind blew in a north-easterly direction and the lowest humidity was when the wind blew in a west south westerly direction over the trial period.

Table 3. Pearson correlations between total counts of moths at each distance from the release point (pooled for all sites) and specific weather parameters. There was one observation of weather parameters for all sites at a specific time (the same weather readings applied to all release sites).

Distance		Temp Max	Temp min	Rainfall	RH	Wind speed
30	Correlation	**0.330**	0.302	-0.086	0.073	0.092
	P-value	**0.033**	0.052	0.589	0.645	0.562
60	Correlation	**0.354**	**0.394**	-0.058	0.084	0.144
	P-value	**0.021**	**0.010**	0.716	0.599	0.364
100	Correlation	**0.350**	**0.381**	-0.010	0.072	0.088
	P-value	**0.023**	**0.013**	0.949	0.649	0.579
150	Correlation	0.276	**0.332**	-0.047	0.144	0.120
	P-value	0.077	**0.032**	0.770	0.361	0.450
Tot	Correlation	**0.354**	**0.357**	-0.070	0.085	0.113
	P-value	**0.021**	**0.020**	0.661	0.593	0.474

It is clear that in the vast majority of cases there is a significant positive correlation between moths trapped (both at each distance from the point of release and overall) and maximum and minimum temperature (Table 3). Temperatures were always only recorded between dusk and midnight, when the moths would have been active (Rob Stotter 2009). There was no correlation between moth dispersal and rainfall, relative humidity and wind speed (Table 3).

4.2 Release of the moths

4.2.1 Weighing the moths

The results of the determination of the average mass of a sterile FCM moth are given in Table 4. The number of sterile moths released on every occasion was determined by dividing the total mass of moths that release by the average mass of a moth.

Table 4. Mass of the moths for determining numbers released

Replicate		Number of Moths	Mass	Average Weight/moth
2013/09/16	1	10	0.220	0.022
	2	10	0.263	0.026
	3	10	0.227	0.023
	4	10	0.233	0.023
	5	10	0.275	0.028
2013/04/11	6	10	0.244	0.024
	7	10	0.320	0.032
	8	10	0.170	0.017
	9	10	0.250	0.025
	10	10	0.260	0.026
Overall average mass per moth			**0.246**	0.025

The average mass per sterile FCM moth was determined to be 0.246 g.

An average weight per moth of 0.025 g was determined.

4.2.2 Dyeing the moths

The results of the comparison between recaptures of dyed and un-dyed moths is given in Table 5.

Table 5. Numbers of dyed and undyed FCMs trapped

Direction	Sqrt(FCM)
Undyed	0.90a*
Dyed	0.13b
LSD(P=0.05)	0.32

* Means with the same letter are not significantly different

It is clear that significantly more undyed FCMs were trapped than dyed FCMs (Table 5). According to the distribution patterns over the two trial periods there is a significant difference in the total number of marked moths and the unmarked moths trapped, indicating that the marking of the moths with dye powder has a negative effect on moth dispersal.

Table 6. P-values for the analysis of variance showing the factors which had a significant effect on the number of FCMs trapped. Release dates were considered as block replicates for other factors. Factors marked in bold are significant at a ≤5% level of probability

Source of variation	**DF**	**P-value**
Release Date	1	0.1571
Dyed status of moth* Dyed/Undyed	**1**	**0.0206**
Direction	3	0.051
Distance	3	0.0032
Compass direction x Distance	**9**	**0.0268**
Type x Direction	3	0.1143
Type x Distance	3	0.1071
Type x Direction x Distance	9	0.0501

The analysis of variance on the square root of the number of FCMs trapped (Table 6) indicates a significant direction distance interaction effect, which is an indication of varying patterns for the number of FCMs trapped over distance in the different directions

Table 7 Means numbers of FCMs trapped at different directions and distances from the release point

	Direction			
Distance (m)	**North**	**South**	**East**	**West**
30	0.35cd*	0.81abc	1.33a	1.43a
60	0.00d	0.25cd	0.50bcd	0.25cd
100	0.25cd	1.27ab	0.00d	0.25cd
150	0.25cd	0.35cd	0.00d	0.91abc
LSD(P=0.05)	0.78			

* Means with the same letter are not significantly different at the 5% level

There was no significant difference in the number of FCMs trapped at different distances for the northerly direction from release point, while for other directions significant differences were observed over distance (Table 7). For traps placed east of the release point, significantly more FCMs were trapped at a distance of 30 m than any other distance. For traps placed south of the release point the number of FCMs trapped at a distance of 100 m did not differ significantly from the number trapped at 30 m, while for traps placed west of the release point the number of FCMs trapped at a distance of 150 m did not differ significantly from the number trapped at 30 m. Mean numbers of FCMs trapped at different distances and directions from the release point are shown in Table 7. From the totals trapped per direction it indicates that most moths were recaptured on southern and western traps, and the least on the north traps**.**

4.3 Recapture of released moths in dispersal trials

4.3.1 Effect of observation day

Table 8. P-values for an Anova of the cumulative percentage of moths trapped per observation day, indicating which factors had a significant effect.**Error! Not a valid link.**

An analysis of variance on the percentage moths trapped per observation day, and different (Table 8) indicates that distance from the release point and the release date had significant influences on the recaptures of the moths over the seven-day observation periods. It also shows that most moths were trapped on the first day after their release.

