A Study on the Application Technology of the Sterile Insect Technique, with Focus on False Codling Moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), a Pest of Citrus in South Africa

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ABSTRACT

False codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) is considered the most important indigenous pest of citrus in southern Africa. Major concerns such as progressive insecticidal resistance, the negative impact of insecticides on the environment, as well as the influence of consumers opposed to chemical residues on fruit, created opportunities for biological control methods such as Sterile Insect Technology (SIT). This technology is now established in the Western and Eastern Cape provinces of South Africa as an effective, sustainable alternative to conventional FCM control methods. Due to the prevalence of the pest in all citrus producing areas of South Africa, potential for SIT to expand is enormous. Success of an SIT programme is highly dependent on efficient application of the technology to achieve its objectives in a timeous manner. The aim of this study was to advance the application of SIT for control of FCM on citrus in South Africa, by investigating the effect of certain critical stages in the process. The effect of long-distance transportation on fitness of irradiated FCM was determined, showing reduced performance with cold-immobilized transport. A significant decrease in flight ability and longevity of irradiated FCM was found, although critically, realized fecundity was not affected. The effect of two different insecticides in the pyrethroid and organophosphate chemical groups were investigated for their residual effect on mortality of released irradiated FCM, to determine if these pest control programmes could be integrated. Both chlorpyrifos and tau-fluvalinate were effective in killing irradiated FCM for a number of days after application, after which degradation of the active ingredient rendered it harmless. This effect was found to be similar for irradiated and non-irradiated males, consequently ratios of sterile : wild male FCM should be retained regardless of whether sprays are applied or not. The modes for release of sterile FCM in an SIT programme were investigated. Efficacy of ground and aerial release platforms were tested by evaluating the recovery of released irradiated male FCM in these orchards. More irradiated FCM were recovered in orchards released from the ground compared to air. However, an economic analysis of both methods shows application of irradiated insects over a large geographical area is more cost-effective by air. Depending on the terrain and size of the target area, a combination of both methods is ideal for application of SIT for control of FCM in citrus. Development of application technology for advance of the programme is discussed and recommendations for future research and development are offered.

DECLARATION

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

Eugene S Nepgen 05.02.2014

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TABLE OF CONTENTS

Abstract.....ii

Declaration.....iii

Acknowledgements.....iv

Table of Contents.....v

List of Tables.....viii

List of Figures.....x

List of Abbreviations.....xiii

Problem statement.....xvi

Chapter 1: General Introduction.....1

1.1 The Technology.....1

1.1.1 History of SIT.....1

1.1.2 Biological basis for SIT.....2

1.1.3 Radiation technology.....4

1.1.4 Inherited sterility.....5

1.1.5 Population suppression.....6

1.1.6 Environmental considerations.....7

1.2 Impact of SIT Strategies Worldwide.....8

1.2.1 Eradication.....9

1.2.2 Suppression.....10

1.2.3 Containment.....10

1.2.4 Prevention.....11

1.3 The Pest.....12

1.3.1 Taxonomy and distribution.....12

1.3.2 Host range of FCM.....13

1.3.3 Life history of FCM.....17

1.3.4 Economic importance.....18

1.4 Control of FCM.....19

1.4.1 Inspection and monitoring.....19

1.4.2 Cultural control.....20

1.4.3 Biological control.....20

1.4.4 Granulovirus.....21

1.4.5 Chemical control.....21

1.4.6 Mating disruption.....22
1.4.7 Attract and kill.....23
1.4.8 Sterile insect technique.....23
1.5 Aims of Research.....24

Chapter 2: The effect of long distance transportation on the fitness of irradiated FCM.....25
2.1 Introduction.....25
2.2 Materials and methods.....26
2.2.1 Longevity.....28
2.2.2 Flight ability.....28
2.2.3 Realized fecundity.....28
2.2.4 Statistical analyses.....29
2.3 Results.....29
2.3.1 Longevity.....30
2.3.2 Flight ability.....32
2.3.3 Realized fecundity.....33
2.4 Discussion.....35

Chapter 3: Integrating chemical control with releases of irradiated FCM in an IPM programme utilising SIT.....38
3.1 Introduction.....38
3.2 Materials and methods.....42
3.2.1 Leaf substrate.....42
3.2.2 Soil substrate.....43
3.3 Results.....43
3.3.1 Leaf substrate.....45
3.3.2.1 Dose response.....45
3.3.2.2 Time response.....53
3.3.2 Soil substrate.....57
3.4 Discussion.....60

Chapter 4: Efficacy of release technologies for SIT.....63
4.1 Introduction.....63
4.2 Material and methods.....65
4.2.1 Release sites.....65

4.2.2 Release methods……69
4.2.3 Monitoring moth populations……70
4.2.4 Statistical analyses……71
4.3 Results……71
4.4.1 Release methods……71
4.4 Discussion……75
4.5 Appendix……79

Chapter 5: General discussion……80
5.1 The importance of application technology in an SIT programme ……80
5.2 Long distance transportation of irradiated insects for SIT……82
5.3 Integration of insecticides in an SIT programme……83
5.4 Efficacy of different release methods for application of SIT ……85
5.5 The way forward for SIT in South Africa ……86

References……88

LIST OF TABLES

Table 1.1. Cultivated host plants of FCM.

Table 1.2. Wild host plants of FCM.

Table 3.1 Important insect and mite pests of citrus in South Africa.

Table 3.1 Important insect and mite pests of citrus in South Africa.

Table 3.2 T-test statistics for comparison of irradiated and non-irradiated male FCM mortality in water-treated controls.

Table 3.3 Results of the t-test comparison between irradiated and non-irradiated male FCM mortality in the water-treated controls.

Table 3.4 Mortality of irradiated male FCM in dose-response evaluations with tau-fluvalinate residues of different ages.

Table 3.5 Mortality of non-irradiated male FCM in dose-response evaluations with tau-fluvalinate residues of different ages.

Table 3.6 Mortality of irradiated male FCM in dose-response evaluations with chlorpyrifos residues of different ages.

Table 3.7 Percentage mortality (mean ± SE) of irradiated male FCM after 12 h exposure to chlorpyrifos and tau-fluvalinate residues plus a water treated control on a leaf substrate.

Table 3.8 Percentage mortality (mean ± SE) of non-irradiated male FCM after exposure to chlorpyrifos and tau-fluvalinate residues plus a water-treated control on a leaf substrate.

Table 3.9 Logistic regression data for mortality of irradiated male FCM exposed to chlorpyrifos and tau-fluvalinate residues plus a water treated control on a leaf substrate.

Table 3.10 Logistic regression data for mortality of non-irradiated male FCM exposed to chlorpyrifos and tau-fluvalinate residues plus a water-treated control on a leaf substrate.

Table 3.11 Percent mortality (mean ± SE) of irradiated male FCM after 12 h exposure to chlorpyrifos and tau-fluvalinate residues plus a water treated control on a soil substrate.

Table 3.12 Percent mortality (mean ± SE) of non-irradiated male FCM after 12 h exposure to chlorpyrifos and tau-fluvalinate residues plus a water treated control on a soil substrate.

Table 4.1 Number of irradiated male and female FCM released per hectare per week by ground and aerial methods from 10 September 2012 to 25 March 2013.

LIST OF FIGURES

Figure 1.1 The geographical distribution of false codling moth (*Thamatotibia leucotreta*) in Africa and its neighbouring islands.

Figure 1.2 False codling moth (*Thamatotibia leucotreta*).

Figure 2.1 A cardboard container used for storage and shipping of irradiated FCM.

Figure 2.2 Insulated polyurethane cooler box with moth containers and Poly-ice packs used for cooling during transport.

Figure 2.3 Temperature (°C) of irradiated FCM in insulated cooler boxes during transport of six consignments originating in Citrusdal, Western Cape and arriving in Addo, Eastern Cape.

Figure 2.4 Longevity of irradiated (T) and non-irradiated (N), male (♂) and female (♀) FCM with and without transportation, during summer 2013. Bars with different letters denote significant differences.

Figure 2.5 Longevity of irradiated (T) and non-irradiated (N), male (♂) and female (♀) FCM with transportation, during winter and summer 2012/13.

Figure 2.6 Flight ability (grade 0-3) of irradiated (T) and non-irradiated (N), male (♂) and female (♀) FCM with and without transportation, during summer 2013.

Figure 2.7 Flight ability (grade 0-3) of irradiated (T) and non-irradiated (N), male (♂) and female (♀) FCM with transportation, during summer 2013. Bars with different letters denote significant differences.

Figure 2.8 Realized fecundity (number of eggs) for combinations of irradiated (T) and non-irradiated (N), male (♂) and female (♀) FCM as mating pairs, viz. T♀ x T♂, T♀ x N♂, N x T♂ and N♀ x N♂, with and without transportation, during summer 2013. Bars with different letters denote significant differences.

Figure 2.9 Realized fecundity (number of eggs) for combinations of irradiated (T) and non-irradiated (N), male (♂) and female (♀) FCM as mating pairs, viz. T♀ x T♂, T♀ x N♂, N x T♂ and N♀ x N♂, with transportation during summer and winter, 2012/13.

Figure 3.1 Irradiated male FCM on a treated leaf substrate inside a munger cell.

Figure 3.2 Series of 40 Munger cells of which half contain irradiated and half non-irradiated male FCM on treated leaf substrate connected to an air-flow system.

Figure 3.2 Ten irradiated male FCM on treated soil substrate enclosed inside a container.

Figure 3.4 Probit line for tau-fluvalinate residue of different ages against irradiated and non-irradiated male FCM.

Figure 3.5 Probit lines for chlorpyrifos and tau-fluvalinate residues of different ages against irradiated male FCM.

Figure 3.6 Mortality of irradiated male FCM after exposure to leaf-residues of chlorpyrifos and tau-fluvalinate.

Figure 3.7 Mortality of non-irradiated male FCM after exposure to leaf-residues of chlorpyrifos and tau-fluvalinate.

Figure 3.8 Cumulative percentage mortality of irradiated male FCM over 12 hours for different age chlorpyrifos residues.

Figure 3.9 Cumulative percentage mortality of non-irradiated male FCM over 12 hours for different age chlorpyrifos residues.

Figure 3.10 Cumulative percentage mortality of irradiated male FCM over 12 hours for different age tau-fluvalinate residues.

Figure 3.11 Cumulative percentage mortality of non-irradiated male FCM over 12 hours for different age tau-fluvalinate residues.

Figure 3.12 Mortality of irradiated and non-irradiated male FCM after exposure to fresh residues of chlorpyrifos, tau-fluvalinate and water on a soil substrate.

Figure 3.13 Cumulative mortality of irradiated male FCM over a 12 hour period after exposure to fresh residues of chlorpyrifos, tau-fluvalinate and water on a soil substrate.

Figure 3.14 Cumulative mortality of non-irradiated male FCM over a 12 hour period after exposure to fresh residues of chlorpyrifos, tau-fluvalinate and water on a soil substrate.

Figure 4.1 Bernol Farm, Addo, where aerial releases were conducted. Blocks with green boundaries form part of release area while yellow shaded (lemon orchard) block does not.

Figure 4.2 Penhill Estates, Addo, where ground releases were conducted. Blocks with green boundaries form part of release area while yellow shaded (lemons) and green shaded (fallow land) blocks do not.

Figure 4.3 A specially equipped ATV used in the SIT programme for ground release of irradiated FCM in citrus orchards.

Figure 4.4 Specially equipped gyroplanes used in an SIT programme for aerial release of irradiated FCM on citrus orchards.

Figure 4.5 Mean weekly recaptures of irradiated male FCM per trap from orchards where releases were conducted by ground and aerial methods from 10 September 2012 to 25 March 2013. Standard Error bars have been omitted for clarity.

Figure 4.6 Mean irradiated male FCM per trap, with significantly higher recaptures from orchards where releases were conducted by ground compared to aerial method.

Figure 4.7 Mean irradiated male FCM per trap from orchards where releases were conducted at different release rates, regardless of mode of release. Means not followed by the same letter differ significantly.

Figure 4.8 Total amount of irradiated male FCM per trap from orchards where releases were conducted by ground and aerial methods combined at mean minimum night temperatures.

LIST OF ABBREVIATIONS

%	percent
°C	degrees Celsius
µl	micro litre
♀	Female
♂	Male
^{137}Cs	caesium-137
^{60}Co	cobalt-60
ATV	all-terrain vehicle
AW-IPM	area-wide integrated pest management
CGA	Citrus Growers Association
CM	codling moth
cm^2	square centimetre
CRI	Citrus Research International
d	days
DAFF	Department of Agriculture, Fisheries and Forestry
DNA	deoxyribonucleic acid
EPN	entomopathogenic nematodes
EU	European Union
F_1	first filial generation
FCM	false codling moth
GPS	global positioning system

Gy	gray
ha	hectare
h	hours
IGR	insect growth regulator
IOBC	International Organisation for Biological Control
IPPC	International Plant Protection Convention
km	kilometre
km^2	square kilometre
kph	kilometre per hour
L	litre
L/ha	litre per hectare
LAB	Los Angeles Basin
LC_{50}	medial lethal concentration
LT_{50}	medial lethal exposure time
m	metre
m/s	metres per second
Medfly	Mediterranean fruit fly
MeV	Million Electron Volts
ml	millilitre
ml/m^2	millilitre per square metre
mm	millimetre
N	non-irradiated

n	number of replicates
NWS	new world screwworm
P_1	first parental generation
PBW	pink bollworm
PPECB	Perishable Products Export Control Board
PVC	polymerizing vinyl chloride
RBE	relative biological effectiveness
RH	relative humidity
SIT	sterile insect technique
SRV	Sunday's River Valley
T	treated
US$	United States Dollar
USA	United States of America
UV	ultraviolet
ZAR	South African Rand

PROBLEM STATEMENT

In order to suppress a very mobile, polyphagous pest, with high reproductive potential such as false codling moth (FCM), all control measures must be implemented with great thoroughness and timeliness. Margins for error are very small. The proposed focus of this study will be the improvement of application technology for the sterile insect technique (SIT) on FCM in South Africa.

Improvement of SIT for suppression of FCM and expansion of the programme to other citrus producing areas in southern Africa is of major importance to the citrus industry. Recent results verified the efficacy of SIT for control of FCM by release of irradiated moths originating from geographically distinct areas (Hofmeyr *et al*. 2010). Irradiated FCM are produced in Citrusdal, Western Cape province, and transported over a long distance to the release site in Addo, Eastern Cape province. By sufficient cooling, irradiated FCM go into a quiescent state in which energy output is minimised and aging is decelerated. The physiological effect transport has on irradiated insects needs to be addressed to improve quality of released insects. The commercial release of irradiated FCM over citrus orchards is currently conducted by aircraft. The concern is that stress caused by high temperature and high insect density in the release mechanism impacts on insect quality and therefore efficacy of SIT. Additional factors such as release altitude and accuracy of distribution play a role in efficacy of release methods. Application of broad spectrum insecticides remains the most effective control for certain problematic pests in citrus. Some insecticides appear to influence recaptures of irradiated FCM and possibly the efficacy of SIT. In particularly, certain pyrethroids and organophosphates could kill moths on contact with a leaf or soil substrate, justifying integration of chemicals in an area-wide integrated pest management (IPM) programme utilising SIT. This thesis attempts to fill these knowledge gaps.

1

GENERAL INTRODUCTION

1.1 The technology

1.1.1 The History of SIT

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: Drosophilidae) at the Moscow State University in the 1930s and 1940s, supported the principles of Mendelian genetics for advance of Soviet Agriculture by the use of chromosomal translocations to cause inherited partial sterility for pest population suppression (Robinson 2002). Ultimately he determined the extent to which sterility would appear as an inherited trait in a population after a single release of translocation homozygotes, developed means of promoting the level of sterility by the use of different translocations and suggested releasing only male insects to circumvent the problem of temporary increase in the breeding population (Curtis 1968). Vanderplank (1947) worked at a tsetse field research station in Tanzania when he discovered a method to induce sterility by crossing two tsetse fly species, *Glossina swynnertoni* (Austen) and *Glossina morsitans* (Westwood) (Diptera: Glossinidae), consequently producing hybrids of those crosses. He determined that the genitalia from hybrid flies were different from both parent species, that the males were sterile and the females partially sterile. His discovery led to the virtual elimination of *G. swynnertoni* through release of *G. morsitans* in Tanzania. Hybrid sterility advanced, based on Haldane's rule, which states when two subspecies are crossed it is always the heterogametic sex that is sterile (Orr 1997). Today this system remains an option for control of tsetse flies, because many *Glossina* species are allopatric, enabling the release of male insects from a certain species for the control of a geographically distinct target species (Krafsur 1998). Knipling (1955) primarily researched mating behaviour of the New World screwworm, *Cohliomyia hominovorax* (Coquerel) (Diptera: Calliphoridae). He soon realised the potential for population suppression through sterilisation and release of vast numbers of the insects and developed a mathematical model that determined the probability of eradication, provided a significantly high ratio of sterile:fertile screwworms is sustained over several successive generations (Barclay 2005, Klassen 2005). Runner (1916) demonstrated X-ray induced sterility in the cigarette beetle, *Lasioderma serricorne* (Fabricius)

(Coleoptera: Anobiidae) in 1916 and was subsequently followed by H.J. Muller's discovery of induced mutagenesis through ionic radiation in 1946. The first radiation and release of screwworm pupae was performed in the late 1940s on Sanibel Island, near Tampa, Florida, USA (Bushland & Hopkins 1953). Their work preceded SIT programmes for area-wide application to eradicate screwworm populations in the United States of America (USA), Mexico and Central America by aerial releases of sterile pupae shipped from rearing facilities in Florida, Texas, Panama and Mexico. More recently, SIT was effectively applied worldwide for control of insect populations from the order Diptera, including the Mediterranean fruit fly (medfly), *Ceratitis capitata (*Wiedemann*)* (Diptera: Tephritidae) in Guatemala and Mexico, the tsetse fly *Glossina austeni* (Newstead) (Diptera: Glossinidae) in Zanzibar, the onion fly, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae) in the Netherlands and the Melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) in the Ryukyu Islands of Japan (Enkerlin 2005). Successful SIT programmes for control of insects from the order *Lepidoptera* are ongoing in the Central Valley of California, USA, against Pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechidae), codling moth (CM), *Cydia pomonella,* (Linnaeus) (Lepidoptera: Tortricidae) in British Columbia, Canada, and false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) in the Western Cape province, South Africa (Bloem *et al.* 2005).

1.1.2 Biological Basis for SIT

Numerous fundamental biological factors should be considered when deciding to implement SIT for control of a given insect population. Assessment of important factors such as the role of the insect in the agro-ecosystem, pest ecology, mating systems, sterile insect production and some post-copulatory factors will eventually determine whether SIT will be appropriate for control of the pest population. Reduction of the target species should have a significant effect, for example the eradication of a vector for serious diseases, *Glossina* spp. as a vector of Trypanosomiasis (Feldmann *et al.* 2005), the reduction in phytosanitary risk for international trade, FCM as a pest of citrus (Hofmeyr *et al.* 2005), or to prevent establishment of a key pest in a new area, medfly in California, USA (Enkerlin 2005). SIT is especially feasible when applied against small numbers of a pest that attacks high value crops such as CM as a pest of apples and pears (Bloem *et al.* 2010). In principal, success of SIT is based on the dynamics of the target pest population and the intended over flooding ratio of sterile to wild male insects that is to be sustained continuously over time to guarantee suppression of the population. The relationship is inversely density-dependant given the fact that the frequency of sterile matings will increase as the wild population becomes smaller and eventually collapses. Biological characteristics such as sexual reproduction and a low intrinsic rate of increase are essential for the success of SIT while parthenogenesis is a potential pitfall (McInnis *et al.* 1996).