Table 9. Total number of moths trapped (cumulative over time - percentage of those released) at the different distances from the different release points in citrus and veld

Release Point	Dist	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
Veld	30	0.1328	b	0.2438	b	0.2641	bc	0.2793	bc	0.2895	bc	0.2948	bc	0.2948	bc
Veld	60	0.0590	bcd	0.0955	cdef	0.1568	bcdef	0.1750	bcdef	0.1955	bcdef	0.2261	bcdefg	0.2261	bcde
Veld	100	0.0308	bcd	0.0952	cde	0.1259	cdef	0.1517	cdef	0.1568	cdef	0.1695	bcdefgh	0.1695	bcde
Veld	150	0.0155	d	0.0230	ef	0.0692	ef	0.0951	def	0.0951	def	0.1028	efgh	0.1131	cde
Lemons	30	0.1206	bc	0.2130	bc	0.2412	bcd	0.2591	bcd	0.2669	bcd	0.2694	bcde	0.2771	bc
Lemons	60	0.0846	bcd	0.1590	bcde	0.1719	bcdef	0.1924	bcdef	0.2104	bcdef	0.2130	bcdefgh	0.2258	bcde
Lemons	100	0.0154	d	0.0257	cdef	0.0385	f	0.0540	f	0.0565	f	0.0617	h	0.0668	e
Lemons	150	0.0334	bcd	0.0514	ef	0.0668	ef	0.0822	ef	0.0873	ef	0.0899	fgh	0.0899	de
Navels	30	0.0540	bcd	0.2274	bc	0.3048	b	0.3332	b	0.3435	b	0.3460	b	0.3486	b
Navels	60	0.0205	cd	0.0542	def	0.0721	ef	0.0824	ef	0.0824	ef	0.0824	fgh	0.0824	de
Navels	100	0.0233	cd	0.0336	def	0.0491	f	0.0619	f	0.0619	f	0.0646	gh	0.0646	e
Navels	150	0.0182	cd	0.0311	def	0.0414	f	0.0466	f	0.0568	f	0.0671	gh	0.0671	e
Softcitrus	30	0.0232	cd	0.1010	bcdef	0.1242	cdef	0.1294	cdef	0.1294	cdef	0.1372	cdefgh	0.1398	cde
Softcitrus	60	0.0593	bcd	0.1698	bcd	0.2237	bcde	0.2571	bcd	0.2673	bcd	0.2854	bcd	0.2983	bc
Softcitrus	100	0.0342	bcd	0.0625	def	0.0889	def	0.0966	def	0.1043	def	0.1068	defgh	0.1222	cde
Softcitrus	150	0.0103	d	0.0154	f	0.0284	f	0.0515	f	0.0566	f	0.0592	gh	0.0592	e
Valencia	30	0.3841	a	0.5090	a	0.5784	a	0.5990	a	0.6041	a	0.6222	a	0.6350	a
Valencia	60	0.1083	bcd	0.1493	bcdef	0.2136	bcde	0.2367	bcde	0.2393	bcde	0.2472	bcdef	0.2600	bcd
Valencia	100	0.0282	cd	0.0410	def	0.0436	f	0.0693	ef	0.0745	ef	0.0796	fgh	0.0873	de
Valencia	150	0.0077	d	0.0154	f	0.0282	f	0.0361	f	0.0387	f	0.0438	h	0.0438	e
LSD(P=0.05)		0.1037		0.1435		0.1591		0.1680		0.1745		0.1789		0.1863	

*Values followed by the same letter are not significantly different at the ≤5% probability level.

In the navel and Valencia orchards, significantly the highest percentage of moths was trapped at 30 m from their release points than any other distance. The difference in dispersal over distance for the different release points is indicated in Table 9. In the veld observations there was no significant difference in the percentage of moths trapped over all the distances. In the lemon orchard there was no significant difference in percentage for the moths trapped at 30 m and 60 m from their release point, but a significantly more of moths were trapped at 30 m than at 100 m and 150 m. In the Mandarin orchard the highest percentage of moths was trapped at 60 m from their release point. This did not differ significantly from recaptures at 30 m and 100 m, but were significantly higher than catches at 150 m from their release point.

4.3.2 Effect of distance from release point

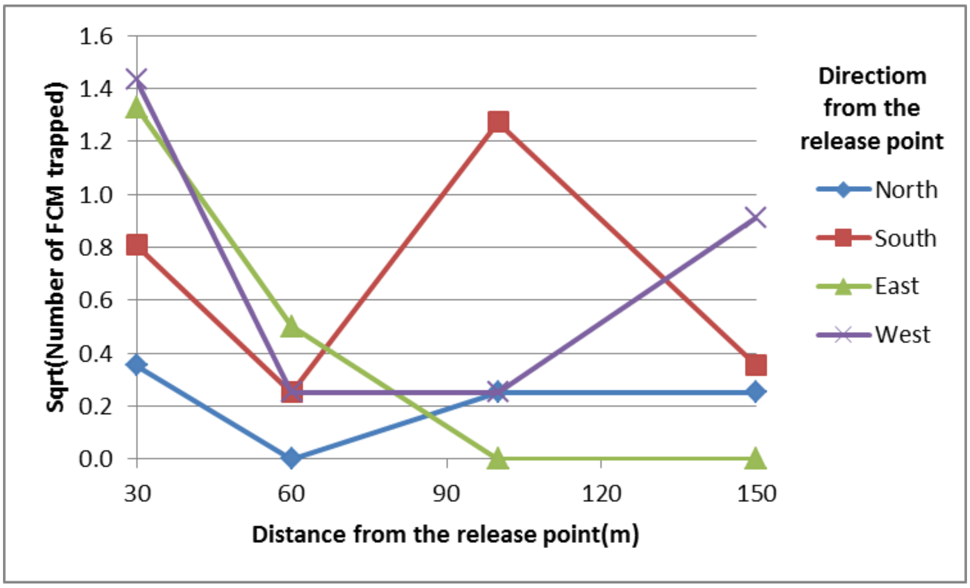
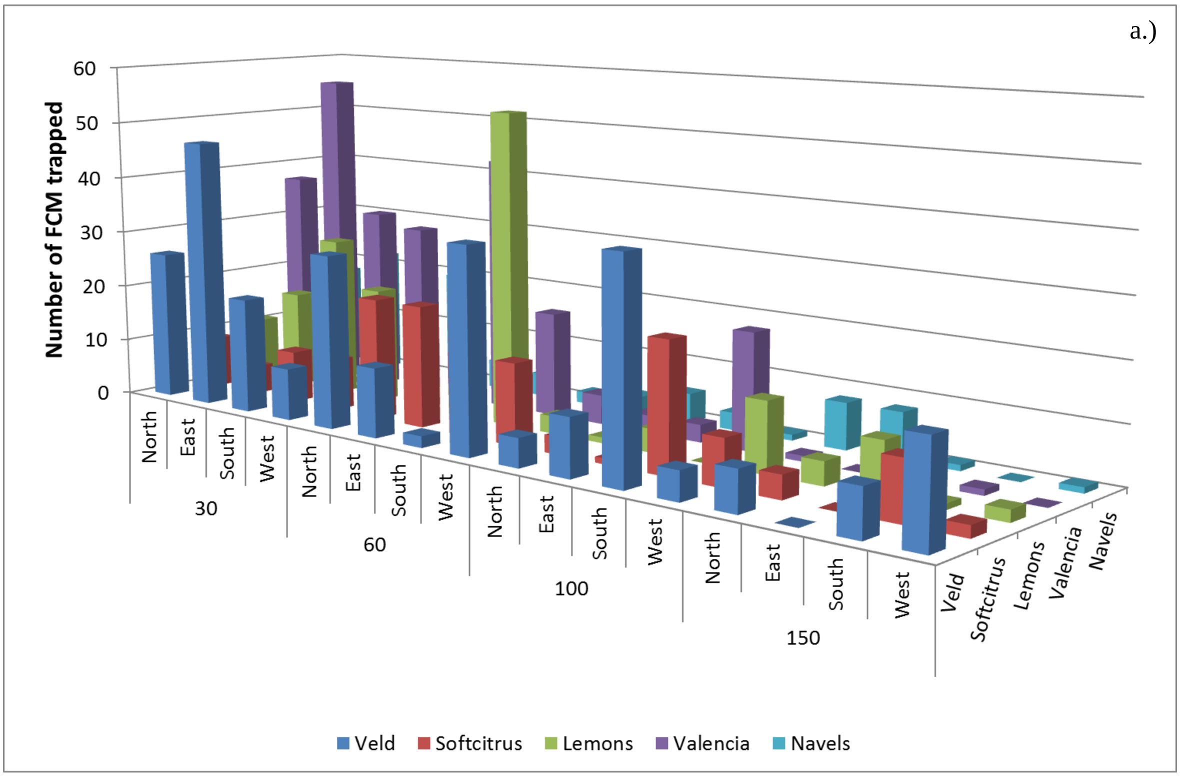


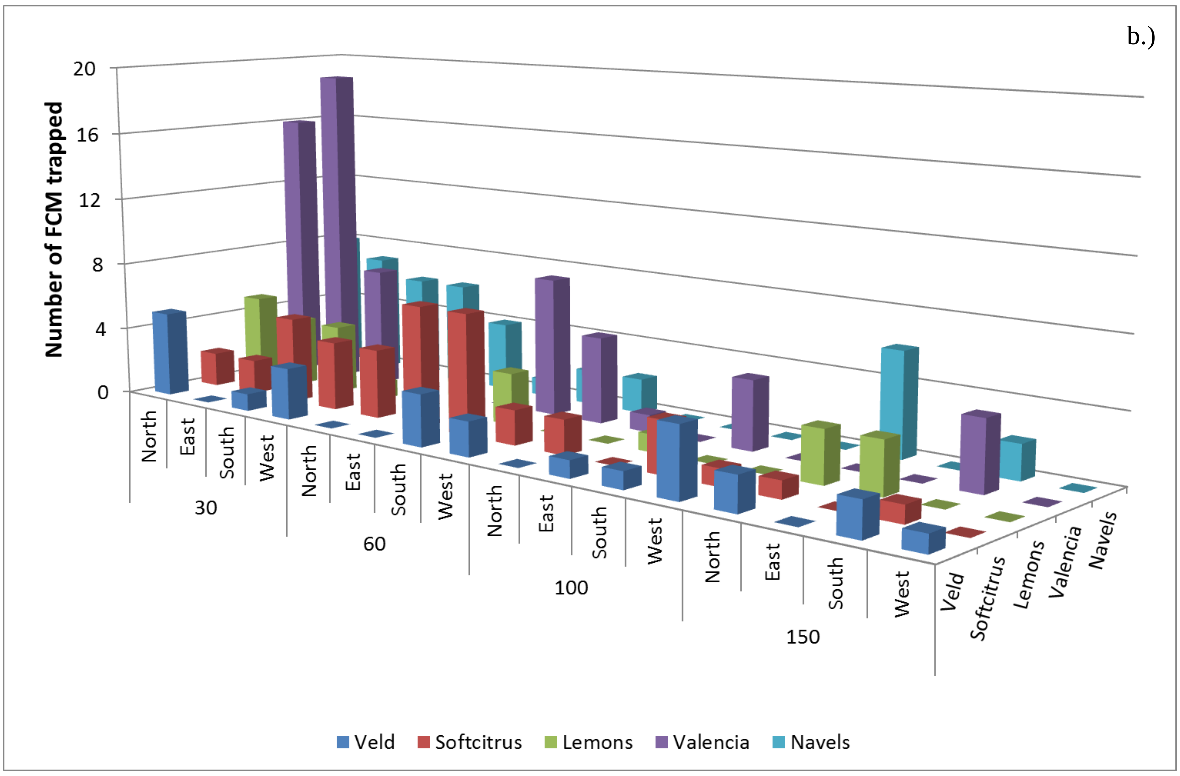
Figure 19. Number of FCM trapped 1 week after release at the different distances and direction from the release point (data transformed to square roots).