Seasonality and the target insects' thermal tolerance have major implications for both the laboratory reared- and wild insects. Microclimate temperatures will influence the reproduction rate, longevity and survival of insects and consequently could affect the ability of sterile insects to sufficiently compete for wild females in the field. For example, wild FCM has a greater tolerance to low temperatures and a lower minimum threshold for flight activity than the facility-reared moths, which places severe constraints on the SIT programme (Stotter & Terblanche 2009). The dispersal of insects over a given area as well as the dispersion ability of individuals plays a significant role in SIT. An evenly distributed target population, sparsely dispersed over a wide area will increase the efficacy of an SIT programme. 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). Chemical communication has important implications for SIT. Instraspecifically, the sterile male should be able to recognize and find a potential mate using the semiochemicals produced by females. In addition, synthetic sexual attractants can be used for the benefit of SIT, to monitor the released sterile and wild population for assessment of distribution, sterile to wild male ratio and sterile male quality (Vreysen 2005).

1.1.3 ***Radiation technology***

Holometabolous species are favoured over hemimetabolous species since the presence of a quiescent pupal stage facilitates mass rearing and in some species the sterilization and transportation of large quantities of sterile insects (Parker 2005). Dormancy periods, obligatory diapause and length of the lifecycle must also be taken into account when developing a feasible rearing system. Environmental differences between the rearing facility and field influence the insect phenotype and competitiveness. Other aspects of the mass-rearing environment such as the artificial diet, handling methods and irradiation will have an impact on sterile insect quality (Calkins & Parker 2005). Sterility induced by X-rays, electron beams or gamma rays cause chromosomal damage and is greatly influenced by the insect species, stage of development during radiation and the radiation dosage. The effective dosage varies between species even though the aim in general is to achieve the highest level of sterility without sacrificing insect quality (Bakri *et al.* 2005). The ability of released sterile males to compete for a mate is the cornerstone of SIT and should be assessed and quantified to ensure its compatibility and disposition is maintained (Calkins & Parker 2005). Characteristics of a simple or complex mating system, such as male courtship ritual, the female choice of mates, sex pheromones, and male-male competition determines the outcome of SIT. Post-copulatory factors such as female receptiveness and quantity or quality of sperm transferred can limit effectiveness of mating between sterile males and wild females.

The International Plant Protection Convention (IPPC) (FAO 2007) defines a sterile insect as: 'an insect that, as a result of a specific treatment, is unable to reproduce'. Although ionizing radiation is currently the preferred treatment to induce dominant lethal mutations in the released male insect's sperm, many chemical mutagens have been tested as alternatives. Chemical substances such as alkylating or deaminating agents raise the frequency of mutation greatly beyond the spontaneous background level by causing substitution of the alkyl or amine group for active hydrogen in a DNA molecule (Hoy 2003). Although chemosterilants were very effective for sterilizing mass-reared insects, by either adding them to insect diet or applied topically (Moursy *et al.* 1988), their use is no longer practical due to the carcinogenic, mutagenic and/or teratogenic nature of the compounds leading to environmental and human health issues (Hayes 1968). Sterilizing insects by ionizing radiation is a reasonably simple process as long as the dose is distributed evenly. The vital parameter is the radiation absorbed dose, expressed in SI units as gray (Gy) (1 Gy = 100 rad), in which 1 Gy is equivalent to 1 joule of energy absorbed by 1 kg of a specified material (1 Gy = 1 J/kg) (Bakri *et al.* 2005b). Parker & Mehta (2007) confirmed advantages of using radiation to sterilize insects are the insignificant temperature rise during the process enabling immediate release into the field, the absence of residues that could be harmful to humans or the environment and allowing sterilization after packaging seeing that radiation can pass through packaging material. Bakri *et al*. (2005a) determined properties such as Relative Biological Effectiveness (RBE), penetrability, availability, safety and cost play a role in suitability of radiation type. The RBE of radiation is defined as the ratio of the dose of 200-250 kV X-rays required to produce a specific biological effect to the dose of radiation required to produce a similar effect. Gamma rays, high-energy electrons and X-rays have a similar RBE in insects and are all acceptable radiation sources used in SIT programmes worldwide (Bakri *et al*. 2005a).

In general gamma rays from cobalt-60 (^{60}Co) (photon energies of 1.17 and 1.33 MeV) and caesium-137 (^{137}Cs) (0.66 MeV), electrons generated from accelerators with less than 10 MeV energy emitted, and X-rays generated from electron beams with energy below 5 MeV, are acceptable for sterilizing insects (Bakri *et al*. 2005a). The measurement and distribution of the absorbed dose delivered to the insect are vital for effective sterilization of insects (Parker & Mehta 2007). Reference-standard dosimetry systems are used to accurately measure absorbed radiation dose to determine the dose rate at a specific reference position inside a self-contained gamma irradiator (ISO/ASTM 2005). Using dosimeters that exhibit a quantifiable change in some property after radiation, e.g. colour, together with measuring instruments to read the change in dosimeters, it is possible to calculate the dose-distribution and determine the dose variation within the radiation batch. The optimal sterilizing dose for a specific insect species is of particular importance in SIT programmes (Robinson 2002). As the

radiation dose increases, sterility increases, but quality and competitiveness decrease (Calkins & Parker 2005).

In preparation for irradiation, the ideal development stage is determined by maturity of insect reproductive organs, handling suitability during irradiation and shipping as well as sensitivity to somatic change. In most holometabulous species germ tissues have formed by the late pupal stage or early adult stage, which is consequently suitable timing for irradiation. Early irradiation could have adverse effects on the somatic tissues producing inferior quality insects and irradiation too late in the development cycle will cause viable eggs to be formed, regardless of having been irradiated, given that oocytes have formed in the female at that stage.

1.1.4 Inherited sterility

The IPPC (FAO 2007) describes SIT as: 'a method of pest control using area-wide inundative release of sterile insects to reduce reproduction in a field population of the same species'. 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 to a certain extent can 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 (F_1 generation) show a higher level of sterility than their parents and in addition the level of sterility in F_1 females is lower than in F_1 males. This unique genetic phenomenon, termed inherited sterility, typically exists in Lepidoptera and a small number of other arthropods. Two significant consequences of radiation induced sterility are sex-specific and positive correlation with treatment dose. Gamma-irradiated males of Lepidoptera are better able to survive with a high number of chromosomal breaks than females and consequently the sex-ratio of the F_1 generation is highly in favour of the males (Tothova & Marec 2001). A significant increase in aberrations was found in the F_1 generation when studies on the Mediterranean flour moth, *Ephestia kuehniella (*Zeller) (Lepidoptera: Pyralidae) was done with an increasing dose of radiation. Multiple translocations forming complicated configurations were observed that increased linearly with dose (Tothová & Marec 2001). The high level of radio-resistance is possibly attributed to molecular mechanisms such as inducible cell recovery and DNA repair. The holokinetic nature of lepidopteran chromosomes and the presence of a localized kinetochore plate, to which microtubules attach during cell division, ensures that most radiation induced breaks will not lead to the loss of chromosome fragments as is the case for species with monocentric chromosomes (Wolf *et al.* 1997). In addition these large kinetochore plates reduce

lethality caused by formation of dicentric chromosomes, acentric fragments and other unstable aberrations (Tothová & Marec 2001). At cytological level, a dominant lethal mutation causes production of imbalanced gametes and consequently, inhibition of mitosis and death of embryos. Lepidopterans, and to a lesser degree hemipterans, require radiation doses as high as 350-500 Gy to induce the multiple chromosome rearrangements that will manifest as dominant lethal mutations and consequently full sterilization in the parental (P_1) generation (Lachance & Graham 1984). In the F_1 generation the main chromosomal mechanism of inherited sterility is represented by various types of translocations (non-reciprocal, reciprocal and multiple) that produce genetically unbalanced gametes (Tothova & Marec 2001). Inherited sterility provides advantages in pest control, specifically because egg hatch is significantly reduced and the radiation induced deleterious effects are inherited by the F_1 generation which are both highly sterile and predominantly male. The reduced radiation dose improves the released sterile insects' ability to disperse, its realized fecundity and sperm competitiveness (Bloem *et al.* 2001, Carpenter *et al.* 1997, North 1975).

1.1.5 Population suppression

The level of suppression by SIT is determined by the intrinsic rate of increase of the wild population and the degree of sterility introduced into the population by releasing sufficient amounts of sterile males to overcome the reproductive success of the wild females (Knipling 1968). For effective population suppression the sterile insects should have an advantageous numerical ratio to induce sterility and consequently stabilize the density of the target population. Insect species are unique in their capacity to increase, which means the ratio of sterile to fertile insects required to keep the natural population stable varies substantially. Additionally, population density of insects vary greatly in a given area and due to this heterogeneous distribution a significant over-flooding ratio must be maintained to be generally certain that a reduction in the population will occur in all parts of the environment. In Lepidoptera, partially sterile moths create higher levels of sterility in the F_1 generation in comparison to release of 100% sterile moths (Knipling 1970). Irrespective of the greater competitiveness due to lower doses of radiation received by the partially sterilized insects, four times the amount of completely sterile moths should be released to achieve a similar suppressive effect as a result of inherited sterility hypothetically based on 40% survival per generation (Knipling 1970). It is highly beneficial to resume release of sterile insects when the wild population is at a seasonal low, or immediately after a decline in numbers due to adverse weather events, to take advantage of the immense power of SIT against sparse populations of pests (Kniping 1979). Supplemental methods to reduce the target pest population, before- or in conjunction with the release of sterile insects have been used in many successful SIT programmes as a precursor for

suppression. In an area-wide integrated pest management system (AW-IPM) a combination of tactics is used to suppress a local population, to suppress a total population, to eradicate a well-established pest population or to contain and prevent an invasion. An efficient AW-IPM system combines each control tactic considering the relationship of the density of the pest to the efficiency of the control tactic. SIT is a highly species-specific method for pest suppression, targeting the reproductive system of sexually reproducing pests that plays a role in implementing all of these strategies. The use of a selective insecticide or releases of a pest specific parasitoid, both effective at high pest densities, along with release of sterile insects, effective only at sparse pest densities, are mutually attractive tactics. The synergy between inherited sterility and natural enemies is tremendously effective for suppression of Lepidoptera (Carpenter 2005). A combination of additive tactics should be integrated to achieve more reliable suppression than would be achieved from a single method, particularly when the economic threshold or quarantine status of a pest is moderately high (Knipling 1979).

1.1.6 Environmental considerations

Sterile insects are non-invasive, living- yet non-reproductive biological agents that are released into the environment and could be easily incorporated and processed within food networks. The only possible direct adverse effect of release sterile insects on non-target biota is related to changes in interactions amongst species (Nagel & Reveling 2005). Most area-wide SIT programmes are aimed at suppression or containment not eradication. Nevertheless, the possibility of eradication of a pest from or in elected areas holds the immediate economic and environmental benefit of reduction in pesticide use (Kinley 1998). Overwhelming economic, health and environmental benefits from eradication of primary targets using SIT, such as fruit flies (Enkerlin 2005), moths (Bloem *et al.* 2005), screwworm flies (Vargas-Terán *et al.* 2005) and tsetse flies (Feldmann *et al.* 2005) to some degree, overshadows the risks . In the event of total elimination of a target species, a decline in the genetic diversity for that species will occur (Myers *et al.* 2000). Eradication of native species from their complete natural range is a concern for biodiversity conservation and creates the possibility of destabilizing ecosystems if the ratio of extinction to speciation shows imbalance towards extinction. Nagel & Reveling (2005) showed this is not relevant for eradication of exotic species that have invaded regions outside of their natural range, although the impact on other species should be considered to minimize disruption to the environment. The risk of area-wide control varies among ecosystems to such an extent that removal or addition of elements from the food network could result in overabundance of others. This phenomenon was observed with impact of New World screwworm eradication resulting in the intense increase in numbers of white-tailed deer, *Odocoileus virginianus* (Zimmerman) (Artiodactyla: Cervidae), in the United States (Reichard 2002) after which

the deer parasitizing Gulf-coast tick, *Amblyomma maculatum* (Koch) (Ixodida: Ixodidae) emerged as a subsequent pest of cattle in the area (Kettle 1993). In addition, where species-specific-flower-pollinator systems or host-specific-parasitoid systems exist, SIT is not necessarily the acceptable method for pest control. Although limited, environmental hazards due to additional insecticide sprays as well as large scale trapping with artificial attractive devices designed for detection, monitoring or control of a pest population can pose negligible to high risks depending on the actual type and combination (Nagel & Reveling 2005). In general, ground applications of selective biological larvicides have less ecological effect and consequences for biodiversity conservation than the alternative aerial application of broad spectrum insecticides based on calendar dates (Pimentel 2005). Potential adverse effects such as disruption of natural biological control agents, poisoning of terrestrial and aquatic non-target invertebrates, by direct over-spray or run-off, and accumulation in the food chain triggering secondary effects can produce shifts in community structure and trepidations in ecological services (Nagel 1995). Knipling (1976) explained, the environmental risk of artificial trapping devices to non-target organisms increases with trap density. Certain species may be attracted coincidentally, by visual traits, odours or by mode of placement. However the use of species specific pheromones ensures local populations are unlikely to be affected. In this case, adverse effects will most likely occur in non-target organisms from the same genus or family as the target insect.

1.2 Impact of SIT strategies worldwide

The aim of area-wide integrated pest control (AW-IPM) is to suppress an entire pest population within a defined geographical area in order to prevent infestation of agricultural commodities and prevent the re-establishment of destructive densities of the pest population (Klassen 2005). SIT is an integral component of an AW-IPM system whereby continuous, successive and methodical release of sterile insects could effectively eradicate a pest population within a few generations (Knipling 1955). Several countries have profited from pest intervention programmes utilising SIT by reducing crop losses, minimizing pesticide use and facilitating international trade by providing better options for technical obstacles to phytosanitary issues (Hendrichs 2000). By following either one of four different strategies, namely eradication, suppression, containment or prevention, SIT has been used with unquestionable success to control a number of horticultural pests of great importance (Hendrichs 2005).

1.2.1 Eradication

Eradication is the removal of every single individual of a species from an adequately isolated geographical area where reinvasion is unlikely to occur (Myers *et al.* 1998). SIT has proven effective to eradicate an established pest species from its native habitat or following an introduction of the pest in an exotic habitat. The New World screwworm fly (NWS) is a myiasis causing fly that lays its eggs in the wounds of warm-blooded animals and the cause of severe losses to cattle and livestock producers in infested areas (Vargas-Terán *et al.* 2005). In 1954 on the island of Curaçao, Dutch Antilles, SIT was applied continuously for 6 months in a pilot project before the pest was eliminated and Curaçao was declared screwworm free (Baumhover *et al.* 1955). Wyss (2000) recalled successful eradication attempts, from the late 1950s, when NWS was applied on commercial scale in Florida, USA, declaring the area NWS free by 1960. By 1966 the last endemic population of NWS was eradicated in the south eastern United States which prompted the opening of a rearing facility in Mexico in 1976 with the capacity to produce 600 million sterile screwworm flies per week. By 1982 the entire USA received NWS free status. Eradication of NWS advanced to the Isthmus of Tehuantapec in 1984, and the rest of Mexico in 1991 (Hendrichs 2000). In subsequent years eradication was achieved in Belize and Guatamala in 1994, El Salvador in 1995, Honduras in 1996 and Nicarugua in 1999 (Wyss 2000). The releases of sterile screwworm flies are continuing over Costa Rica and Panama, where the aim is to maintain a permanent barrier of sterile flies in the Darien Gap between Panama and Colombia (Hendrichs 2000). After this destructive insect was introduced into Libya in 1988 with a shipment of sheep from South America, it quickly became established, rapidly spreading to a 25 000 km^2 area and threatening to disperse even further into the Near East, Mediterranean and Sub-Sahara (Klassen *et al.* 2005). After a decision was taken to eradicate the outbreak by using SIT in 1990, sterile flies were release by air over a treatment area of 40 000 km^2 and the population was eradicated by 1991 (Hendrichs 2000).

The efficacy of NWS eradication by SIT is partly due to its association with mammals, especially domesticated animals where major populations are likely to be associated, which enables monitoring of the insect's distribution (Galvin & Wyss 1996). Other desirable characteristics contributing to the successful control of NWS are that the flies can be trapped, are easily reared and sterilized in the laboratory, and above all else its distribution, behaviour and genetics are well characterized (Myers *et al.* 1998). NWS is primarily a tropical insect and consequently eradication on the edge of its natural range where conditions are unfavourable could provide an advantage for eradication due to the fact that SIT exhibits increased efficiency with a decreasing target population density (Klassen 2005).

1.2.2 Suppression

Encouraged by the success of the NWS eradication in the USA and Central America, other programmes were initiated for eradication of established exotic insect species. The codling moth *C. pomonella* is endemic to Europe but has established in many countries worldwide as a serious economic pest of apples. A pilot project was started in apple and pear orchards in the Similkameen Valley of British Columbia, Canada, from 1976 to 1978 to explore the possibility of eradicating CM using SIT (Bloem et al. 2005). After several years the population was reduced significantly and eradication was achieved in some localized areas even though at a high cost compared to conventional insecticide control (Proverbs 1982). A full scale eradication programme was initiated in 1989 in the Okanagan Valley, British Colombia, Canada, to eradicate CM using mainly SIT, before insecticidal sprays, mating disruption and tree wraps were incorporated in the operational AW-IPM strategy during 1992 (Vreysen 2010). The mass rearing facility in Osoyoos was instated in 1994 and by 2004 produced 16 million moths per week (Bloem et al 2007). Results in the initial treatment area of 3200 hectares (ha) looked promising with a reduction in wild moth trap catches of 13 and 2.5 wild male CM per trap per week in the first and second generation in 1995, to 0.08 wild male CM per trap per week for the first and second generation in 2000. In addition, orchards where no CM damage was recorded increased from 42% in 1995 to 95% in 2000 and the sales of organophosphate insecticides dropped to 10% of the 1995 levels (Bloem et al 2005). Mainly due to poor implementation of quarantine measures, the SIT programme in the Okanagan Valley re-evaluated feasibility of eradication and decided it was no longer necessary, consequently changing it to suppression in the winter of 1998 (Bloem *et al.* 2007). Threats of shrinking budgets and varied land use including urban areas, abandoned orchards and multiple authorities such as native Americans contributed to the decision to change strategy (Myers *et al.* 1998). The population size for potential eradication ultimately determines the feasibility of either strategy for the control of established exotic pests (Sharov & Liebhold 1998). Area-wide suppression is often a more achievable alternative to eradication, especially if the goal is to reduce species with high fecundities such as CM to such low densities that populations are eliminated. The coordinated use of mating disruption, microbial insecticides and natural enemies within an AW-IPM programme can reduce the impact of an exotic species severely over time as well as reduce the selection for resistance that occurs if only chemical products were used.

1.2.3 Containment

The aim of containment in an SIT programme is to avoid the spread of established exotic pests or to consolidate progress made in a continuous eradication programme. In areas where the wild pest

population is too high for effective control, various reduction methods should be integrated in order for the SIT to be effective (Hendrichs *et al.* 2005). In 1976 'Programa Moscamed' was established with its primary goal being containment of medfly in Guatamala and prevention of spread into neighbouring Mexico and the USA, thereby protecting the horticulture industry in all three countries (Hendrichs 2000). The construction of a mass rearing facility in Tapachula, Chiapas, Mexico, enabled production of 500 million sterile flies for release over a 6 400 km^2 area in Chiapas that was already invaded (Enkerlin 2005). Between 1977 and 1982 the medfly was eradicated from Chiapas by integrating the release of sterile flies with several other control measures including quarantine measures, chemical insecticides as well as cultural and mechanical methods (Villaseńor *et al.* 2000). Strict legal measures threatening Mexico's multi-million dollar fruit and vegetable export trade with the USA was the driving force behind the continuation of the programme (Hendrichs 2000). After the initial success of eradication in 1982, a sterile medfly barrier was sustained from southern Belize through Guatamala to southern Mexico to guarantee fly-free status of Mexico, USA and half of Guatamala.

An on-going SIT programme for the containment of pink bollworm (PBW) in the San Joaquin Valley of California has proven successful for area-wide control of an invasive exotic species (Staten *et al.* 1993). The PBW was detected in Mexico in 1911 and in Texas, USA, by 1917 after this destructive pest of cotton spread through New Mexico into Arizona by 1926 and ultimately to the cotton growing area of southern California in 1965 (Burrows *et al.* 1982). A cooperative effort from the state and federal governments and growers was realised as a mass rearing facility was built in 1968 in Phoenix, Arizona, producing sterile PBW adults that could be released over approximately 400 000 hectares of cotton on a daily basis. The integration of SIT with extensive cultural control and mating disruption prevented the high population of PBW occurring in the adjacent regions of southern California, Arizona and northern Mexico from becoming established in the San Joaquin Valley (Staten *et al.* 1993).