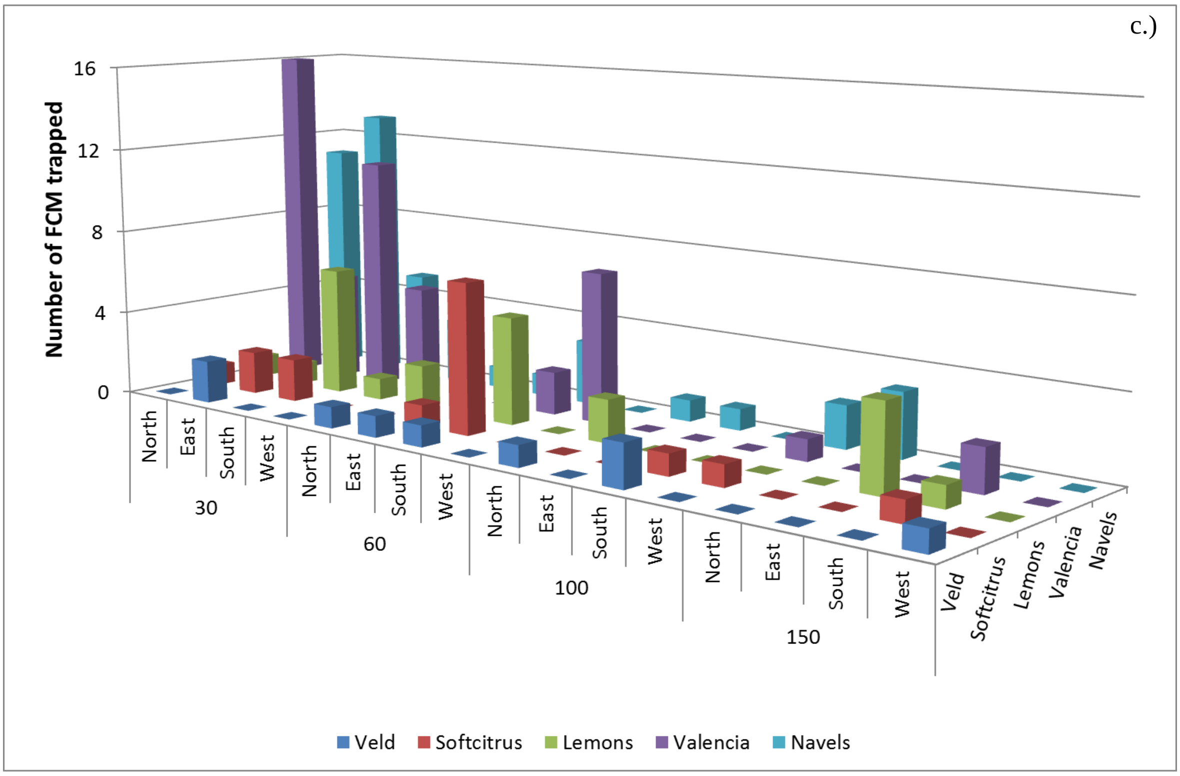
Figure 19 shows the number of moths trapped one week after release at different distances and directions of each release point from each of the release dates. From these results it is clear that there is no constant pattern for the number of FCMs trapped over distance or direction**.**

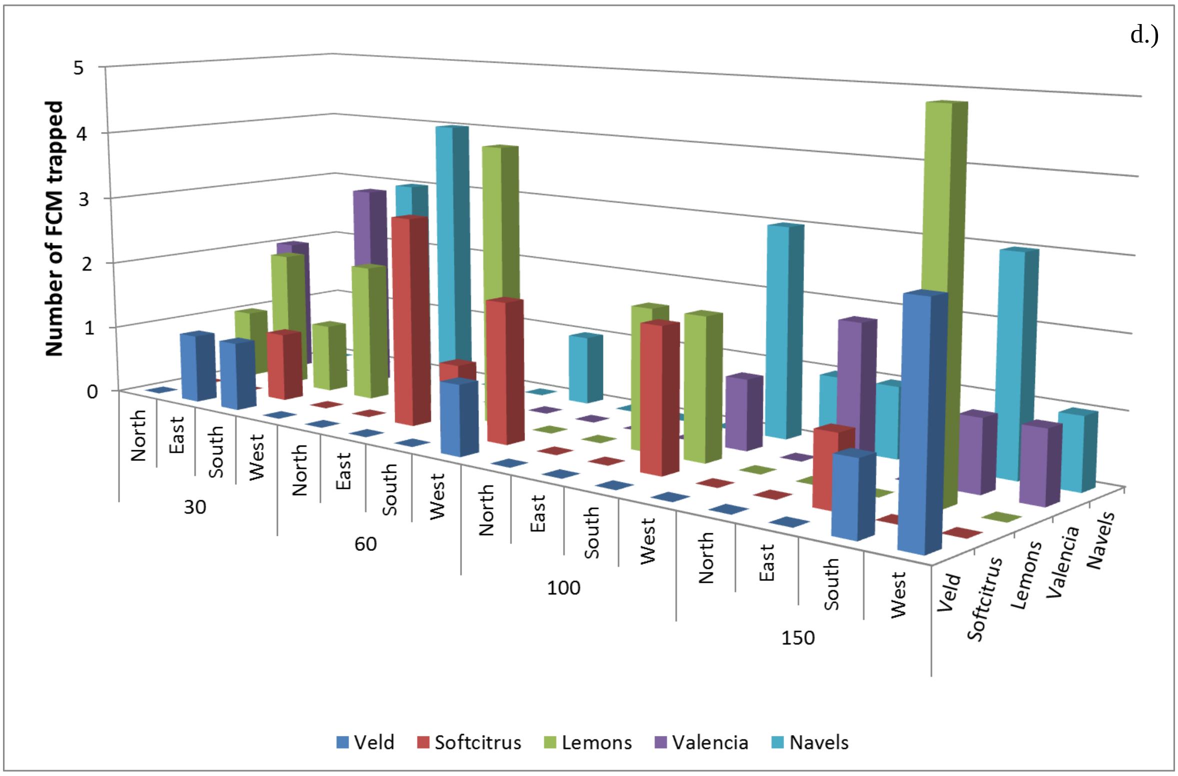
4.3.3 Effect of citrus cultivar or release site