1.2.4 Prevention

Several examples exist where SIT is being applied as preventative control to avoid establishment of an exotic species. Once medfly was detected in California in 1975 and again in 1980, large scale eradication campaigns were launched to eliminate medfly with malathion-bait sprays (Carey 1996). Recurring infestations in 1980 and 1982 exemplified the ineffectiveness of chemical insecticides for eradication of medfly in Florida (Penrose 1995). Successful eradication was declared in 1989 and the programme was concluded. In 1989 however, 25 medflies were trapped in the eradication zone. In the following years an attempt at eradication of medfly utilising SIT, also failed after 100 flies were

captured in the original eradication zone (Myers *et al.* 1998). In view of these failed attempts as well as public opposition to regular aerial bait-sprays over urban areas, authorities entered into an area-wide SIT programme against medfly in 1994 covering the entire Los Angeles Basin (LAB). In 1996 the medfly was successfully and cost-effectively eradicated in the LAB and continued area-wide release of sterile medflies over 5 500km^2 of high risk areas prevented further outbreak of the pest (Dowell *et al.* 1999, Hendrichs 2000). From 1996 to 2000 there was a 97% reduction in medfly infestation in the treatment area (CDFA 2000). Constant monitoring for detection of confined medfly populations within the LAB proved that California is under constant threat of invasion. However, the preventative SIT approach is a highly effective environmentally-friendly method to prevent the development of medfly populations from these invasions (Hendrichs 2000). A major weakness in the evaluation of medfly population eradication was the use of ineffective lures for detection of low population levels (Kaneskro 1993). Trap catches from California in the 1980s and early 1990s imply the presence of an extensive medfly population that increases intermittently, possibly linked with favourable climatic conditions, to densities that are high enough to be detected in the traps. In support of this hypothesis it was found that 95% of the medflies were trapped in summer and autumn and only 5% in winter and spring, illustrating a seasonal pattern that might influence the declaration of eradication (Carey 1996).

1.3 The Pest

1.3.1 Taxonomy and distribution

Fuller (1901) originally defined FCM after he found an infested citrus fruit in the Natal province (now KwaZulu-Natal), South Africa. He placed it in the *Carpocapsa* genus and named it the Natal codling moth (Schwartz 1981). Howard (1909) made reference to the 'orange codling moth', *Enarmonia bactrochopa*, before Meyrick redefined the species as *Argyroploce leucotreta* (Eucosmidae: Olethreutidae) in 1913 (Newton 1998). Kelly (1914) was the first to refer to the insect as the false codling moth after he discovered it in acorns near Pietermaritzburg, South Africa. Clarke (1958) moved it under the *Cryptophlebia* genus, whereafter Komai (1999) removed it and placed it in *Thaumatobtibia*. At present the accepted classification of FCM is *Thaumatotibia leucotreta* (Venette *et al.* 2003). Codling moth can be confused with FCM because of appearance and damage, even though the pest primarily attacks apples and pears (Venette *et al.* 2003). The macadamia nut borer, *Thaumatotibia batrachopa* (Leidoptera: Tortricidae) (Meyrick) and the litchi moth *Cryptophlebia peltastica* (Lepidoptera: Tortricidae) (Meyrick) are close relatives of FCM that overlap in distribution and host range in southern Africa, which could cause possible misidentification (Newton 1998, Timm 2007). False codling moth is endemic to sub-Saharan Africa and is particularly prevalent in Ethiopia,

Senegal, Ivory Coast, Togo and Upper Volta. The climate in the area occupied by the pest is tropical, dry or temperate (Venette 2003). It has also been found in neighbouring islands Mauritius, Madagascar and Cape Verde (Figure 1.1) (Stibick 2008, Hill 1975). Wyoski (1986) reported the moth as an agricultural pest in Israel when he found it infesting macadamia nuts, possibly after accidental introduction into the country.

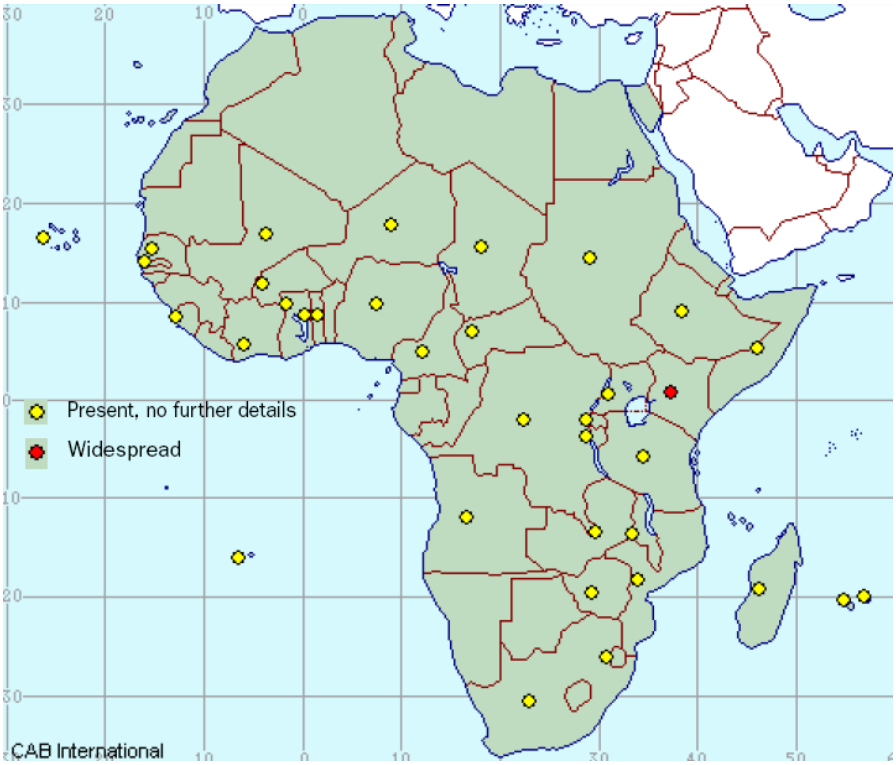

Figure 1.1 The geographical distribution of false codling moth (*Thamatotibia leucotreta*) in Africa and its neighbouring islands.

1.3.2 Host range of FCM

FCM feeds on a wide range of cultivated host plants (Table 1.1). It is a particulary problematic pest of cotton in most equatorial areas (Reed 1974, Newton 1998), citrus in southern Africa (Catling & Aschenborn 1974, Newton 1998) and more recently macadamias in Malawi (La Croix & Thindwa 1986, Newton 1998). Other cultivated hosts include walnuts, almonds (Annecke & Moran 1982), maize (Schulthess *et al.* 1990), tea seeds, olives (Annecke & Moran 1982), avocados (Erichsen & Schoeman 1992), litchis (Newton & Crause 1990) and some deciduous fruit species (Daiber 1978). All citrus cultivars produced in southern Africa are susceptible to infestation (De Villiers & Grove 2006). Although navel varieties are most prone to attack, mandarins, Valencias and grapefruit are also

vulnerable to FCM (Hofmeyr 1998). Lemons are generally not considered a suitable host but could harbour significant levels of FCM in some cases (Anonymous 2011). A wide range of plants in the wild can be hosts to FCM infestation (Table 1.2), creating added problems for control of the pest in an area where these plants are predominant, as reinvasion of crops could arise. However, it is possible that the listed host range has been exaggerated, as several of these associations appear to have been forced in the laboratory, rather than recorded in the field, such as is the case with pineapple.

Table 1.1. Cultivated host plants of FCM (Reed 1974, Newton 1998, Catling & Aschenborn 1974, La Croix & Thindwa 1986, Annecke & Moran 1982, Schulthess *et al.* 1990, Erichsen & Schoeman 1992, Newton & Crause 1990, Daiber 1978, Venette *et al.* 2003).

Common Name	**Species**
Avocado	*Persea americana*
Almonds	*Prunus dulcis*
Apricot	*Prunus armeniciata*
Banana	*Musa paradisiacal*
Bean	*Phaseolus spp.*
Cacao	*Theobroma cacao*
Citrus	*Citrus sinensis, Citrus spp.*
Coffee	*Coffea arabica, Coffea spp.*
Cola	*Cola nitida*
Maize	*Zea mays*
Cotton	*Gossypium hirsutum*
English Walnut	*Juglans regia*
Grape	*Vitis spp.*
Guava	*Psidium guyajava*
Litchi	*Litchi chinensis*
Loquat	*Eribotrya japonica*
Macadamia nut	*Macadamia ternifolia*
Mango	*Mangifera indica*
Olive	*Olea europaea subsp. Europaea*
Pepper/pimento	*Capsicum spp.*
Persimmon	*Diospyros spp.*

Plum	*Prunus spp.*
Pineapple	*Ananas comosus*
Pomegranate	*Punica granatum*
Sorghum	*Sorghum spp.*
Tea	*Camellia sinensis*

Table 1.2. Wild and commercial host plants of FCM (Schwartz 1981, Venette *et al.* 2003, Kirkman & Moore 2007).

Common Name	**Species**
Bur weed	*Triumfeta spp.*
Bluebush	*Diospyros lycoides*
Bloubos	*Royena pallens*
Boerboon	*Schotia afra*
Buffalo thorn	*Zizyphus mucronata*
Carambola	*Averrhoa carambola*
Castorbean	*Ricinnus communis*
Chayote	*Sechium edule*
Cowpea	*Vigna unguiculata, Vigna spp.*
Custard apple	*Annona reticulata*
Elephant grass	*Pennisetum purpureum*
Governors plum	*Flacourtia indica*
Indian mallow	*Abutilon hybridium*
Jakkalsbessie	*Diospyros mespiliformis*
Jujube	*Zizyphus jujuba*
Jute	*Abutilon spp.*
(Wild) Kaffir plum	*Harpephyllum caffum*
Kapok/copal	*Ceiba pentranda*
Kei apple	*Dovyalis caffra*
Khat	*Catha edulis*
Kudu-berry	*Psuedolachnostylis maprouneifolia*
Lima bean	*Phaseolus lunatus*
Mallow	*Hibiscus spp.*
Mangosteen	*Garcinia mangostana*

Marula	*Sclerocarya caffra, Sclerocarya birrea*
Monkey pod	*Cassia petersiana*
Oak	*Quercus spp.*
Okra	*Ablemoschus esculentus*
Peacock flower	*Caesalpinia pulcherrima*
Pride of De Kaap	*Bauhinia galpini*
Raasblaar	*Combretum zeyheri*
Red milkwood	*Mumisops zeyheri*
Rooibos / Bushwillow	*Combretum apiculatum*
Sida	*Sida spp.*
Snot apple	*Azanza garckeana*
Stamvrugte	*Chrysophyllum palismontanum*
Sodom apple	*Calotropis procera*
Soursop	*Annona muricata*
Stemfruit	*Englerophytum magaliesmontanum*
Surinum cherry	*Eugenia uniflora*
Suurpruim / large sour plum	*Ximenia caffra*
Water-bessie	*Syzygium cordatum*
Wag'n'bietjie	*Capparis tomentosa*
Weeping boerboon	*Scotia brachypetala*
Wild fig	*Ficus capensis*
Wild medlar	*Vangueria infausta*
Wing bean	*Xeroderris stuhlmannii*
Yellow-wood seeds	*Podocarpus falcatus*
Yellow-wood, real	*Podocarpus latifolius*

1.3.3 Life history of FCM

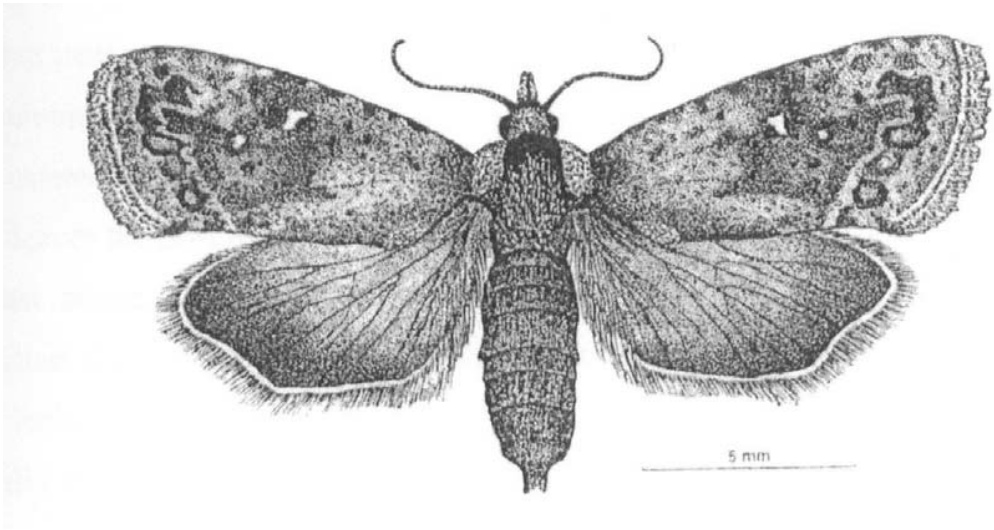

Figure 1.2 False codling moth (*Thamatotibia leucotreta*) (Pinhey 1975).

Female moths (Figure 1.2) lay their eggs one at a time, often in large numbers, on the rind of the fruit of the host plant. The eggs are translucent, hemispherical discs with a granulated surface, 0.77 mm in length and 0.6 mm in diameter (Daiber 1979a). Egg maturation varies between 9-14 days in winter and 4-8 days in summer, generally dependant on temperature, before the larva hatches (Schwartz 1981). The neonate larvae are white with a black head capsule and first thoracic segment, measuring approximately 1.4mm in length (Annecke & Moran 1982, Stofberg 1948). Within a short period of time the larvae will bore into the fruit and begin to feed on the inner rind of the fruit (Pinhey 1975). The young larva is cannibalistic towards eggs and larvae, which could be the reason why rarely more than one larva is found inside a fruit under normal circumstances (Catling & Aschenborn 1974). The larval stage consists of 5 instars (Daiber 1979b) transforming from a white, cream colour in the first instar to a characteristic pink colour as a mature fifth instar larva, measuring 15 – 20 mm in length. Development of larvae from first to fifth instar takes 25 to 67 days, both dependant on temperature (Stofberg 1948) and food quality (Daiber 1979b). The mature larvae will exit the fruit through a noticeable frass-filled hole in the fruit, either from a silken thread while the fruit is still hanging on the tree, or after the fruit has dropped to the ground (Moore 2002). The larvae will stop feeding at this point and begin to spin a cocoon from silken threads and soil particles as it enters the pupal stage (Stofberg 1948). The pupal stage is preceded by a pre-pupal stage, a soft-skinned version lasting nearly 2 days, after the chitin hardens and becomes dark brown (Daiber 1979c). The female FCM pupae are larger than the males and in addition, take 11 days to maturity compared to 12 days for the male at 25°C (Daiber 1979c). In the field, varying temperature plays a role in duration of the pupal stage, lasting anywhere between 21 to 80 days (Daiber 1979c). The

adults are small, unremarkable, brown to grey-coloured moths with a wingspan of 16-20 mm (Newton 1998). They have mottled forewings, while their hind wings are more evenly coloured grey and fringed (Newton 1998, Moore 2002). Males can be distinguished from females due to their smaller size, densely elongated scales on their hind tibia, an anal tuft of scales and scent organs near the anal angle of each hind wing (Newton 1998). Within 2-3 days of emergence from pupae, females will begin to mate (Stofberg 1954), although the pre-oviposition period could also be influenced by temperature for up to 22 days (Daiber 1980). It was reported that females could lay up to 300 eggs each (Stofberg 1948) or even as many as 456 eggs per female (Daiber 1979a), of which the largest portion occur in the first 5 days after mating (Moore 2002). The entire life-cycle of FCM will take 45-60 days to completion during warm summer months, from August to March, and 68-100 days during cooler winter months, April to July (Stofberg 1954). Favourable conditions, such as climate and food quality, will cause the number of generations to vary between 5 and 6 per year (Newton 1998).

1.3.4 Economic importance

In 2012, South Africa was the thirteenth largest producer and the second largest exporter of citrus fruit globally with 70% of the total production destined for lucrative overseas markets, 22% for the local markets whilst the remainder was processed (CGA 2012). In 2010, approximately 1.5 million tons of citrus was exported to Africa, the Americas, Asia and Europe, yielding a value of close to ZAR 6.6 billion (DAFF 2010). Annual losses of more than ZAR 100 million (US$ 14 million) to the southern African citrus industry is attributable to 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. As a result of FCM larvae that penetrate fruit shortly prior to harvest, detection with the naked eye is exceptionally difficult and many infested fruit pass unnoticed during initial sorting of fruit delivered to the pack house. In a survey done by the Perishable Products Export Control Board (PPECB) in 2011, FCM was identified as the foremost reason for Navel and Valencia orange as well as Grapefruit rejections at port, and only secondary to decay for rejections of Clementine and Satsuma cultivars across the board. In addition, the phytosanitary status of FCM creates an immeasurable risk to the South African citrus industry. Its polyphagous nature, resulting in the attack of a variety of different crops, is a concern for the USA and Europe that could result in major problems if it was accidentally introduced (Carpenter *et al.* 2004). The detection of a single larva in an export consignment on arrival can lead to the entire consignment being rejected, resulting in fruit the being rerouted to less sensitive markets for a considerable reduction in income (Moore 2002). The increased risk and potential threat of

establishment outside of southern Africa recently resulted in a zero tolerance policy enforced at pack houses that send fruit to sensitive markets.

1.4 Control of FCM

1.4.1 Inspection and monitoring

All different life stages of FCM are important to monitor the potential threat of FCM infestation and to determine FCM population levels in particular orchards. Fruit should be inspected for viable FCM eggs in order to determine the fluctuation in population size, although there is a poor relationship with fruit drop due to factors such as cannibalism, predation and accidental removal of larvae by brushing leaves and wind (Moore 2011a). Inspecting fruit for eggs can prove problematic, especially at an advanced ripening stage when the fruit begin to colour, as they are small and transparent. Dropped fruit should be dissected and inspected weekly from the beginning of January until harvest for FCM larvae or signs of infestation. Fruit drop however is not an accurate reflection on present levels of FCM due to drop occurring a few weeks after an increase in moth activity (Hofmeyr 2003). Data generated from fruit drop analysis can be used to gauge the extent of the FCM situation in a specific orchard and consequently the risk for post-harvest decay and infestation (Moore 2011a). Hofmeyr (2003) also determined the only effective means of monitoring FCM population levels is a pheromone based trapping system. A dispensers loaded with a synthetic female pheromone is housed inside a yellow delta trap or PVC (polymerizing vinyl chloride) pipe trap and hung in a tree, on the upwind side of an orchard, with a distribution of 1 for every 4 to 6 hectares of citrus. Male moths are attracted to the pheromone source and ensnared on a sticky floor inserted in the bottom of the trap. The trapping system is important to predict FCM infestation, however trap counts should be evaluated in conjunction with fruit drop and infestation for each orchard in order to establish a relationship and apply control measures optimally (Hofmeyr 2003). Historic thresholds determined that 10 male FCM trapped per week should equate to 1 infested fruit per tree in that orchard, and consequently justifies a corrective measure (Hofmeyr 2003). These threshold values do not apply anymore due to the phytosanitary status of FCM and as a result corrective measures should be applied regardless of the population levels in traps and fruit inspection points (Moore 2011a). Trap data can be used as an early warning management tool, but not directly for control of FCM (Hofmeyr 2003). 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 1 to 2 weeks afterwards (Moore 2011a).

1.4.2 Cultural control

Orchard sanitation to eliminate larvae and rotting fruit from orchards is the foundation of FCM control in citrus (Moore 2011a). A recent survey by Citrus Research International (CRI) showed that it is possible to remove as much as 75% of the larvae from an orchard by conducting orchard sanitation once per week. However, it is highly recommended that sanitation be conducted more frequently, particularly in warm summer months, due to the temperature dependant development cycle of FCM (Moore 2011a). Good cultural practice includes removal of fruit from the orchard floor as well as visibly infested fruit from trees due to larvae often leaving the fruit prior to them falling to the ground (Moore 2011a). Fruit collected from the orchard should be buried under a layer of soil or destroyed mechanically to ensure no larvae escape (Hepburn & Bishop 1954). Regular weekly orchard sanitation should commence before fruit are marble-sized, October to November, and continue throughout the harvesting period, above all making sure there are no fruit left in the orchards after harvesting in an orchard is complete and that could potentially be a source of FCM infestation for the next fruit crop setting in spring (Stofberg 1954, Schwartz 1974, Hofmeyr 2003).

1.4.3 Biological control

Suppression of FCM populations by biological methods has proven successful through a number of different parisitoids, pathogens and predators. Moore (2002) reported 25 natural enemies of FCM, of which 12 are known to occur in citrus orchards in South Africa. The naturally occurring egg parasitoid, *Trichogrammatoidea cryptophlebia* (Nagaraja) (Hymenoptera: Trichogrammatidae) remains the most effective biological control agent for FCM and if undisrupted can parasitize over 80% of eggs in the latter stage of the season (Moore 2011a). Moore and Fourie (1999) showed that inundative augmentation of parisitoids at 100 000 per hectare of citrus, beginning as early as October, reduced fruit infestation by 49%. Several species of flies and wasps are parasitoids of FCM larvae and can play a major role in suppression of FCM populations. The wasp, *Agathis bishopi* (Nixon) (Hymenoptera: Braconidae) seems to be the most effective of these, parasitizing up to 40% of FCM larvae in the Eastern Cape (Gendall *et al.* 2006, Moore 2011a) and was investigated for effectiveness as a commercial biological control agent (Gendall *et al.* 2006). Predators such as *Orius* beetles that prey on FCM eggs and assassin bugs which attack FCM larvae can reduce infestation, yet the most effective predators are ants, that are known to attack FCM pupae and thereby suppress FCM populations (Bownes 2002). Bioassays by Malan *et al.* (2011) showed the potential of entomopathogenic nematodes (EPN) as possible biological control agents for FCM. Two species of EPN have been suggested for the potential control of the soil-borne life stages of FCM, *Heterohabditis bacteriophora* (Poinar) (Rhabditida: Heterorhabtidae) and *Steinernema feltiae* (Filijev)

(Nematoda: Steinernematidae). *Heterohabditis bacteriophora* was recently registered (Act 36 of 1947) by River Bioscience as a pesticide for FCM control (Chambers, River Bioscience pers. comm.).

1.4.4 Granulovirus

The *Cryptophlebia leucotreta* granulovirus (CrleGV) is a naturally occurring indigenous pathogen registered as a biological control agent for the control of FCM (Moore 2002). Both Cryptogran (River Bioscience, South Africa) and Cryptex (Andermatt, Switzerland) are virus-based products available for FCM control and have identical modes of action. The product is sprayed onto the fruit and ingested by the larvae as they attempt to feed, consequently infecting the entire body, causing it to rupture and die. Upon death, large magnitudes of virus particles are released back into the environment, which could potentially be ingested by other larvae (Moore 2011a). Both products are similar in composition although Cryptex is registered at a lower concentration and application rate. Virus-based products are highly species-specific with no effect on beneficial insects and are therefore well-suited to an IPM programme (Moore 2011a). They also have the advantage over other biological agents, being fully compatible with a chemical control programme without any negative effects on the virus (Moore 2011a). Optimal efficacy of a virus is obtained during the neonate larva's first attempt to feed. As a result, the timing of the application should correspond with egg hatch, ideally one to two weeks after a peak in trap catches have been observed. Moore *et al.* (2004a) reported 70% reduction in FCM infested Navel oranges for up to 17 weeks in a trial with Cryptogran in Sundays River Valley (SRV), Eastern Cape. Subsequent trials showed diminished efficacy of 60% for up to 5 weeks, possibly due to high levels of ultra-violet (UV) radiation causing the breakdown of virus particles and leading to a shorter residual activity (Hofmeyr & Hofmeyr 2003).

1.4.5 Chemical control

Triflumuron (Bayer, Germany) and teflubenzuron (Cyanamid, South Africa) are chitin synthesis inhibitors that belong to the benzoyl urea chemical group. Both products are registered for the control of FCM, targeting the eggs and so aim to disrupt embryonic development and prevent hatch. Thorough coverage of the fruit is of the utmost importance and should be applied prior to oviposition, (Moore 2011a). Results from laboratory trials indicate good residual action for suppression of egg hatch in addition to a notable reduction in fruit fall from Navel orange trees (Hofmeyr 1984). However, the use of triflumuron and teflubenzuron have been restricted recently because FCM has developed tolerance where these products where used extensively in the Western Cape (Hofmeyr & Pringle 1998) and Mpumulanga (Moore 2002). Additional restrictions are caused

by the harmful effect of triflumuron on the egg parasitoid, *T. cryptophlebia* (Hattingh & Tate 1997) and for this reason the product is not compatible with an IPM programme (Moore 2002). Alternatively, there are two synthetic pyrethroids registered for control of FCM, namely cypermethrin (Agropharm, South Africa) and fenpropathrin (Sanachem, South Africa). These chemicals have an extensive effect on FCM including an inhibitory effect on the female's egg-laying ability, as well as a direct contact and residual activity on eggs. Fruit infestation by FCM larvae can be avoided for several months before harvest with a single application of a suitable pyrethroid (Hofmeyr 1983a). Pyrethroids are classified as broad spectrum insecticides that are potentially toxic to a wide range of beneficial insects and as a result will cause repercussions of red scale, mealybug and soft scales (Moore 2011a). Neither fenpropathrin nor cypermethrin is widely recommended for FCM suppression because of disappointing results due to resistance development in certain areas as well as low tolerance of pesticide residues in specific markets (Moore 2011a). Spinetoram (Dow AgroSciences, USA) can be used as a more IPM-compatible approach to FCM control. It is a contact and stomach insecticide in the spinosyn group, targeting the nicotynic asetylcholine receptor of the insect causing death by rapid twitching of involuntary muscles, convulsions and paralysis. The best results were achieved in a two-spray programme, eight and four weeks before harvest (Moore 2011a). Recently another new chemistry has been developed for control of FCM and introduced to the market in the form of chlorantraniliprole (DuPont, France). Its novel mode of action, activating the ryanodine receptor, prevents muscle contractions and causes death by paralysis. Trials in 2010 by CRI in the Eastern Cape Province showed a 53% reduction in Navel fruit infestation (Moore & Kirkman 2010). It is also considered a softer chemical because of its harmless nature to most natural enemies and humans (Moore 2011a). Chlorantraniliprole showed comparable efficacy to all the chemical or biological products that are currently available for control of FCM with a residual efficacy of eight weeks (Moore & Kirkman 2010). The most recent registration of a chemical product for the control of FCM is methoxyfenozide (Dow AgroSciences, USA). It has a moulting acceleration mode of action that is mainly effective when ingested, with minimal contact effect on the eggs and second instar larvae. Relatively little research has been reported on citrus although results look promising.

1.4.6 Mating disruption

Mating disruption controls FCM by prevention of mating and as a result suppresses egg-laying on fruit. It relies on a high density of synthetic female sex pheromone distributed over a large homogenous area in such a way that males become confused, repelled or habituated to such an extent that they are unable to find females for mating (Hofmeyr 2003, Carde & Minks 1995). Isomate

(Bioglobal Limited, Austria) is a registered mating disruption product that distributes the synthetic pheromone as an aerosol through thin polyethylene tube dispensers into the environment. The dispensers are hung inside the tree twice per season, at 500 per hectare in October and 300 per hectare in January (Moore 2011a). Checkmate (Suterra, USA) is a pheromone containing capsule suspension formulation that is applied as a foliar spray every 21-28 days to the top third of the tree (Moore 2011a).

1.4.7 Attract and kill

The attract and kill (A&K) approach to controlling FCM is similar to the mating disruption technique, but instead of disrupting mating it kills the male insect (Moore 2011a). Last Call FCM (Insect Science, South Africa) is a product registered for the control of FCM on citrus. It consists of a synthetic female pheromone and a pyrethroid combined into a gel like material. It is distributed onto the leaves of a tree by hand with a special pre-calibrated applicator at 3000 droplets per hectare in 50 microliter (µl) drops, three to four times per season. The male moth is attracted to the pheromone and is killed soon after making contact with the pyrethroid active ingredient. Last Call FCM appears less effective than the mating disruption products and is mostly recommended for areas with a low pest population density (Hofmeyr 2003).

1.4.8 Sterile insect technique

The sterile insect technology for area-wide suppression of FCM was initially investigated in Citrusdal, Western Cape province. A reduction in fruit fall of 94.4% in Navel orange orchards was achieved in the preliminary study (Hofmeyr & Hofmeyr 2004). In 2007 the technology was commercialised by X Sterile Insect Technique Pty (Ltd) (XSIT) for release of sterile insects over 1500 hectares of citrus planted in Citrusdal. Over a period of six years a significant reduction of in the wild FCM population was achieved (Groenewald, XSIT, pers. comm.). Consequently trials were conducted by CRI in the Sunday's River Valley, Eastern Cape province, and Letsitele, Limpopo province, to determine the efficacy of SIT after long distance transportation of irradiated insects. Initially, poor results were obtained in Limpopo (Hofmeyr & Hofmeyr 2010), however, 80% reduction was eventually achieved in the third year of such trials (Moore 2011b). In the Eastern Cape, around 80% reduction in Navel fruit infestation was also achieved (Hofmeyr & Hofmeyr 2010). XSIT started commercial releases of irradiated FCM in the Eastern Cape over 1800 hectares of citrus in 2011. First season results revealed a 50% reduction in the wild FCM population and 35% reduction in FCM infested Navels where SIT was used in combination with sprays compared to the control where sprays were applied as the only method of control. Subsequently expansion was imminent, largely driven by the increasing

phytosanitary pressure from sensitive European Union (EU) markets. The SIT programme in the Eastern Cape is currently being conducted in 3400 hectares of citrus. However, there are still a number of knowledge gaps that need to be filled, especially regarding application technology of SIT on FCM in South Africa. Therefore this thesis has three main research aims:

- To evaluate the physiological effect of long distance transportation (to Addo, Eastern Cape province) under low temperature conditions on sterile moth quality, particularly regarding flight ability, longevity and realized fecundity of irradiated FCM with their wild counterparts after recovering from the quiescent state, thereby determining ideal transportation conditions (Chapter 2).
- To determine optimal timing for release of irradiated FCM within a chemical pesticide spray programme, by confirming the effect of pesticides on adult FCM males through exposure of the insects to residual doses of commercially available insecticides belonging to the organophosphate and pyrethroid chemical groups and measuring mortality as the response over time (Chapter 3).
- To assess the comparative efficacy of aerial and ground release methods by evaluating recaptures in specific areas within the commercial SIT area, with special regard to the accurate distribution of irradiated FCM over the designated area whilst considering release height above ground, high moth-density and high temperature inside the release mechanisms on moth fitness (Chapter 4).

2

THE EFFECT OF LONG DISTANCE TRANSPORTATION ON THE FITNESS OF IRRADIATED FALSE CODLING MOTH

2.1 Introduction

Importing countries demand pesticide residue-free fruit even though introductions of exotic pest insects through increased trade is growing (Myers *et al.* 2000). SIT may expand to countries intent on establishing pest-free areas and eradicating invasive pests (Robinson & Hendrichs 2005). Extraordinarily high costs and time required to construct a mass-rearing facility for production of sterile insects is a major concern for expansion of existing SIT programmes globally. Costs of production and release of moths in SIT programmes for suppression of species such as codling moth (CM), *Cydia pomonella,* (Linnaeus) (Lepidoptera: Tortricidae) and pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechidae) are especially high relative to traditional methods of control (Blomefield *et al.* 2011). If initial capital input could be eliminated, expansion of current SIT programmes to geographically distinct areas would become more feasible (Bloem *et al.* 2010). In a situation where an exotic pest is introduced into an area of international trade, the time for development and implementation of a control programme to prevent the establishment and spread of the pest is limited, let alone the construction of an expensive mass-rearing facility (Blomefield *et al.* 2011). Importation of sterile moths from an existing production centre would be a more logical and cost effective approach.

Long distance transportation of insects as pupae or adults is a routine part of SIT globally, particularly in SIT programmes for area-wide control of Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Dowell *et al.* 2005) and screwworm, *Cohliomyia hominovorax* (Coquerel) (Diptera: Calliphoridae) (Rull *et al.* 2011) populations. However, there is little experience in transportation of Lepidoptera as adult moths over long distances (Blomefield *et al.* 2011). Transportation of moths prior to release involves important logistical requirements to result in optimum survival and behavioural characteristics that make them sufficiently competitive with their native relatives (Dowell *et al.* 2005). In the PBW, CM (Dowell *et al.* 2005) and false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (Hofmeyr & Pretorius

2010) commercial SIT programmes, moths are collected inside the insectary and temporarily immobilized by cold temperature for easier handling during packing, transport and subsequent loading of release mechanism (Dowell *et al.* 2005).

The purpose of this study was to examine the effects of transportation between Citrusdal and Addo on the fitness of immobilised, irradiated FCM.

2.2 Materials and Methods

Irradiated FCM adults and pupae were obtained from the XSIT rearing facility in Citrusdal, Western Cape province and transported over a distance of 729 kilometre (km) to Addo, Eastern Cape province, where the study was conducted. The FCM culture was two years old at the time and comprised FCM collected from orchards in the Citrusdal area. The rearing process at this facility involved FCM eggs on wax paper inoculated onto a maize-meal based artificial diet, originally developed by Moore *et al.* (2002c). The addition of Calco Red, a product that dyes the gut lining of the moth pink, enabled distinction of irradiated and feral FCM males in the field (Stotter 2009).

Original rearing protocols developed by Schwartz (1971) were modified to suit the commercial production of 11 generations per year at XSIT. After the diet was cooked in a glass jar, it was inoculated with an estimated 1200 FCM eggs on a piece of wax paper. Trolleys with jars were pushed into walk-in larval rooms, where a relatively constant temperature (≈25-28°C) and photoperiod (12:12; L:D) were maintained. Although no humidity control existed in larval rooms, the relative humidity (RH) was generally between 45 and 55 %. On day 14 after inoculation, cardboard honeycomb pupation trays were inserted under the jars for pupation to take place. On day 21, trays with pupae were removed from the larval rooms and left to mature for approximately two days at room temperature and subsequently placed inside moth emergence cabinets, also maintained at 25-28˚C and 45-55 % RH. As the moths emerged they dropped down into collection pipes where an air stream (14 m/s) carried them via a plenum braking chamber into troughs in a cold room (Hofmeyr & Pretorius 2010). Inside the cold room, moths were kept in a chilled state at 2-5°C. While in this immobile state moths were placed into cardboard containers, 110 mm x 110 mm x 55 mm (Figure 2.1). Thirty two boxes, each containing approximately 8 000 moths were then exposed to gamma irradiation (150 Gy) from a ^{60}Co panoramic point source radiator. Boxes destined for the Eastern Cape were stored in a cold room at 2-5°C for a minimum of 2 hours before packing commenced.

For experiments with non-irradiated FCM, a cardboard pupal tray was placed in an emergence container in Citrusdal or transported to Addo inside a cooler box with minimal cooling, so as to prevent moths emerging in transit. Sufficient pupae were removed from the cardboard after which

male and female pupae were separated and placed in containers for emergence. In so doing, all experiments were conducted with virgin irradiated and non-irradiated moths from the exact same batch and thus the same age.

Figure 2.1 A cardboard container used for storage and shipping of irradiated FCM.

Insulated polyurethane cooler boxes were packed with 32 moth-boxes at 2-5°C inside the cold room at XSIT, Citrusdal. Two types of cooler boxes were used with dimensions of 330 mm x 330 mm x 330 mm (holding 16 moth containers) and 590 mm x 590 mm x 330 mm (holding 32 moth containers) (Figure 2.2). Poly-ice® packs (Techni-ice, Australia) wrapped in brown paper were used for cooling. These commercially available ice packs consist of layered plastic that encapsulates a cross-linked polyacrylate polymer. The ice packs were pre-cooled for 36 hours to approximately minus 14°C, after which they were packed in a single layer at the bottom, top and in a 20-30 mm gap between the stacks of moth boxes. Each cooler box contained an electronic data logger (HOBO U10 RH logger), placed inside a centrally-located moth box and totally covered with moths. The logger was programmed to record temperature and relative humidity at 10 minute intervals.

Cooler boxes were transported overnight enclosed inside a canopy on a utility vehicle, to minimise exposure to extreme temperatures. Upon arrival in Addo, data were recorded for time in transit, temperature fluctuations in the cooler boxes during transport and general appearance and activity of the moths. General appearance of moths was determined by removing a sample of 100 male and female moths and determining the amount of deformed, damaged and dead moths. Pre- and post-transport moth quality was evaluated by examining moth longevity, realized fecundity and flight ability. Samples from three consignments were considered for this study. The entire experiment was replicated on two occasions i.e. in February and June, the hottest and coolest months of the year.

Figure 2.2 Insulated polyurethane cooler box with moth containers and Poly-ice packs used for cooling during transport.

2.2.1 Longevity

Twenty irradiated (T) and non-irradiated (N), male (♂) and female (♀) FCM were placed individually in a small petri dish, 60 mm diameter x 15 mm high. The moths were incubated at 25°C, 75 % RH in 100% darkness in the laboratory, without water, and mortality was recorded daily until all the moths were dead.

2.2.2 Realized fecundity

Twenty pairs of moths were used in each of the following combinations, *viz.* T♀ x T♂, T♀ x N♂, N x T♂ and N♀ x N♂. The moths were placed in a petri dish, 60 mm diameter x 15 mm high, and supplied with water through soaked cotton wool. Moths were removed and placed inside a new dish after 24 hours, for a total of five days. Fecundity was measured by recording the number of eggs laid over the entire area of the petri dish. Dishes were incubated at 25°C, 75% RH and 100% darkness in the laboratory and duplicated for pre- and post-transport trials.

2.2.3 Flight ability

Twenty irradiated (T) and non-irradiated (N), male (♂) and female FCM (♀) were used to perform flight tests indoors in an enclosed area. Transported moths were separated in a small petri dish, 90 mm diameter x 15 mm high, and left to recover from the immobile state for 15 minutes at ambient temperature. Active moths were released individually from a height of 2.5 m above ground level. A grading system of 0-3 based on dispersal distance was used to record flight ability. Moths unable to gain height and invariably descending involuntarily, or falling directly to the ground, were regarded as unable to fly and were recorded as a 0, whereas moths dispersing more than 3 m were regarded as flight capable and recorded as a 3. A grade of one was awarded to moths that were considered lethargic but able to fly up to one meter upon release, while a grade of two was awarded to moths that were able to fly a distance of two meters from the point of release.

2.2.4 Statistical analyses

Mean longevity, and fecundity of transported and non-transported irradiated and non-irradiated, male and female FCM were compared using a Factorial ANOVA and the post-hoc Tukey test using Statistica version 11 (StatSoft, Inc. 1984-2012). A general non-linear model with a Poisson distribution was used to compute Factorial ANOVA on data flight ability data.

2.3 Results

The total time needed for packing of moths in Citrusdal for transportation to Addo, duration of transportation and subsequently unpacking of chilled irradiated moths in Addo was similar for six consignments (11.5-12 hours) (Figure 2.3). There was a notable difference in temperature at packing in Citrusdal during summer and winter. A slight temperature increase during the first hour of transport is visible for all shipments with the exception of consignment 3, showing an initial decrease in temperature. Relatively small variation in temperature inside moth boxes was recorded for consignments 1, 2 and 3 transported during winter, compared to consignments 4, 5 and 6 that were transported in summer (Figure 2.3). A difference of 20°C in temperature between consignment four and six compared to the rest of the consignments was recorded, which could be the cause of the difference in performance of the moths. The temperature profile for consignment 4 and 6, transported in summer, varied considerably with a maximum at 38.6°C and 37.5°C respectively. These moths were active upon arrival in Addo where visual inspection indicated 10% dead, 10% damaged and 30% deformed for consignment 4 and 0% dead, 0% damaged but 50% deformed moths for consignment 6. The high percentage deformities were evident throughout the production

batch and could be traced back to the moth emergence cabinets where temperature was unusually high for this particular period. Moths from other consignments were inactive and in good condition.