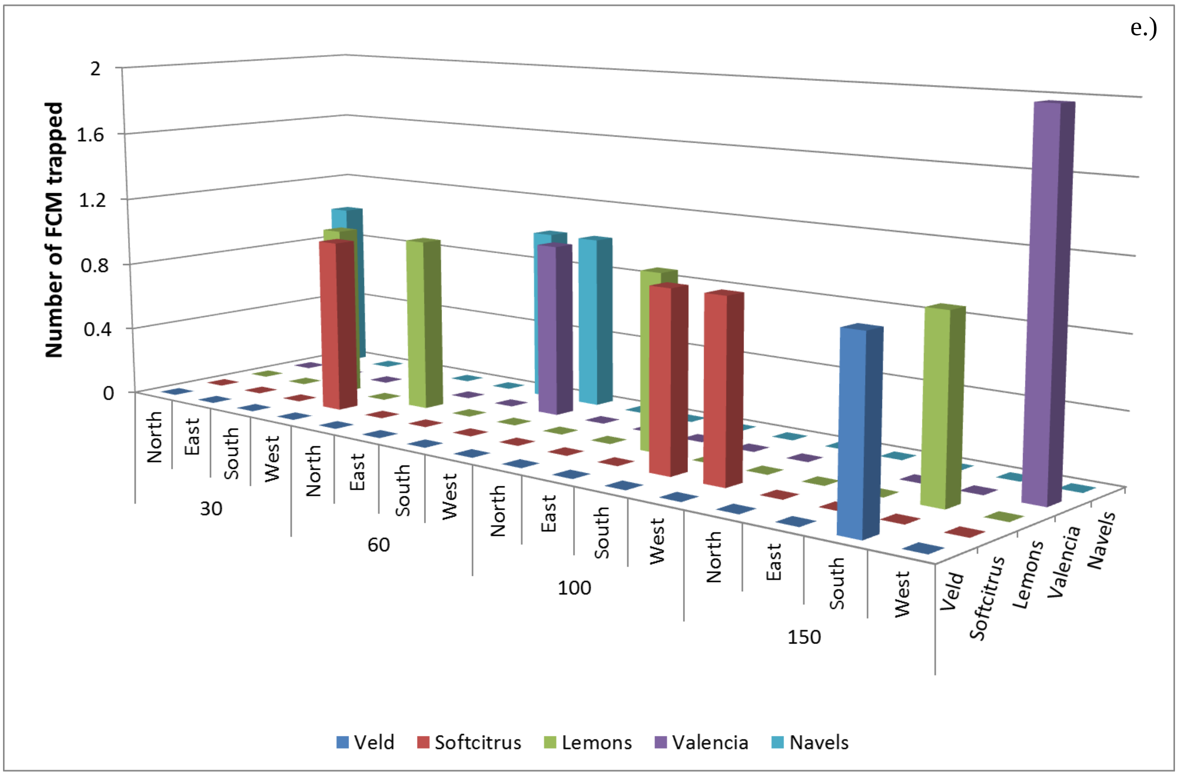
Fig 20 (a-f) shows the number of sterile FCM recaptured from different directions and at different distances from the release point from all six release dates.




b.)
Number of FCM trapped
20
16
12
8
4
0
North
East
South
West
30
North
East
South
West
60
North
East
South
West
100
North
East
South
West
150
Veld
Softcitrus
Lemons
Valencia
Navels
Veld Softcitrus Lemons Valencia Navels


c.)
Number of FCM trapped
16
12
8
4
0
North
East
South
West
30
North
East
South
West
60
North
East
South
West
100
North
East
South
West
150
Veld
Softcitrus
Lemons
Valencia
Navels
Veld Softcitrus Lemons Valencia Navels


d.)
Number of FCM trapped
0
1
2
3
4
5
North
East
South
West
30
60
100
150
Veld
Softcitrus
Lemons
Valencia
Navels


e.)
Number of FCM trapped
0
0.4
0.8
1.2
1.6
2
North
East
South
West
30
60
100
150
Veld
Softcitrus
Lemons
Valencia
Navels