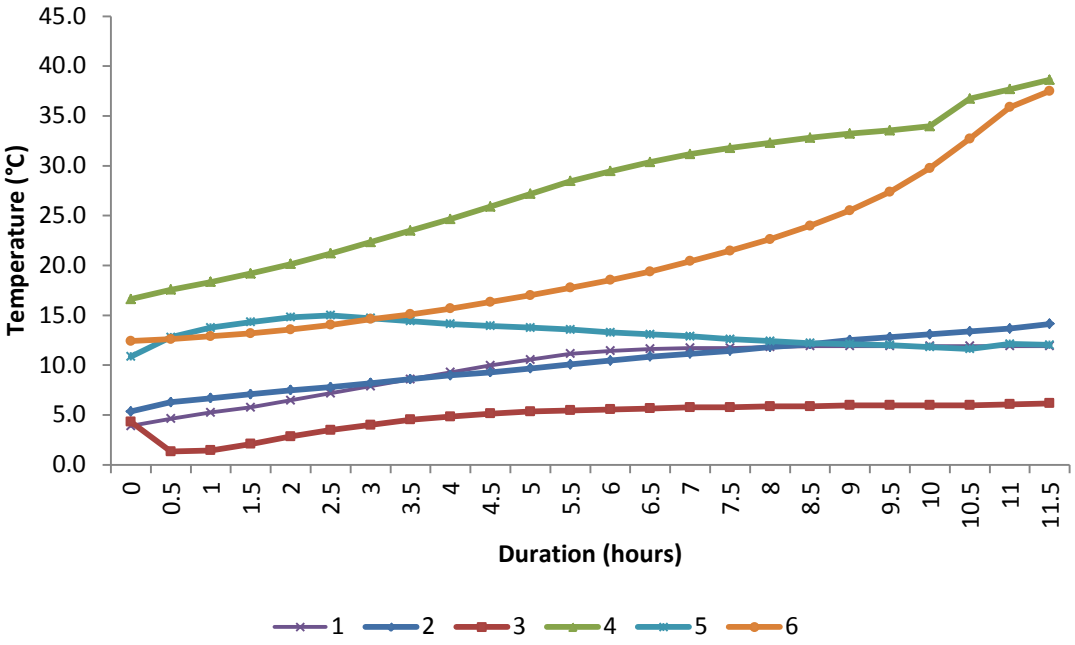

Figure 2.3 Temperature (°C) of irradiated FCM in insulated cooler boxes during transport of six consignments originating in Citrusdal, Western Cape and arriving in Addo, Eastern Cape.

2.3.1 Longevity

Irradiation (150 Gy) had no significant impact on the longevity of non-transported male or female FCM ($F = 17.719$; $P < 0.001$) (Figure 2.4). Transportation significantly effected longevity of male and female FCM ($F = 6.424$; $P = 0.001$), regardless of whether moths were irradiated or not (although not significantly in the case of non-irradiated females) (Figure 2.4). Transport also significantly effected longevity of non-irradiated male and female FCM that were transported as pupae from Citrusdal to Addo ($F = 182.537$; $P < 0.001$). Irradiated females proved to be the most vigorous with a mean age of 9.02 ± 2.53 days in summer and 9.78 ± 4.70 days in winter (Figure 2.5). The undesirable effect of transportation on the lifespan of moths was significantly greater for irradiated male compared to irradiated female FCM as well as non-irradiated FCM ($F = 18.880$; $P < 0.001$). Additionally, only life expectancy of irradiated male FCM was significantly reduced with transport in summer compared to winter, which does not appear to be true for the other three groups $F = 44.269$; $P < 0.001$). Irradiated male FCM had a mean age of 3.97 ± 1.34 days in summer and 6.88 ± 3.10 days in winter (Figure 2.4), possibly due to the extreme variation in transport temperature particularly for consignment four and six compared to the rest of the consignments (Figure 2.3).

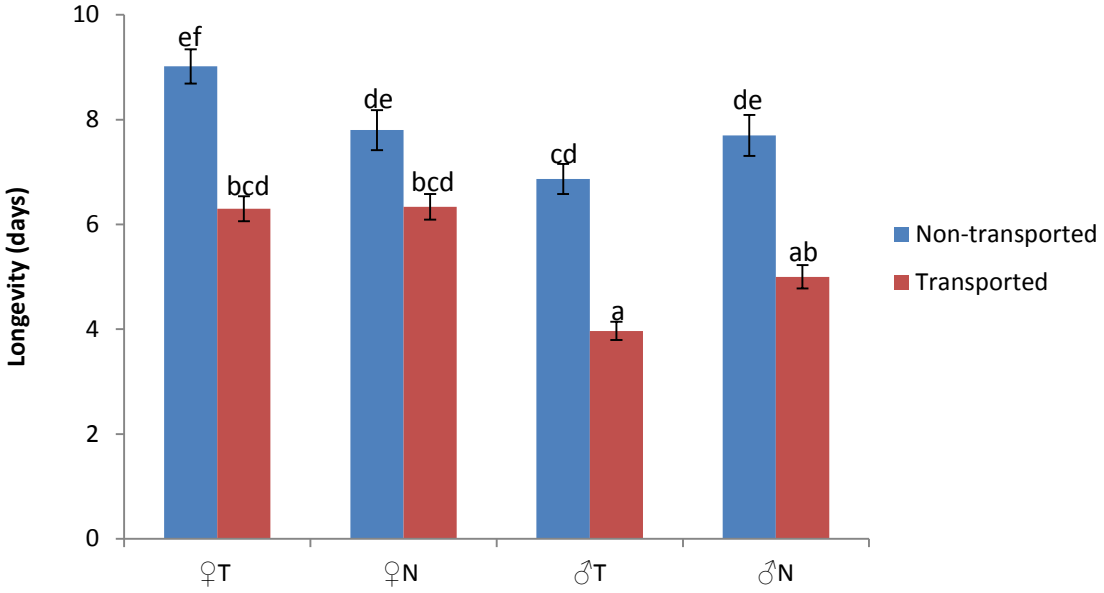

Figure 2.4 Longevity of irradiated (T) and non-irradiated (N), male (♂) and female (♀) FCM with and without transportation, during summer 2013. Bars with different letters denote significant differences ($P < 0.05$, Tukey HSD test).

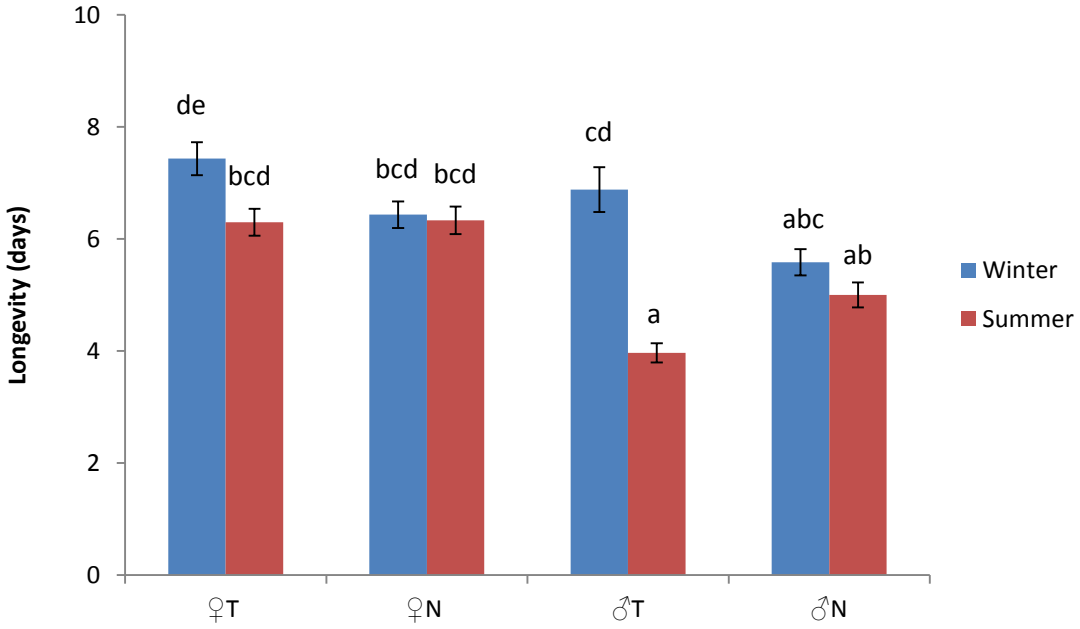

Figure 2.5 Longevity of irradiated (T) and non-irradiated (N), male (♂) and female (♀) FCM with transportation, during winter and summer 2012/13. Bars with different letters denote significant differences ($P < 0.05$, Tukey HSD test).

2.3.2 Flight ability

Flight ability of adult FCM was significantly affected by long distance transportation (X^2 =120.125; P < 0.001) (Figure 2.6). Interestingly, no difference in flight ability of male and female irradiated moths was recorded without transportation. A negative effect of irradiation on flight ability of both male and female FCM regardless of transportation was apparent (X^2 = 174.302; P < 0.001). Sex appears to have been a major factor in flight ability of FCM with transportation due to irradiated females being significantly less flight capable than irradiated males (X^2 = 58.569; P < 0.001). Flight ability of irradiated male and female FCM was further reduced when transportation took place in summer compared to winter (X^2 = 114.745; P < 0.001) (Figure 2.7). The non-irradiated female FCM, transported as pupae, were also less flight capable after transportation in summer compared to winter displaying the combined effect of transport and temperature (X^2 = 99.194; P < 0.001). This phenomenon is likely to be the result of a change in temperature during transport (Figure 2.3).

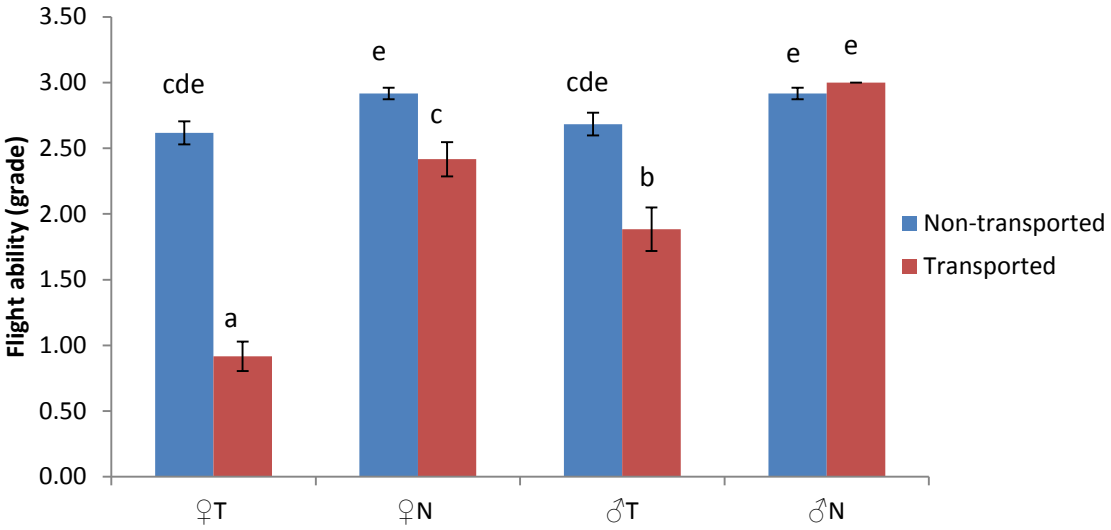

Figure 2.6 Flight ability (grade 0-3) of irradiated (T) and non-irradiated (N), male (♂) and female (♀) FCM with and without transportation, during summer 2013. Bars with different letters denote significant differences (P < 0.05, Tukey HSD test).

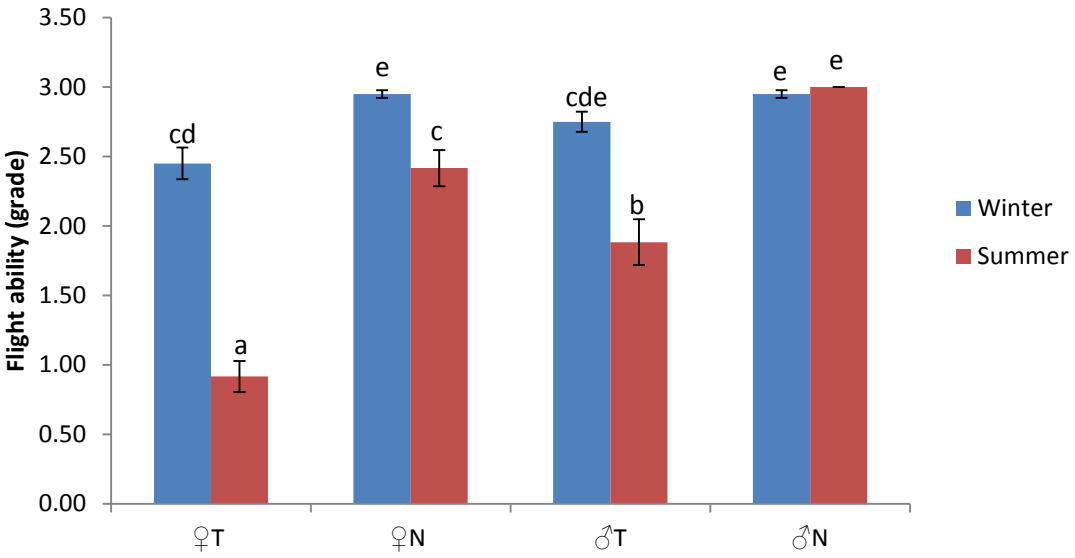

Figure 2.7 Flight ability (grade 0-3) of irradiated (T) and non-irradiated (N), male (♂) and female (♀) FCM with transportation, during summer 2013. Bars with different letters denote significant differences ($P < 0.05$, Tukey HSD test).

2.3.3 Realized fecundity

Realized fecundity of irradiated male FCM was not affected by transport which is particularly relevant to the SIT programme (Figure 2.8). Although taking into account that the vast majority of eggs will not hatch, there was an increase in amount of eggs laid when irradiated male FCM were paired with non-irradiated female FCM that were transported, 176.9 ± 159.7 compared to non-transported, 150.9 ± 140.3. The impact of transportation on fecundity of irradiated female FCM did not appear significant, although a reduction in the number of eggs occurred when paired with irradiated males ($F = 69.879$; $P < 0.001$) compared to non-irradiated males ($F = 4.269$; $P < 0.001$). In fact, irradiated female FCM laid on average 285.9 ± 114.7 eggs when paired with irradiated males, compared to only 176.9 ± 159.7 eggs when they were paired with non-irradiated male FCM, with transportation in summer. Transport temperature during summer did not significantly impact realized fecundity of any potential mating pair that involved non-irradiated FCM, although a significant reduction in eggs were recorded for irradiated male and female FCM with transport ($F = 10.790$; $P < 0.001$) (Figure 2.9). A trend of increased mating with reduced transport temperature during winter was observed in both pairings with non-irradiated female FCM, although in neither case was it significant (Figure 2.9).

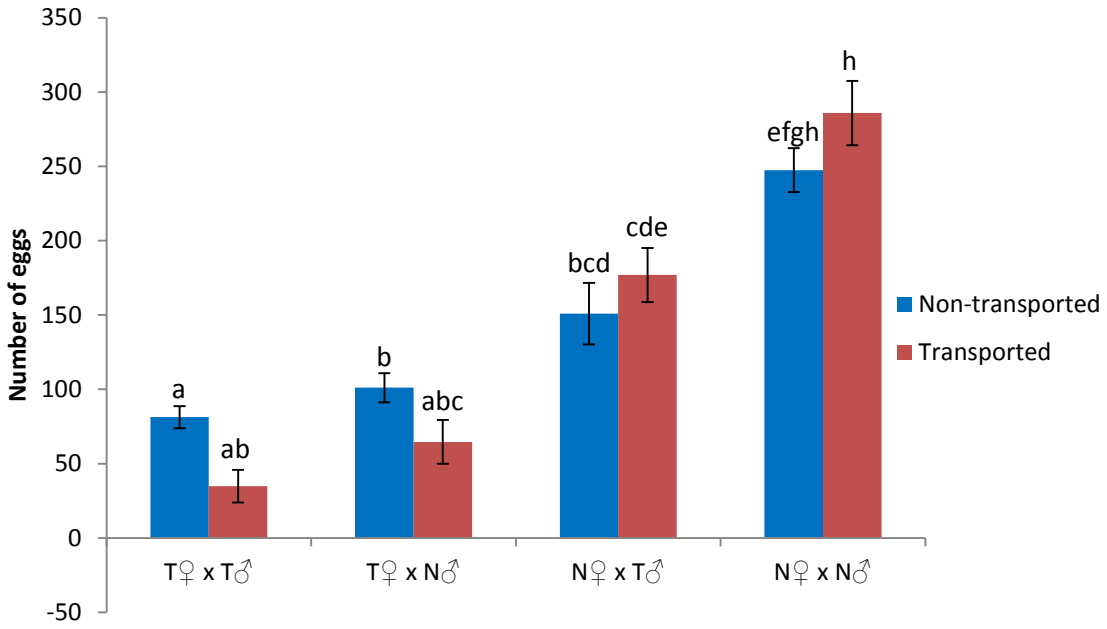

Figure 2.8 Realized fecundity (number of eggs) for combinations of irradiated (T) and non-irradiated (N), male (♂) and female (♀) FCM as mating pairs, viz. T♀ x T♂, T♀ x N♂, N x T♂ and N♀ x N♂, with and without transportation, during summer 2013. Bars with different letters denote significant differences ($P < 0.05$, Tukey HSD test).

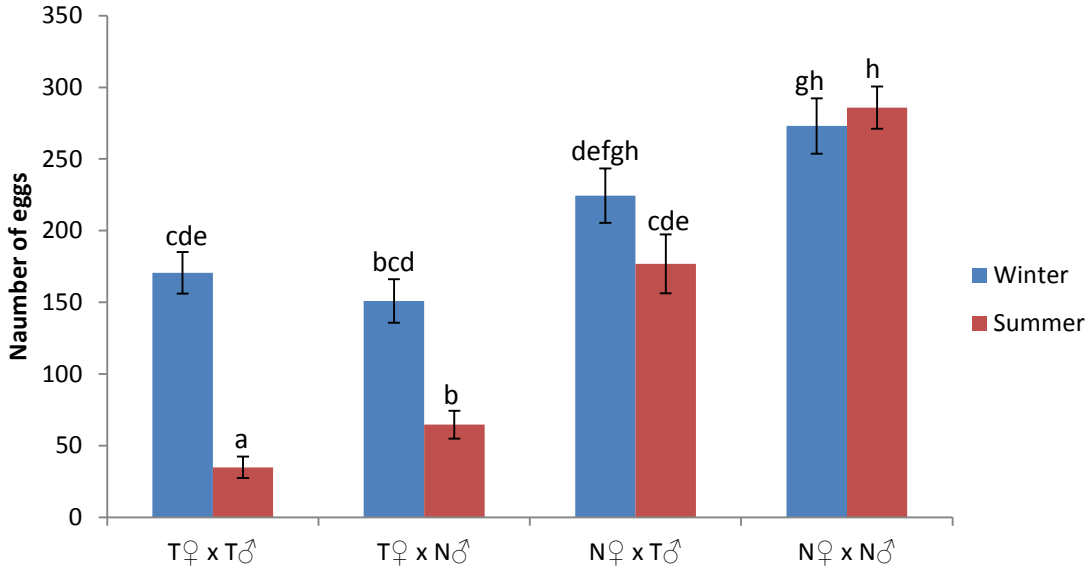

Figure 2.9 Realized fecundity (number of eggs) for combinations of irradiated (T) and non-irradiated (N), male (♂) and female (♀) FCM as mating pairs, viz. T♀ x T♂, T♀ x N♂, N x T♂ and N♀ x N♂, with

transportation during summer and winter, 2012/13. Bars with different letters denote significant differences ($P < 0.05$, Tukey HSD test).