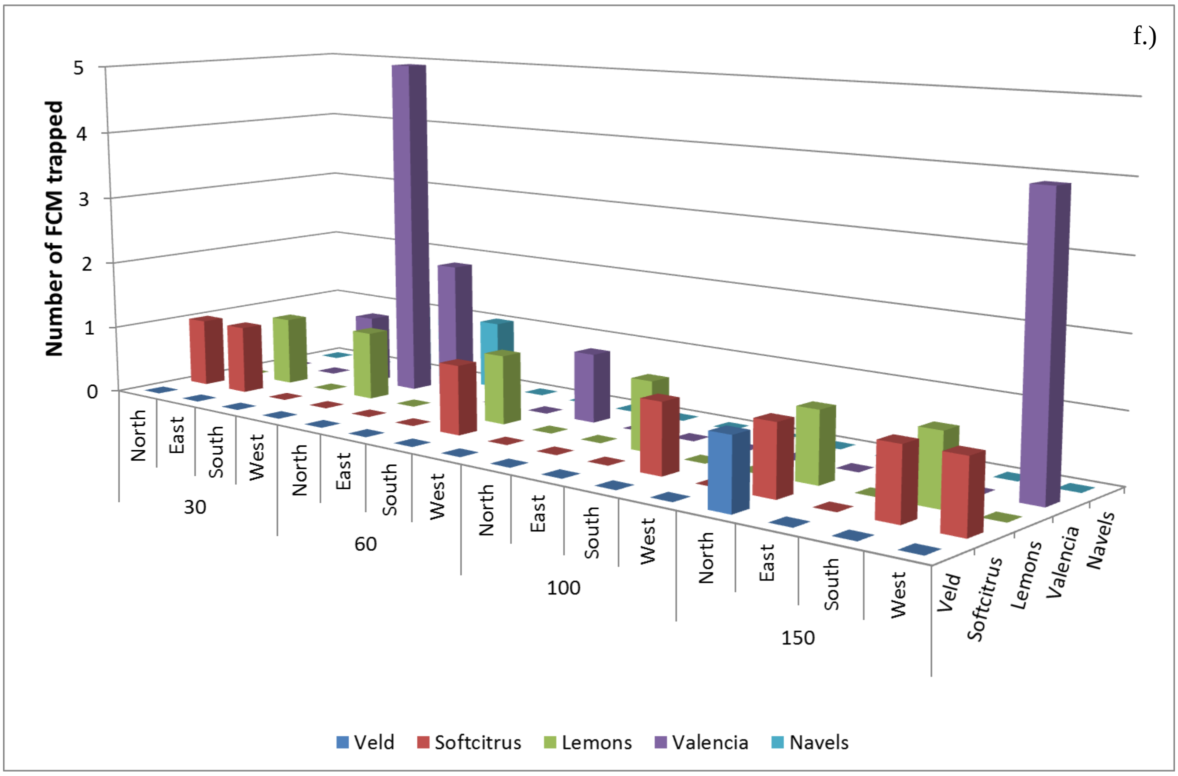


Figure 20. Numbers of moths trapped at different distances and directions from each release point for the seven-day period following each release date: a.) 19/12/2012, b.) 30/01/2013, c.) 12/03/2013, d.) 07/05/2013, e.) 17/09/2013, and f.) 05/11/2013

Mean numbers of FCMs trapped at different distances and directions from the release point are shown in Figure 20. It also indicates the recapture of the released moths over the six release dates for the five release sites, and shows that most moths were recaptured over the first two release dates which were December 2012 and January/February 2013. There was no significant difference in the recapture of the released moths on the other release dates in March, May, September and November 2013.

4.3.4 Effect of wind direction on percentage moths trapped in each direction.

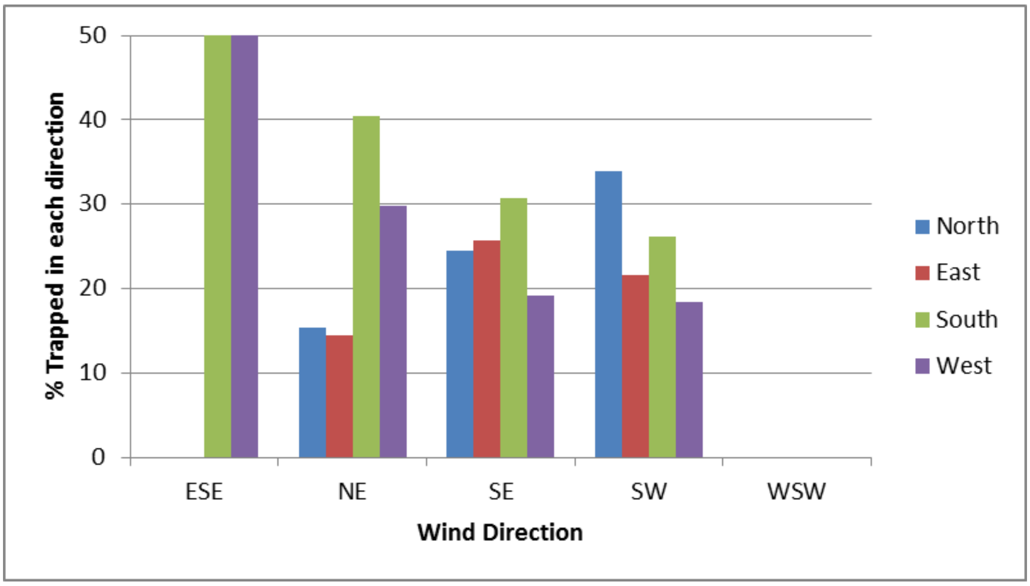


Figure 21. Percentage of moths trapped in each direction from the release point for each wind direction

When the wind blew in a north-easterly or south-easterly direction, the highest numbers of moths were trapped south of the release point (Fig. 21). When the wind blew in a south westerly direction, most moths were trapped north of the release point (Fig. 21). Distribution patterns were less evident for other wind directions. No moths at all were caught when the wind blew in a west-south westerly direction. Wind speed in this direction was only 0.6 m/s (lower than for any of the other wind directions). This may have been a disincentive for moths to fly, as it would have been more difficult to follow any pheromone plume or it may have simply been too difficult to locate pheromone traps with so little wind movement. However, wind was recorded in a west south westerly direction during only one day and hence it is difficult to draw certain conclusions.

4.3.5 Effect of maximum and minimum temperature on moths dispersal

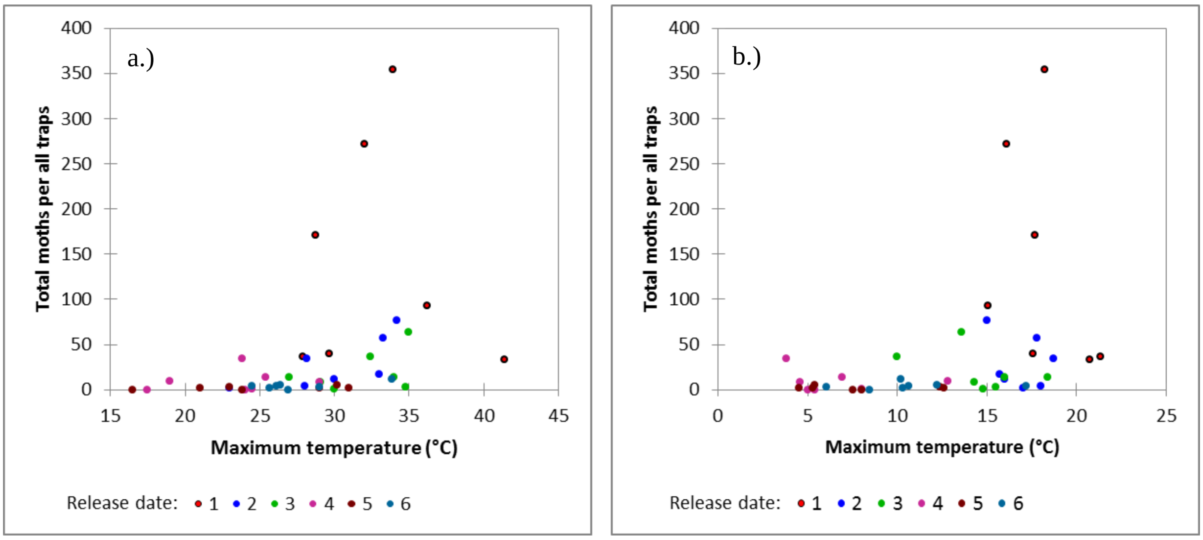


Figure 22. Scatterplots of total moth counts (in all traps at all distances at all sites) against a.) maximum temperature and b.) minimum temperature. Temperatures were captured between dusk and and midknight.

As temperatures rose above 25°C, moth movement began to increase (Fig. 22a). When minimum temperatures rose above 10°C, moth movement also began increasing (Figure 22b).

Chapter V DISCUSSION

It is important to understand the biology and behaviour of the FCM to assist in the correct planning and execution of control strategies for the pest. In the lemon orchard and the veld there was no significant difference in the percentage moths trapped at the various distances from the release points. This may be due to the lack of suitable hosts in these two environments. Lemons are not considered to be a host of the FCM (Georgala, 1968). Other citrus cultivars with the exception of limes are, however, susceptible to FCM attack, and there were no known hosts of the FCM in the veld (Kirkman & Moore, 2007). As indicated in the trial data, if the FCM finds a suitable host, it would not move around more than 30 m to 60 m. If, however, it cannot find a suitable host it can move around as far as 150 m and this is a likely conclusion based on the different observations and recordings in the different environments.

It has been asserted that navel oranges are more prone to the FCM attack than Valencia oranges (Schwartz, 1981). However, similar moth movement in the Valencia and navel orchards in this study may indicate that they are similarly suitable hosts. Consequently, it may be necessary to pursue similar control efforts against the FCM in navel and Valencia orchards. This finding is supported by Stotter (2009), who found that it is apparent that Valencia orange cultivars may support significant populations of male FCMs within orchards, particularly in late June/July when the fruit is ripening. The similar movement of the released sterile FCMs in the navel and Valencia orchards in this study can also be attributed to the difference in tree size and tree density, as the trees were two metres high and sparse in the Valencia orchards and three metres high and dense in navel orchards. This means that tree size and density within orchards may also restrict dispersal. The higher and more dense the trees, the better the chances of hampering moth movement, and if trees are lower and sparse, moth movement is easier. Since the sterile FCM moths moved around more in the Mandarin orchards it is possible that Mandarins are less susceptible to the FCM attack (Hofmeyr, 1998) than Navels and Valencia’s. If Mandarins and Valencia orange varieties are less vulnerable, grapefruit even less and lemons not at all to the FCM attack, it indicates that if there are no or low host availability the dispersal may be more.

Regarding the effect of the weather data on FCM behaviour, maximum and minimum temperatures had the most significant influence on the FCM dispersal. When minimum temperatures dropped below 10°C and maximum temperatures dropped below 25°C it had a negative influence on moth movement (fig.22 a and b). Trap catches were similar to those in a trial by Stotter (2009) in Citrusdal on the FCM dispersal where trap catches were low in the winter months when temperatures were low.

Rainfall is also negatively associated with moth movement Table 3. As far as wind direction is concerned, most released moths were trapped in southern traps when the wind blew in a north-easterly and south-easterly direction, whereas most moths were trapped in northern traps when the wind blew in a south-westerly direction, indicating that the moths are actively flying into the wind (Fig 21). Similar moth flight was found by Carde and Minks (1995) on dispersal work done on codling moths. The minimum and maximum temperatures were more ideal for the FCM dispersal when the wind blew in a south-easterly direction. Distribution patterns were less evident for other wind directions.