2.4 Discussion

The shipment of sterile insects is a critical link for any pest management programme utilising SIT. Eradication of invasive pest species such as medfly (Dowell *et al.* 2005), NWS (Baumhover 1955) or PBW (Vetter and Baker 1990) in geographically distinct areas by obtaining sterile insects as pupae or adults from a centralized rearing facility for preparation and release in the target area is a logical, cost effective approach (Blomefield 2011). SIT remains a highly effective control method, providing wild and mass reared colonies are sexually and behaviourally compatible and competitive (Hendrichs *et al.* 2002, Enkerlin 2005). Sterile medfly have been shipped successfully as pupae from Mexico and Guatemala to South Africa, Argentina, Chile, Austria, Israel and continental USA without serious loss in quality of adult flies (Ohinata *et al.* 1978, Rull *et al.* 2011). Emergence was delayed by shipping pupae under hypoxia in a low-oxygen atmosphere even though this could result in reduced quality insects if prolonged, often due to security issues at airports, limiting the distance sterile insects can be shipped (Dowell *et al.* 2005).

Several lepidopteran species have been investigated as candidates for SIT programmes utilising inherited sterility. Two of these programmes currently operational are the PBW containment programme in California, USA, and the CM suppression programme in British Columbia, Canada (Knipple 2013). Lepidoptera reared for an SIT programme are usually packed and shipped as chilled adults (Dowell *et al.* 2005). Sterile PBW moths transported via commercial airlines were found to be rather similar in pheromone emission and courtship competences compared to wild moths and were consequently declared entirely capable of contributing to population suppression in a SIT programme (Vetter & Baker 1990). Temperatures lethal to insects are a combination of the magnitude of variation and the duration of exposure, although thermal physiology varies between species while some even alter their thermal physiology and its plasticity, consequently promoting survival under unfavourable conditions (Stotter & Terblanche 2009). Short term fluctuations (diel thermal history), rate of temperature change, magnitude and duration of exposure affects survival of adult CM, as a result affecting fitness and performance of moths in an SIT programme that uses cold-induced immobilisation for shipment of insects (Chidawanyika & Terblanche 2011). However, CM transport has been refined over time so that the risk of adverse effects on insect quality at the point of delivery becomes negligible, mostly due to rigorous standard operating procedures for handling, packaging and transport (Enkerlin 2007, Simmons 2007). Transport of CM in air freight and normal commercial transport routes from British Columbia, Canada, to Stellenbosch, South Africa,

indicated that transport of moths or pupae under low temperature for extended periods had little effect on quality at the point of delivery (Blomefield 2011). Earlier studies established that CM mobility was not significantly influenced by storage at 2°C for up to 72 hours, while Chidawanyika and Terblanche (2011) found exposure to -7°C for 4 hours did not impact on survival of transported moths. Lower temperature thresholds for pheromone response of mass-reared CM estimated to be 14.7°C while a lower temperature threshold for pheromone trap catches of wild moths is 15.6°C (Judd & Gardiner 2006).

High lethal temperatures for FCM have not been established, although transport in the commercial SIT programme aims to sustain temperatures between 3 and 5°C in the collection facility and subsequent transport to Addo, Eastern Cape province (Groenewald, XSIT, pers. comm.). Lower lethal temperatures for FCM have been investigated by Stotter and Terblanche (2009) in order to determine the effect of rapid chilling during transport on subsequent extreme temperatures experienced by mass-reared FCM on field performance and thus the SIT programme. FCM is nocturnal and present throughout the year in South African citrus orchards (Stofberg 1954). Additionally, it remains unclear whether mass-reared individuals have lost the ability to cold-harden relatively to wild individuals. Data presented in this study showed that transport as road freight significantly impacted flight ability and longevity of mass reared, irradiated male and female FCM as chilled moths in insulated cooler boxes. Unfavourable high temperatures in consignments four and six caused irradiated moths to become active in transit. A thick layer of dorsal scales was observed in a box holding 8000 moths, possibly contributing to the relatively high percentage deformity and mortality upon arrival of these particular shipments. The increase in temperature during transit was of a gradual nature, although short term fluctuation at packing or opening could theoretically have a shock impact on the sterile insects (Chidawanyika & Terblanche 2011). The magnitude and rate of temperature change inside cooler boxes is an important factor, where temperature increased to 38.6 and 37.5°C at unpacking for consignments four and six respectively, fitness and performance of irradiated moths were adversely affected. Additionally, duration of exposure to unfavourable temperatures probably impacted insect quality in consignments four and six since irradiated moths spent the entire 11.5 hour trip from Citrusdal to Addo enduring temperatures exceeding 10°C. The increase in temperature during transport could have been caused by a high internal temperature at packing as well as insufficient freezing of ice-packs used for transport. Packing for transport of sterile FCM should be done inside a cold room at 5°C or below to ensure moths are completely immobilized. Ice packs, such as those used for this trial, should be frozen for 36 hours before packing commences to ensure they remain completely frozen for the entire duration of the trip. This is

particularly important for transport during summer when ambient temperature could escalate above 40°C during the day and between 30°C and 40°C at night (ARC 2012).

Longevity and flight ability of irradiated insects is critical to the success of SIT on account of survival and performance in the field influencing frequency and number of insects released in order to obtain the required sterile : wild male ratios. The fact that male moths only live for an average of 3.97 days in summer and 6.88 days in winter, should set the standard for release frequency at every 3 days during summer and every 7 days during winter. However, international standards recognize successful interaction of sterile males with wild females of the target population as the most important quality indicator (Hendrichs *et al.* 2002). Data from this study show realized fecundity of irradiated male FCM was not significantly affected by transportation, particularly when paired with non-irradiated females. Flight ability and realized fecundity of irradiated male FCM seem to be less affected than females. This finding is important since irradiated males are regarded as the more important component of the SIT or inherited sterility programmes (Blomefield 2007). However, both males and females are released, meaning some benefit could amass from irradiated females which could attract native males (van Steenwyk *et al.* 1979). Henneberry and Clayton (1980) suggested sterile PBW females may be greater contributors to the success of the SIT programme than sterile males, due to increased copulation length meaning native males will be occupied with sterile females for longer, thus enhancing the success of an SIT programme.

Adverse effects of long distance transport on moth quality should be addressed by defining exact temperature thresholds at various quality-control points from production to release. Development and implementation of stringent standard operating procedures for production, handling, packaging and transport of irradiated FCM for application in an SIT programme is of the utmost importance, especially for programmes in areas that are geographically separated from the production facility. The adverse effect of moth transport on fitness and performance could be partly overcome by releasing higher numbers where transportation is involved. The direct effect of unfavourable temperatures on insect quality should be addressed by investigating alternative mode of transport whereby duration of exposure could be minimised while identification of alternative atmospheres and environments for extended shipment or holding time should remain a priority for satellite SIT programmes or trans boundary shipments of insects.

3

INTEGRATING CHEMICAL CONTROL WITH RELEASES OF IRRADIATED FALSE CODLING MOTH IN AN IPM PROGRAMME UTILISING SIT

3.1 Introduction

A wide range of insect and mite pests are associated with citrus in South Africa (Table 3.1). A number of species cause serious direct damage resulting in significant economic losses in primary production and export trade. These pests are controlled using a wide range of insecticides with varying levels of efficacy. In areas where a sterile insect release programme is implemented, the impact of a conventional pest management approach on released sterile insects needs to be assessed to determine compatibility for the correct application of Area-Wide Integrated Pest Management (AW-IPM) programmes. In an SIT programme on citrus grown in Spain, pesticides tested for their effect on Vienna-8 strain sterile male Mediterranean fruit fly (medfly) *Ceratitis capitata* (Diptera: Tephritidae) (Wiedemann) proved harmless with the exception of chlorpyrifos and spinosad (Juan-Blasco *et al*. 2013). The impact of certain insecticides and their residues on natural enemies of false codling moth (FCM) *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) (Meyrick) have been evaluated and are not recommended for use in an IPM programme (Moore 2004b, Morse and Grafton-Cardwell 2012). Numerous organophosphates and pyrethroids are registered for the control of citrus pests in South Africa (Nel *et al*. 2002, Vermeulen *et al.* 1990). Chlorpyrifos and tau-fluvalinate are commonly applied in spring for control of pests such as thrips, mealybug and African bollworm. Broad spectrum pesticides are generally not used later in the season than spring, particularly within an IPM programme; all pesticides used later in the season are specific to FCM. Chrolpyrifos and tau-fluvalinate were selected as being representative of two groups of chemicals namely organophosphates and pyrethroids, considered as being potentially disruptive.

The aim of this chapter was to investigate the residual effect of chlorpyrifos and tau-fluvalinate on survival of wild and released, irradiated male FCM in citrus orchards. If chemical residues are effective in killing released moths on contact, timing of releases could be altered until efficient breakdown renders residues harmless, thereby also aiding SIT through pre-season reduction of the native population.

Table 3.1 Important insect and mite pests of citrus in South Africa ((Annecke & Moran 1982, Bedford 1998, Smith & Peña 2002, CRI 2013).

Citrus pests	**Common name**	**Scientific name**	**Authority name**
Ants	Pugnacious ant	*Anoplolepis custodiens*	Smith
	Black pugnacious ant	*Anoplolepis steingroeveri*	Forel
	Carpenter ant	*Amponotus rufoglaucus*	Jerd
	Dull black ant	*Polyrhachis shistacea*	Gerstaecker
	Brown house ant▲	*Pheidole megacephala*	Forel
	Brown house ant	*Pheidolemegacephala punctulata*	Mayr
	Brown house ant	*Pheidole tenuinodis*	Mayr
	Argentine ant	*Linepithema humile*	Mayr
	Ubiquitous brown ant	*Technomyrmex albipes (Sm.) foreli*	Emery
Armoured scale	Red scale*	*Aonidiella aurantii**	Maskell
	Circular purple scale ▲	*Chrysomphalus aonidium* ▲	Linnaeus
Australian bug	Cottony cushion scale ▲	*Icerya purchasi* ▲	Maskell
Rose beetle	Weevil	*Pantomorus cervinus*	Boheman
Mussel scales	Citrus mussel scale	*Cornuaspis beckii*	Newman
	Long mussel scale	*Lepidosaphes gloverii*	Packard
Soft scale	Brown soft scale	*Coccus hesperidum*	Linnaeus
	Green soft scale	*Pulvinaria aethiopica*	De Lotto
	Black scale	*Saissetia oleae*	Olivier
Wax scale	Citrus wax scale	*Gascardia brevicauda*	Hall

	White wax scale	*Gascardia destructor*	Newstead
Powdery scale	Powdery scale	*Cribrolecanium andersoni*	Newstead
Mealybugs	Citrus mealybug *	*Planococcus citri* ▲	Risso
	Karoo thorn mealybug	*Nipaecoccus viridis*	Newstead
	Oleander mealybug	*Paracoccus burnerae*	Brain
	Long-tailed mealybug	*Pseudococcus longispinus*	Targioni Tozzetti
	Citrophilous mealybug	*Pseudococcus calceolariae*	Maskell
	Striped mealybug	*Ferrisia virata*	Cockerell
Aphids	Black Citrus aphid ▲	*Toxoptera citricidus* ▲	Kirkaldy
	Cotton aphid	*Aphis gossypii*	Glover
	Adult spiraea aphid	*Aphis spiraecola*	Patch
	Brown citrus aphid	*Toxoptera aurantii*	Fonscolombe
Leafhoppers	Citrus leafhopper	*Penthimiola bella*	Stål
	Green Citrus leafhopper	*Empoasca distinguenda*	Paoli
Triozids	Citrus psylla*	*Trioza erytreae**	Del Guercio
Thrips	Citrus thrips*	*Scirtothrips aurantii**	Faure
Caterpillars	Citrus swallowtail	*Papilio demodocus*	Esper
	False codling moth*	*Thaumatotibia leucotreta**	Meyrick
	Apple leaf roller	*Tortrix capensana*	Walker
	Citrus leaf roller	*Archips occidentalis*	Walsingham
	Citrus looper	*Ascotis selenaria reciprocaria*	Walker

	African bollworm ▲	*Helicoverpa armigera*	Hübner
	Citrus flower moth	*Prays citri*	Millière
	Carob moth	*Ectomyelois ceratoniae*	Zeller
	Citrus leafminer	*Phyllocnistis citrella*	Stainton
Fruit flies	Mediterranean fruit fly *	*Ceratitis capitata **	Wiedemann
	Natal fruit fly *	*Ceratitis rosa **	Karsch
	Marula fruit fly	*Ceratitis cosyra*	Walker
Mites	Citrus bud mite ▲	*Aceria sheldoni* ▲	Ewing
	Citrus grey mite	*Calacarus citrifolii*	Keifer
	Citrus rust mite	*Phyllocoptruta oleivora*	Ashmead
	Citrus red mite ▲	*Panonychus citri* ▲	McGregor
	Red spider mite	*Tetranychus cinnabarinus*	Boisduval
	Citrus flat mite	*Brevipalpus californicus*	Banks
	Reddish-black flat mite	*Brevipalpus phoenicis*	Geijskes
	Silver mite ▲	*Polyphagotarsonemus latus* ▲	Banks
	Lowveld citrus mite ▲	*Eutetranychus orientalis* ▲	Klein

* Represents key pests of citrus; ▲ represents major or occasionally important pests of citrus; all other pests are minor or sporadic pests of citrus.

3.2 Materials and Methods

Irradiated FCM adults and pupae were obtained from the XSIT rearing facility in Citrusdal, Western Cape province and transported to Addo, Eastern Cape province, where the study was conducted. Chlorpyrifos 750 WG (750 g/kg) (Dow AgroSciences) was applied at the registered dosage of 48 g per 100 L of water and tau-fluvalinate 240 EW (240 g/l) (Makhteshim Agan) at 30 ml/100 L. Rain water was applied as a control treatment. For a light cover spray an application rate of 5000 L/ha (for mature Navel orange trees) was recommended for control of citrus thrips and African bollworm in particular (van Zyl 2012).

3.2.1 Leaf substrate

Navel orange seedlings, obtained from a local nursery in the Sunday's River Valley were used for each treatment. The seedlings served as a source of leaf substrate after treatment. The chemicals were applied on the seedlings to the point of run-off, with a conventional knapsack sprayer (Kaufmann). The spray tank had a capacity of 5 L, and was equipped with a hand-pump and sprayer that could reach a maximum operating pressure of 0.3 MPa. Three seedlings were sprayed for each treatment to ensure a sufficient number of leaves were available for the experiment. Leaves were collected on day 1, 3, 7, 14 and 21 after application and individually placed inside Munger cells (Morse *et al.* 1986). These cells consist of clear perspex blocks with dimensions 110 mm x 80 mm x 8 mm and a 50 mm hole in the top creating space for the leaf and an individual insect (Figure 3.1). Another 4 mm hole covered with a fine mesh filter in the side allowed a series of Munger cells to be connected to an air-flow system with a flow rate of 50 litres per minute, thereby removing possible build-up of fumigants (Figure 3.2). On each of the five days (d), 20 irradiated males (T♂) and 20 non-irradiated males (N♂) were separated and individually placed on a freshly collected leaf inside a Munger cell. Cells were kept in the laboratory at ambient temperature estimated at 25°C for the duration of the trial. Irradiated (T♂) and non-irradiated male FCM (N♂) in Munger cells were inspected hourly for a period of 12 hours (h) for each time period after application (1, 3, 5, 7, 14 and 21 d). Survival of moths was recorded as the number of individuals alive after set exposure time.

Figure 3.1 Irradiated male FCM on a treated leaf substrate inside a munger cell.

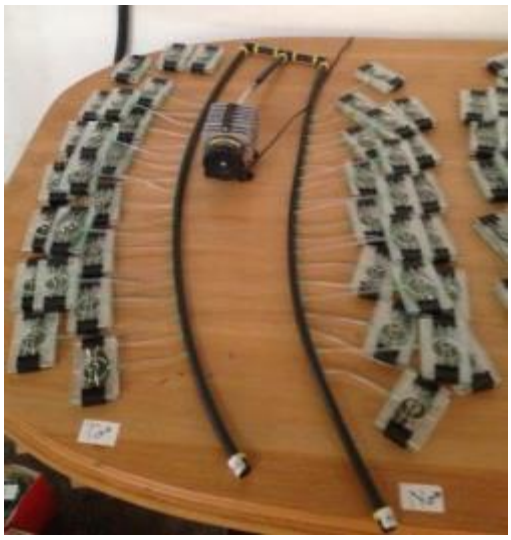

Figure 3.2 Series of 40 Munger cells of which half contain irradiated and half non-irradiated male FCM on treated leaf substrate connected to an air-flow system.

3.2.2 Soil substrate

Soil was collected from an orchard in the Sunday's River Valley and subsequently sieved to remove organic matter and stones. The maximum spray retention for citrus trees in an orchard was estimated to be 2300 L/ha (Cunningham & Harden 1998), therefore a volume of 2700 L/ha was accounted for in the soil as run-off. As trees cover approximately half the area in a hectare of planted citrus, a spray volume of effectively 5400 L/ha (540 ml/m^2) would fall to the ground. This volume was considered as an absolute worst-case scenario; in reality a large volume of the spray drift will not fall under the approximate 0.5 ha which is covered by the trees, but will end up in the

area between the rows. The soil was sprayed with a small atomiser in such a manner that it covered the entire surface area. The area of the soil surface inside each container was 78.54 x 10^{-3} cm^2. Consequently a defined volume of 4.24 ml was applied to the soil surface in each of the containers. Due to the small application volume, 4.24 ml was equated to exactly 23 sprays with the atomiser. Eight containers of evenly distributed soil were sprayed with each insecticide. Ten irradiated males (T♂) and 10 non-irradiated males (N♂) were separated and collectively placed on soil inside eight containers before being covered with cheesecloth to prevent moths escaping (Figure 3.3). Soil containers were placed inside at ambient temperature estimated at 25°C where survival of irradiated (T♂) and non-irradiated male FCM (N♂) were recorded hourly for a period of 12 h on day 1, 3, 7, 14 and 21 after application.

Figure 3.3 Ten irradiated male FCM on treated soil substrate enclosed inside a container.

3.2.3 Statistical analyses

A t-test was done as a direct comparison between survival of irradiated and non-irradiated male FCM exposed to water treated leaves to determine if a significant difference exists in natural mortality between the two independent samples. The analysis was done using XLSTAT version 2013.04.04 (Addinsoft, 1995-2013) (Table 3.2).

Mortality data for different aged residues were subjected to dose-response analysis. Regression analysis was used to compare the dose-response relationship between the log concentration of the insecticide and the empirical probit of mortality. The analysis was done using PROBAN version 2.1 (van Ark 1995). Results were interpreted according to International Organisation for Biological Control (IOBC) – Working Group "Pesticides and Beneficial Organisms" standards (Sterk *et al*. 1999)

where significant differences occurred between treatment and control ($P<0.05$). The classification includes four categories: 1) harmless, mortality lower than 30%; 2) slightly harmful, between 30-79%; 3) moderately harmful, between 80-99%; and 4) harmful, mortality higher than 99%.

Mortality data over a 12 hour period for each age of residue were subjected to time-response analysis. Time-response relationships were evaluated using a logistic version (logit) of a probit analysis (Bliss 1940), suitable for multiple observations over time. Logit is a commonly accepted technique for analysis of time-response data, particularly when the same batch of insects is evaluated over time due to limited resources (Throne *et al.* 1995). The analysis was done using Statistica version 11 (StatSoft, Inc. 1984-2012). The LT_{50} (time to kill 50% of larvae in a sample) and LT_{90} (time to kill 90 % of larvae in a sample) were calculated from these analyses.

A simple regression analysis and a post-hoc Tukey test were used to compare irradiated and non-irradiated male FCM mortality after 12 hours exposure to fresh residues (0 d) of chlorpyrifos and tau-fluvalinate on a soil substrate. Data analysis was done using Statistica version 11 (StatSoft, Inc. 1984-2012).

3.3 Results

3.3.1 Leaf substrate

A direct comparison between survival of irradiated and non-irradiated male FCM on water treated leaves showed no significant difference in natural mortality ($p>0.05$). As a result it should be accepted that gamma irradiation did not play a significant role in mortality of male FCM (Table 3.3). Data was truncated in order for control mortality to be taken into consideration, thereby reducing the total number of individuals considered to be exposed in each replicate as proposed and applied by Bliss (1940) and Moore (2002).