The fact that most moths were trapped in southern traps can be linked to the fact that the wind blew in a south-easterly direction for 57% of the time over the trial period. This means that the moths were used to flying into a south-easterly direction or it could be due to the fact that most of the time when the wind blew into a north-easterly direction, it rained and as indicated by the trial data, moth movement appears to be negatively influenced by rain (Table 3.). When it rained there was no moth movement as indicated by Figure 16. The reason why moths sometimes flew in other directions such as cross wind and downwind rather than up-wind, needs to be studied. Downwind flight of males has also been observed (Witzgall *et al.*, 1999). Although most moths were trapped in south-easterly traps when the wind blew in a south-easterly direction a number of moths were also trapped in other directions. Relative humidity and wind speed were not associated with the FCM dispersal.

A reason for the wind speed not having an influence on the FCM dispersal can be because of the windbreaks planted on the boundaries of the orchards. These windbreaks can reduce the wind speed significantly so that the wind speed does not have an influence on moth movement.

However, when wind speed is really strong the moths (DBM, diamondback moth) may stop flying (Goodwin & Danthanarayana, 1984). Diamondback moths are known to be able to migrate over long distances (Mackenzie, 1958; Lorimert, 1981; Chu, 1986). The average dispersal distances estimated from the recapture data with pheromone traps were 21 m to 35 m and those from Yellow Sticky Buckets 14 m to 18 m for the males and 13m to 24 m for the females. When calculated over all the recaptured moths, the average dispersal range was as low as 17 m, similar to the average dispersal distances ranges obtained in this study. Elsewhere, Caprio and Tabashnik (1992) noted that more than 92% of the marked diamond back moths were caught by traps located within 10 m of their release point in their small-scale and non-replicated mark-recapture experiment. Some indirect data such as seasonal patterns of pheromone trap catches and spatial patterns of resistance levels suggested short distance by residential DBM populations (Shirai & Nakamura, 1994). Observations with night goggles by the authors showed that most moths flew close to the ground and below the plant canopy, again suggesting mostly trivial movements and hence limited dispersal ranges.

The data in table 8 indicates that significantly more moths were trapped over the seven-day period after the December release date than from any other release date. The January release date yielded the second highest number of recaptured moths; however, these numbers as well as those from the rest of the release dates (March, May, Sept and November) were not significantly more than the December recovery. This suggests that summer weather conditions were more ideal for moth dispersal.

The fact that most moths in this dispersed between 30 and 60m suggests that the release of sterile FCM can be made with 50 to 60 m swatches. Sterile FCMs are ground-released quadbikes and aerial releases are done with specially equipped gyrocopters. The variable speed of the quadbikes enables the release of different numbers of moths per hectare and the flow rate of the moth release machine in the gyrocopter can be calibrated to release more or fewer moths (Nepgen, 2014). Thus, applications (the release rate of sterile moths) or distribution patterns need to be intensified (releasing more moths per hectare or at closer transects) from March to September. This period is the end of the harvesting season and temperatures are less ideal for moth movement, and in September the new releases of sterile FCMs and other control practices start.

Moth movement is negatively influenced by minimum and maximum temperatures and in the Sundays River Valley these drop below the levels at which moths are active from March to September. Moths are released with quadbikes equipped with an automated release system for ground releases and by specially equipped gyrocopters. Moth movement increases when maximum temperatures rise above 25°C and minimum temperatures above 10°C as indicated in Figure 15. Temperatures in December and January were recorded to be warmer than during any of the other release months, which is most likely the reason for the higher recaptures during these two months.

SIT for FCM has great potential in the Sundays River Valley, and elsewhere in South Africa where citrus is grown, if it is used in combination with other control practices such as orchard sanitation, granulovirus sprays, chemical control, mating disruption, attract-and-kill and other biological control practices (Bloem *et al*., 2005; Mangan *et al.*, 2005). As the most significant moth movement are at a 30 m distance in navel and Valencia orchards, sterile moths should be released at no more than 60 m transects in these orchards. If releases are conducted in lemon orchards release transects can be much further apart as there is no significant difference in moth movement in these orchards over all distances. Currently, Xsit conducts sterile moth releases in 50 m transects (E. Nepgen pers comm). Results clearly indicate that sterile FCM movement is concentrated within citrus orchards. Similar results were found in Citrusdal (Stotter, 2009). It is thus evident that control practices for the FCM can be concentrated within citrus orchards. As moth dispersal is hampered by lower temperatures from March to November, releases of sterile moths should be intensified during this period.

In Table 5 it is clearly indicated that significantly more un-dyed sterile FCM were trapped over the two trail periods. Indicating that, the marking of the moths with dye powder has a negative effect on moth dispersal.

Table 9 indicated that distance from the release point and the release date had significant influences on the recaptures of moths over the seven-day observation periods, it also shows that most moths were trapped on the after their release.

Releases should also not be conducted during or shortly before rainfall. Releases during periods of cool temperatures are superfluous. Therefore, work on improving the cold tolerance of sterile moths should be conducted. The sterile moth's dispersal ability, or mobility, should also be improved during times when temperatures are not ideal for moth movement, when minimum temperatures drop below 10°C. As wind direction will also affect dispersal patterns, releasing in wind-still conditions will be ideal. Nepgen (2014) showed it is better (more effective measured by recovery of the sterile FCM) to apply SIT on the ground than by gyroplane, but aerial releases are faster, more cost-effective and provide a more uniform distribution of sterile insects over a target area than ground releases.

Acknowledgements

- The Agricultural Research Council is acknowledged for funding the running costs for this project and providing the opportunity to do my thesis on the Addo Research Station in Addo.
- Dr Sean Moore of Citrus Research International for his guidance and supervision of the project and Mr Retief Celliers of NMMU as co-supervisor.
- Marieta van der Rijst of the Agricultural Research Council Infruitec Nietvoorbij for her assistance with statistically analysing the data.
- John Ferreira and Nicola Combrink for their assistance with the graphs and tables.
- Lucio Phindile Zuma, A.K. Mthwisa, C Damaans, L Habana and X. Koloza for field work assistance.

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