3.3.1.1 Dose-response

Analysis of data for irradiated and non-irradiated male FCM on tau-fluvalinate residues of different ages (Table 3.4 and 3.5) presented fitted probit lines in Figure 3.4 and were represented by $y = -3.9299x + 7.9103$ (SE of slope = 0.8111) and $y = -2.7961x + 6.9365$ (SE of slope = 0.7026) respectively. Deviations from the probit line for irradiated male FCM were considered homogenous, making the chi-squared test more applicable than would have been the case if the deviations were heterogeneous (van Ark 1995). Deviation from the probit line calculated for non-irradiated male FCM were considered homogenous by combination of data for residues of ages.

Fiducial limits determine the boundary within which a parameter is considered to be located. A G-value, used to estimate fiducial limits, was calculated to be 0.1803 for irradiated FCM and 0.2465 for non-irradiated FCM (Appendix 1). A value greater than 0.025 means that variations of mortalities are large (van Ark 1995). Nonetheless, experimental procedures or the value of probit lines should only be questioned if G exceeds 0.25. Bartlett's test for homogeneity of residual variance of the lines were determined to be homogenous making the lines comparable. The chi-squared test showed lines to be parallel and the elevations to be comparable. Elevations of the two lines were found not to be significantly different.

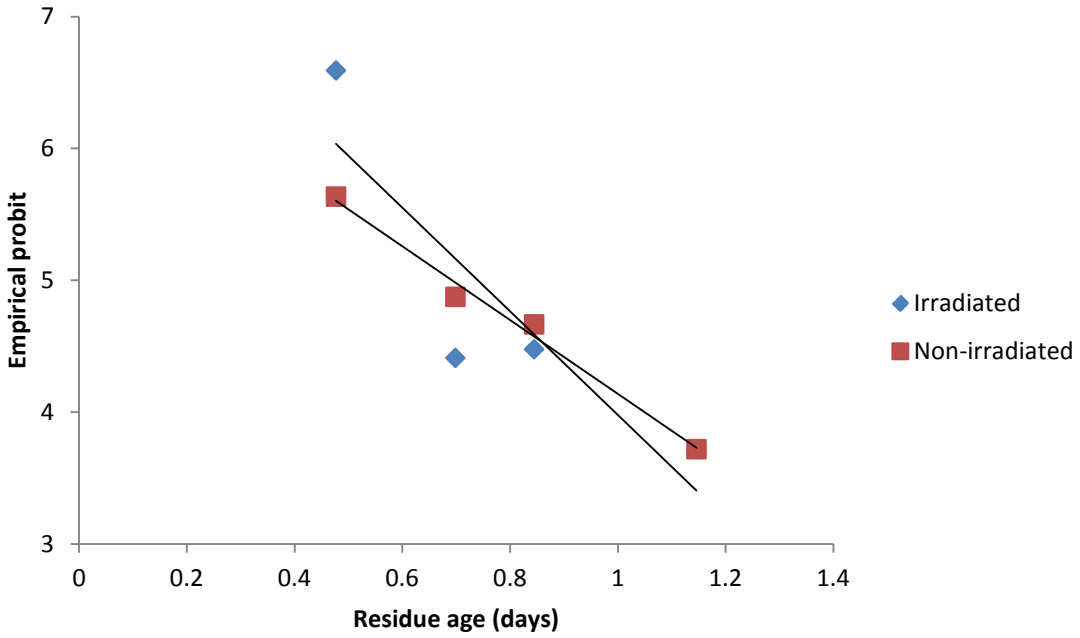

Figure 3.4 Probit line for tau-fluvalinate residue of different ages against irradiated and non-irradiated male FCM.

Table 3.2 T-test statistics for comparison of irradiated and non-irradiated male FCM mortality in water-treated controls.

Variable	Observations	Minimum	Maximum	Mean	Std. deviation
Irradiated	72	0.000	2.000	0.111	0.396
Non-irradiated	72	0.000	1.000	0.069	0.256

Table 3.3 Results of the t-test comparison between irradiated and non-irradiated male FCM mortality in the water-treated controls.

Difference	t (Observed value)	|t| (Critical value)	DF	p-value (Two-tailed)	alpha
0.042	0.750	1.977	142	0.454	0.05

Table 3.4 Mortality of irradiated male FCM in dose-response evaluations with tau-fluvalinate residues of different ages.

Residual age (dose)	Transformed dose	Number exposed	Corrected number exposed	Number responded	% Response	Empirical probit
3	0.48	20.00	18.00	17.00	94.44	6.59
5	0.70	20.00	18.00	5.00	27.78	4.41
7	0.85	20.00	20.00	6.00	30.00	4.48
14	1.15	20.00	20.00	2.00	10.00	3.72

Table 3.5 Mortality of non-irradiated male FCM in dose-response evaluations with tau-fluvalinate residues of different ages.

Residual age (dose)	Transformed dose	Number exposed	Corrected number exposed	Number responded	% Response	Empirical probit
3	0.48	20.00	19.00	14.00	73.68	5.63
5	0.70	20.00	20.00	9.00	45.00	4.87
7	0.85	20.00	19.00	7.00	36.84	4.66
14	1.15	20.00	20.00	2.00	10.00	3.72

Table 3.6 Mortality of irradiated male FCM in dose-response evaluations with chlorpyrifos residues of different ages.

Residual age (dose)	Transformed dose	Number exposed	Corrected number exposed	Number responded	% Response	Empirical probit
5	0.70	20.00	18.00	13.00	72.22	5.59
7	0.85	20.00	20.00	11.00	55.00	5.13
14	1.15	20.00	20.00	1.00	5.00	3.36

LT_{50} values estimated for irradiated and non-irradiated male FCM on tau-fluvalinate were 16.90 h and 16.584 h respectively with upper and lower fiducial limits from 0.595 to 0792 and 0.537 to 0.802. LT_{90} values for irradiated and non-irradiated male FCM were 0.361 and 0.229 respectively, with upper and lower fiducial limits from 0.54 to 0.498 and -0.313 to 0.426.

Data for irradiated male FCM mortality on chlorpyrifos and tau-fluvalinate residues of different ages (Table 3.4 and 3.5) were analysed and fitted to probit lines (Figure 3.5) and were described by the equations y = -5.1293x + 9.2896 (SE of slope = 1.2152) and y = -3.9299x + 7.9103 (SE of slope = 0.8111). Deviations from the probit line for chlorpyrifos were considered homogenous while deviations from the probit line for tau-fluvalinate were considered homogenous by combination of doses. Fiducial limits were calculated to be 0.2280 for chlorpyrifos and 0.1803 for tau-fluvalinate making the lines comparable. Slopes of the two lines were compared since residual variances were found to be homogenous by Bartlett's test. The lines were parallel and the elevations proved comparable by the chi-squared test. Elevations of the two lines were not significantly different. LT_{50} values were assessed for chlorpyrifos and tau-fluvalinate and presented 20.21 h and 16.90 h in that order, with fiducial limits from 0.754 to 0.924 and 0.595 to 0.792. LT_{90} values calculated for chlorpyrifos and tau-fluvalinate were 0.585 and 0.361 respectively, with fiducial limits from 0.321 to 0.692 and 0.054 to 0.498.

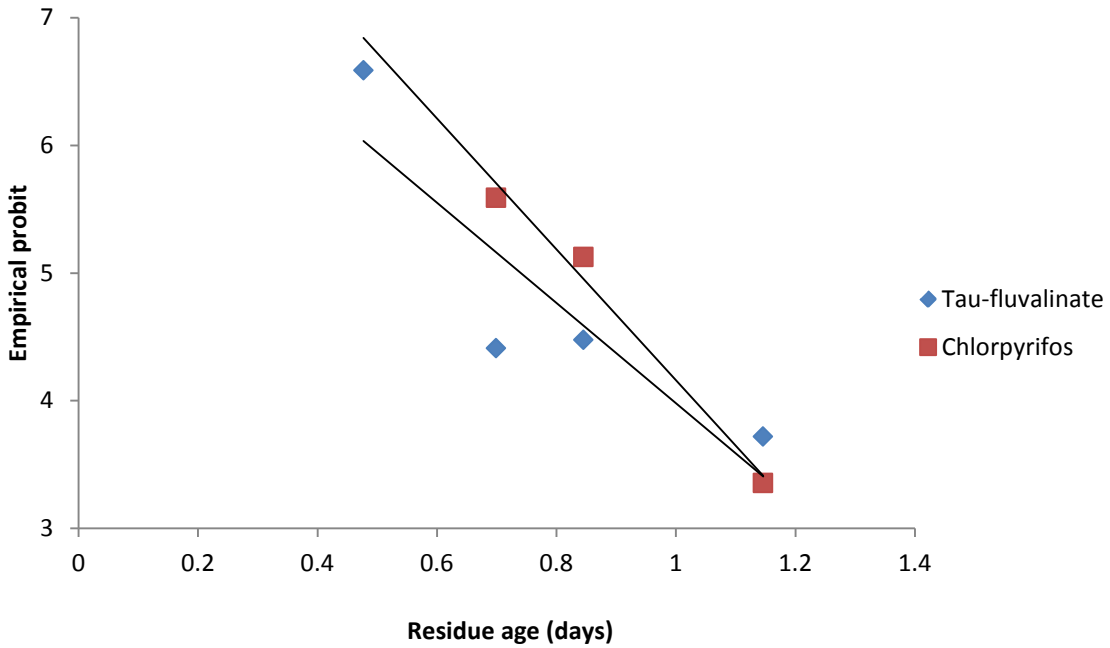

Figure 3.5 Probit lines for chlorpyrifos and tau-fluvalinate residues of different ages against irradiated male FCM.

Twelve hours after exposure to a fresh residue (0 d) of chlorpyrifos and tau-fluvalinate, irradiated male FCM showed high mortality rates compared to those of moths exposed to water treated leaves (Figure 3.6) (Table 3.7). Fresh residues of tau-fluvalinate, as well as fresh and three day-old residues of chlorpyrifos resulted in 100% mortality of irradiated FCM and can be classified as harmful (IOBC 4). Three day-old residues of tau-fluvalinate caused 85% mortality and are as a result considered moderately harmful (IOBC 3). Five and seven day-old residues of chlorpyrifos caused 65% and 55% mortality respectively and are consequently categorised as slightly harmful (IOBC 2). The same age residues of tau-fluvalinate resulted in 25% and 30% mortality of irradiated FCM, indicating a classification of harmless (IOBC 1). Exposure of irradiated male FCM to fourteen day-old residues of chlorpyrifos and tau-fluvalinate caused 5% and 10% mortality, in that order, classifying both as harmless (IOBC 1) (Table 3.7). Twenty-one day-old residues of both chemicals used for experiments in this study resulted in 0% mortality after exposure for 12 h and accordingly, these residues were harmless for irradiated male FCM (IOBC 1).

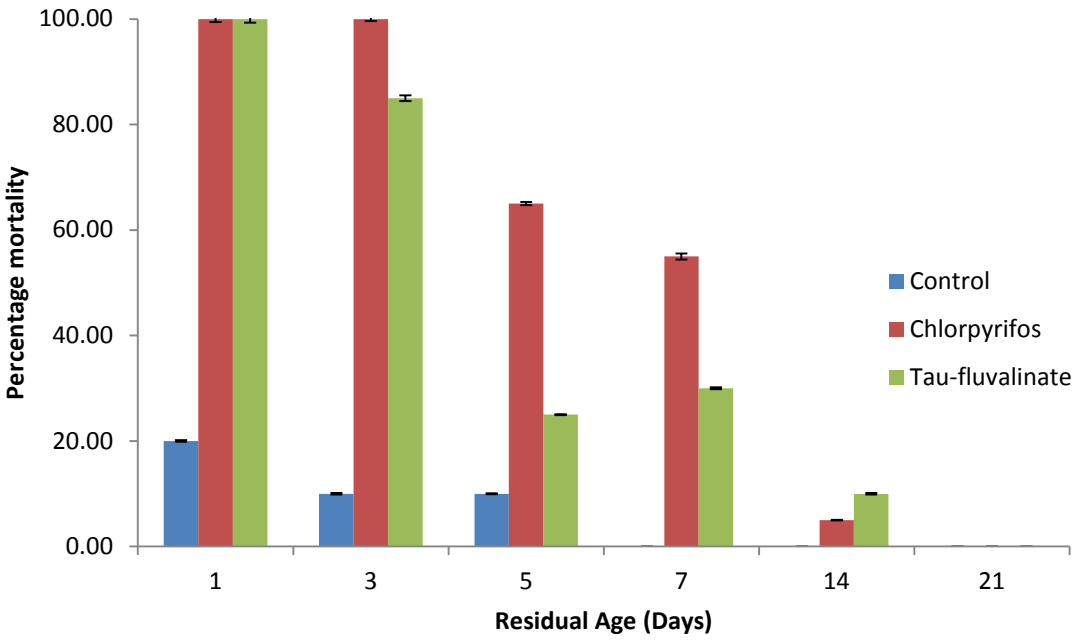

Figure 3.6 Mortality of irradiated male FCM after exposure to leaf-residues of chlorpyrifos and tau-fluvalinate.

Mortality rates of non-irradiated male FCM exposed to chlorpyrifos and tau-fluvalinate on a leaf substrate were significantly different to those of the control (Figure 3.7) (Table 3.8). The fresh and three day-old residues of chlorpyrifos as well as the fresh residues of tau-fluvalinate produced 100%

mortality of non-irradiated male FCM. The three day-old residue of tau-fluvalinate caused a significantly lower mortality rate. A similar trend was also shown for five day-old and seven day-old residues, with chlorpyrifos causing 60% and 50% mortality on respective residues, compared to 45% and 35% mortality for tau-fluvalinate. Fourteen day-old residues of tau-fluvalinate killed more non-irradiated male FCM than the same age residues of chlorpyrifos. Neither chlorpyrifos nor tau-fluvalinate caused mortality of male FCM after exposure for 12 hours on 21 day-old residues on a leaf substrate.

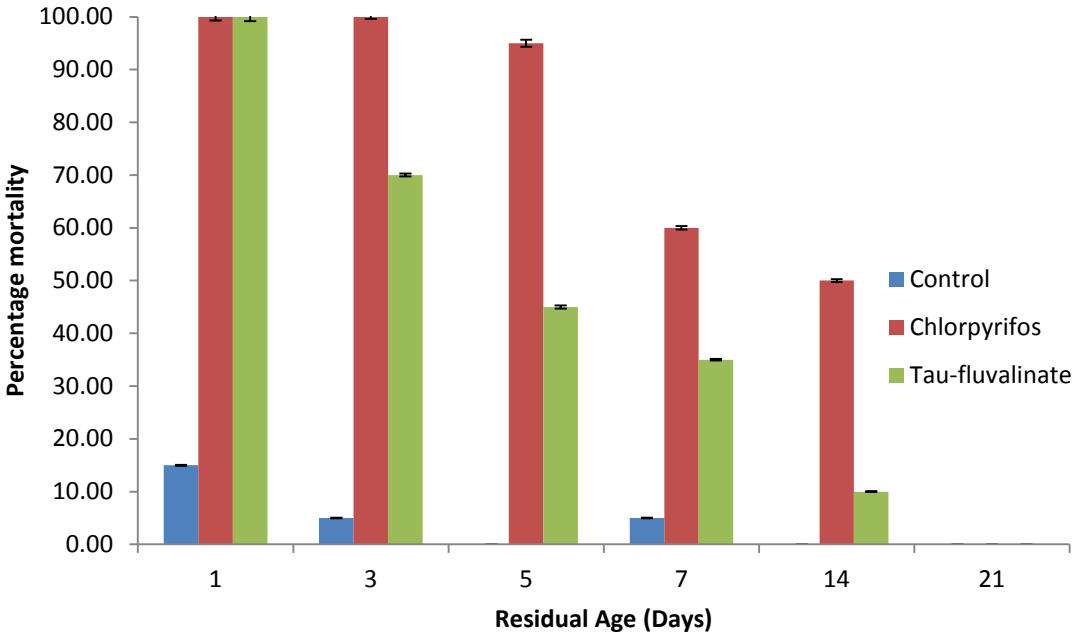

Figure 3.7 Mortality of non-irradiated male FCM after exposure to leaf-residues of chlorpyrifos and tau-fluvalinate.

Table 3.7 Percentage mortality (mean ± SE) of irradiated male FCM after 12 h exposure to chlorpyrifos and tau-fluvalinate residues plus a water treated control on a leaf substrate.

Treatment	**Mortality (%) at each residual age**							
	Fresh	**3-day-old**	**5-day-old**	**7-day-old**	**14-day-old**	**21-day-old**	**LT_{50}**	**LT_{90}**
Chlorpyrifos	100 ± 0.00	100 ± 0.42	65.00 ± 0.31	55.00 ± 0.58	5.00 ± 0.06	0.00	7.20	11.20
Tau-fluvalinate	100 ± 0.00	85.00 ± 0.54	25.00 ± 0.12	30.00 ± 0.18	10.00 ± 0.13	0.00	5.20	10.00
Water	20.00 ± 0.15	10.00 ± 0.13	10.00 ± 0.09	0.00	0.00	0.00	n/a	n/a

* Sample size was 20 individuals

Table 3.8 Percentage mortality (mean ± SE) of non-irradiated male FCM after exposure to chlorpyrifos and tau-fluvalinate residues plus a water-treated control on a leaf substrate.

Treatment	**Mortality (%) at each residual age**							
	Fresh	**3-day-old**	**5-day-old**	**7-day-old**	**14-day-old**	**21-day-old**	**LT_{50}**	**LT_{90}**
Chlorpyrifos	100 ± 0.00	100 ± 0.41	60.00 ± 0.69	50.00 ± 0.34	0 ± 0.27	0.00	6.60	9.30
Tau-fluvalinate	100 ± 0.00	70.00 ± 0.27	45.00 ± 0.29	35.00 ± 0.15	10.00 ± 0.09	0.00	5.60	11.30
Water	15.00 ± 0.10	5.00 ± 0.06	0.00	5.00 ± 0.06	0.00	0.00	n/a	n/a

* Sample size was 20 individuals

3.3.1.2 Time-response

The same batch of insects was evaluated hourly over a period of 12 hours to calculate irradiated and non-irradiated male FCM mortality relative to the time of exposure (hours) to different age residues of chlorpyrifos and tau-fluvalinate. Generally, fresh residues of chlorpyrifos and tau-fluvalinate killed 100% of irradiated and non-irradiated male FCM within five hours after contact. The rate of mortality gradually declined as the residues age, depicted as a decrease in the slope of the line from three day-old to 21 day-old residues (Figure 3.8, 3.9, 3.10 and 3.11). Fresh and three day old residues of chlorpyrifos caused a similar rate of mortality on irradiated and non-irradiated male FCM (Figure 3.8 and 3.9). Respective LT_{50} values of 7 d 4.8 h and 6 d 14.4 h showed that chlorpyrifos residues had a faster impact on non-irradiated compared to irradiated male FCM (Table 3.7 and 3.8). The calculated LT_{90} value showed a significant difference in rate of mortality in favour of non-irradiated (9 d 7.2 h) over irradiated moths (11 d 4.8 h). Tau-fluvalinate showed the opposite, with a slightly higher rate of mortality on irradiated compared to non-irradiated male FCM with respective LT_{50} values of 5 d 4.8 h and 5 d 14.4 h and LT_{90} values of 10 d 0 h and 11 d 7.2 h (Table 3.7 and 3.8) (Figure 3.9 and 3.10).

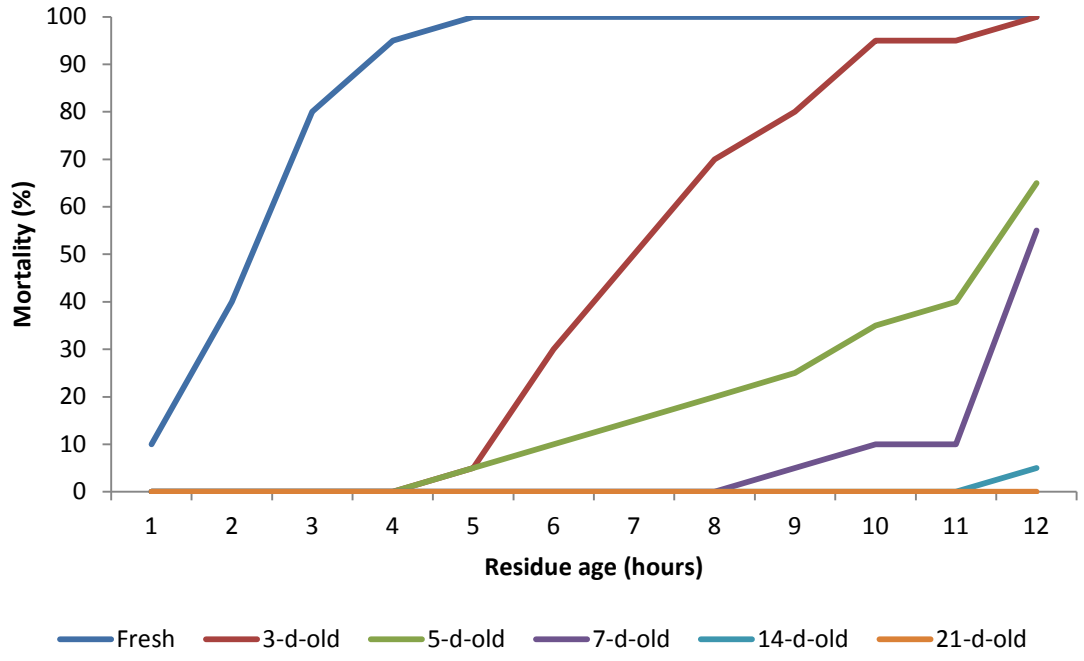

Figure 3.8 Cumulative percentage mortality of irradiated male FCM over 12 hours for different age chlorpyrifos residues.

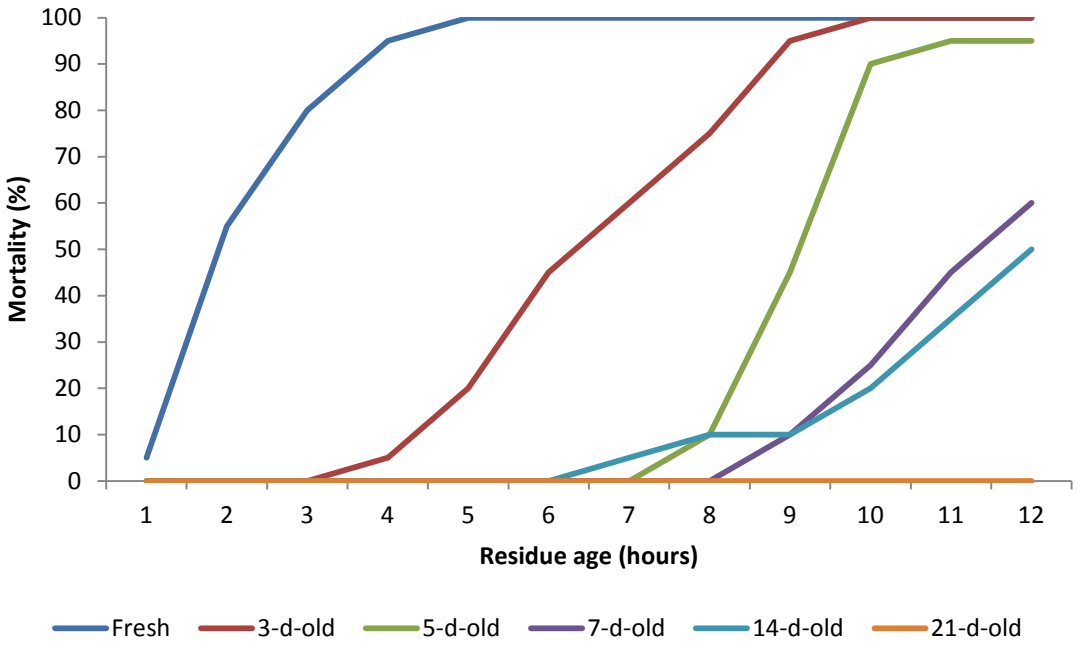

Figure 3.9 Cumulative percentage mortality of non-irradiated male FCM over 12 hours for different age chlorpyrifos residues.

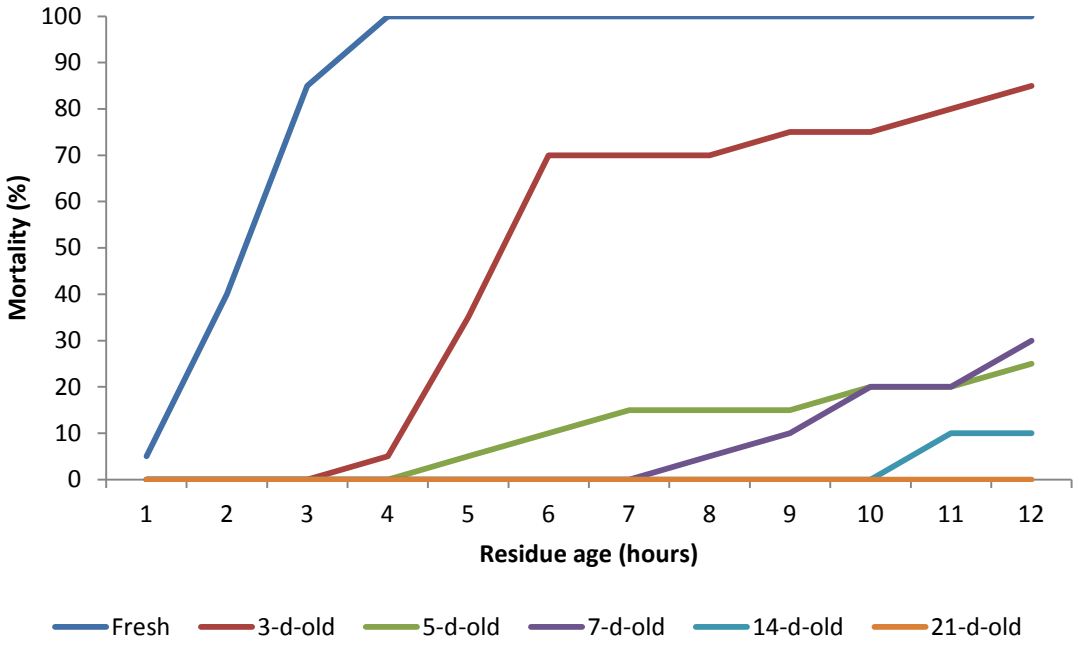

Figure 3.10 Cumulative percentage mortality of irradiated male FCM over 12 hours for different age tau-fluvalinate residues.

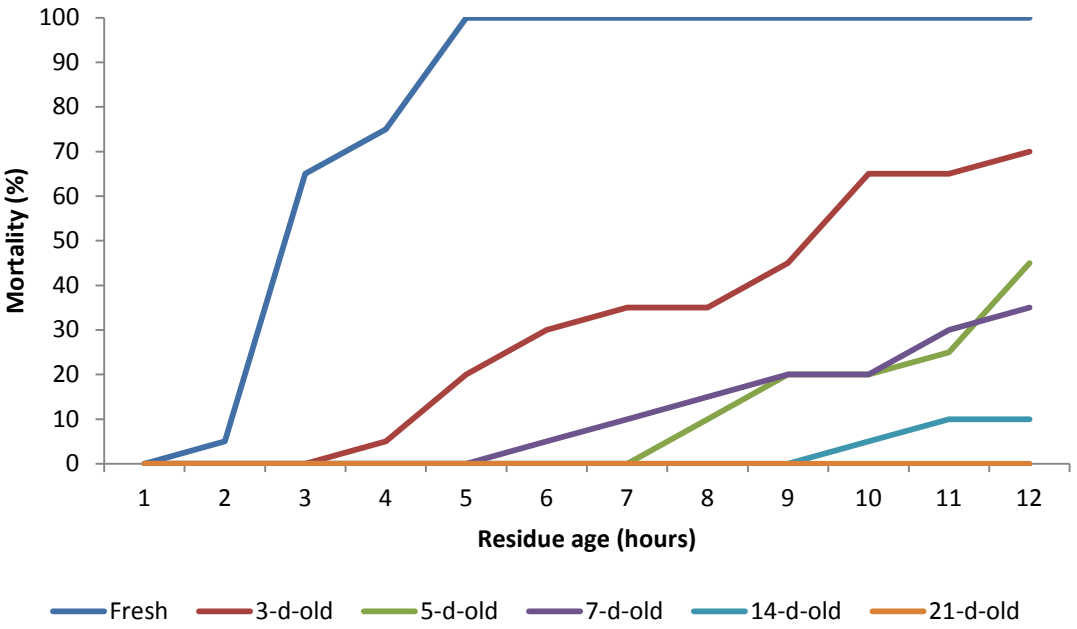

Figure 3.11 Cumulative percentage mortality of non-irradiated male FCM over 12 hours for different age tau-fluvalinate residues.

Table 3.9 Logistic regression data for mortality of irradiated male FCM exposed to chlorpyrifos and tau-fluvalinate residues plus a water treated control on a leaf substrate.

	Chi square	P value	RC	SE	P value	X intercept	SE	P value
Chlorpyrifos	849.160	0.000	2.834	0.077	0.000	-0.247	0.009	0.000
Tau-fluvalinate	563.120	0.000	2.466	0.072	0.000	-0.196	0.009	0.000
Water	79.377	0.000	4.625	0.183	0.000	-0.175	0.021	0.000

Table 3.10 Logistic regression data for mortality of non-irradiated male FCM exposed to chlorpyrifos and tau-fluvalinate residues plus a water-treated control on a leaf substrate.

	Chi square	P value	RC	SE	P value	X intercept	SE	P value
Chlorpyrifos	1505.400	0.000	3.131	0.079	0.000	-0.325	0.010	0.000
Tau-fluvalinate	757.520	0.000	3.049	0.083	0.000	-0.246	0.010	0.000
Water	45.188	0.000	5.294	0.251	0.000	-0.178	0.028	0.000

3.3.2 Soil substrate

Very low mortality was recorded for irradiated and non-irradiated male FCM exposed to either of the insecticides for day 3 to 21 (Table 3.11 and 3.12). It was therefore not possible to conduct dose-response analyses. Fresh chemical residues of chlorpyrifos had a significant effect on mortality of irradiated and non-irradiated male FCM ($F = 15.253$; $P < 0.01$) (Figure 3.12) (Table 3.11 and 3.12). This effect was greater for irradiated moths, albeit not significantly. There was no significant difference in mortality when irradiated or non-irradiated male FCM were exposed to tau-fluvalinate residues compared to the water treated control.

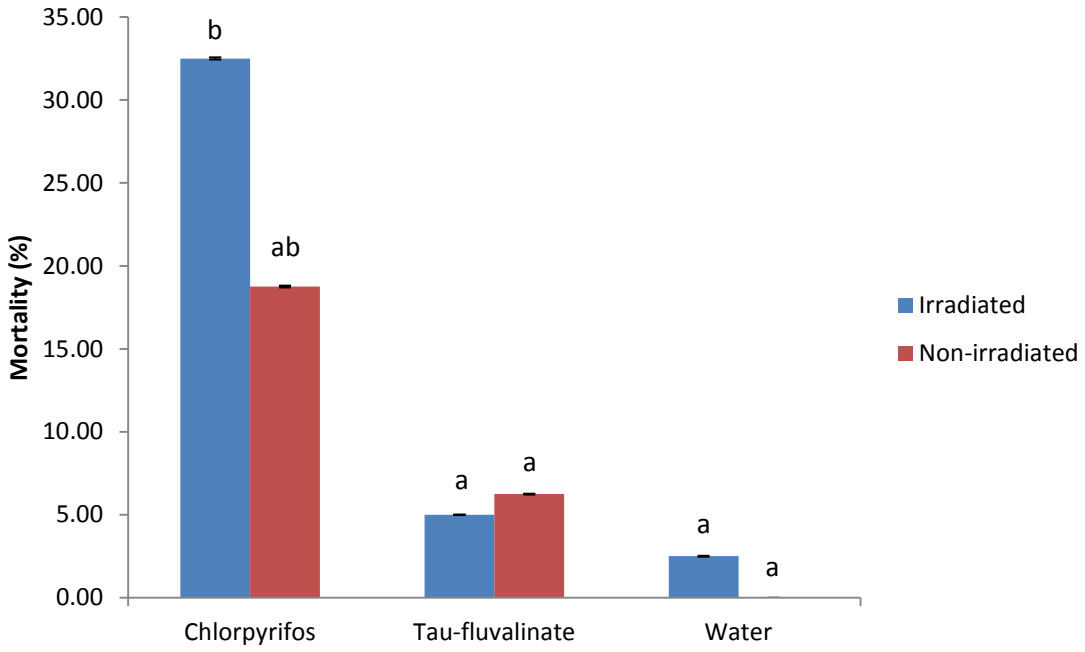

Figure 3.12 Mortality of irradiated and non-irradiated male FCM after exposure to fresh residues of chlorpyrifos, tau-fluvalinate and water on a soil substrate.

Cumulative percentage mortality of irradiated male FCM over a 12 hour period on fresh residues indicated a higher rate of activity for chlorpyrifos compared to tau-fluvalinate for both irradiated and non-irradiated male FCM (Figure 3.13 and 3.14). Additionally, fresh chlorpyrifos residues on a soil substrate killed irradiated FCM slightly faster than non-irradiated FCM. Irradiation did not have a significant effect on the rate of mortality after exposure to fresh tau-fluvalinate residues on soil.

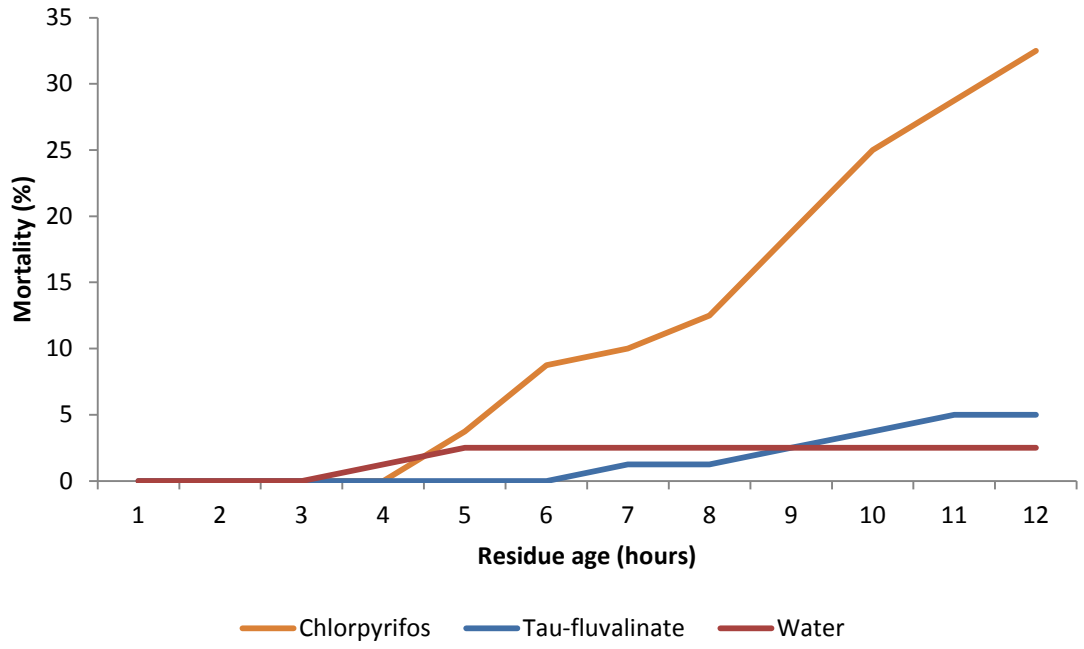

Figure 3.13 Cumulative mortality of irradiated male FCM over a 12 hour period after exposure to fresh residues of chlorpyrifos, tau-fluvalinate and water on a soil substrate.

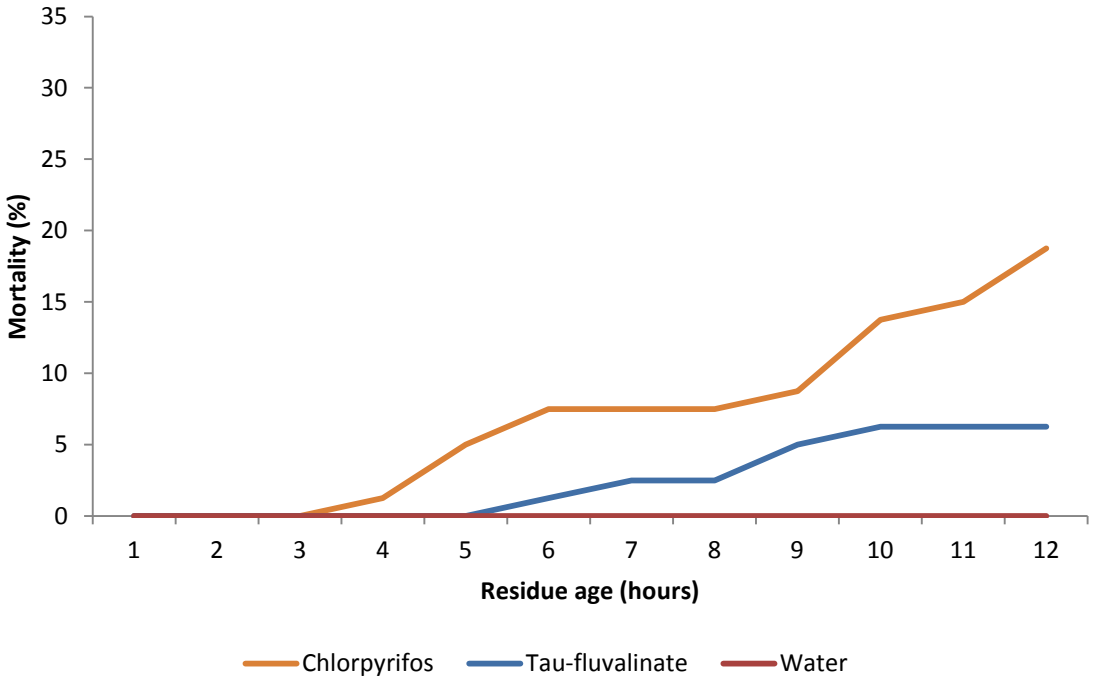

Figure 3.14 Cumulative mortality of non-irradiated male FCM over a 12 hour period after exposure to fresh residues of chlorpyrifos, tau-fluvalinate and water on a soil substrate.

Table 3.11 Percent mortality (mean ± SE) of irradiated male FCM after 12 h exposure to chlorpyrifos and tau-fluvalinate residues plus a water treated control on a soil substrate.

Treatment	**Mortality (%) at each residual age**					
	Fresh	**3-day-old**	**5-day-old**	**7-day-old**	**14-day-old**	**21-day-old**
Chlorpyrifos	32.5 ± 0.07	6.25 ± 0.03	2.5 ± 0.02	1.25 ± 0.01	2.5 ± 0.02	0.00
Tau-fluvalinate	5 ± 0.02	1.25 ± 0.01	2.5 ± 0.02	0.00	2.5 ± 0.02	0.00
Water	2.5 ± 0.02	1.25 ± 0.01	1.25 ± 0.01	0.00	0.00	0.00

Table 3.12 Percent mortality (mean ± SE) of non-irradiated male FCM after 12 h exposure to chlorpyrifos and tau-fluvalinate residues plus a water treated control on a soil substrate

Treatment	**Mortality (%) at each residual age**					
	Fresh	**3-day-old**	**5-day-old**	**7-day-old**	**14-day-old**	**21-day-old**
Chlorpyrifos	18.75 ± 0.06	10 ± 0.04	1.3 ± 0.01	0.00	3.75 ± 0.02	0.00
Tau-fluvalinate	6.25 ± 0.03	3.8 ± 0.02	3.8 ± 0.02	0.00	0.00	0.00
Water	0.00	0.00	1.25 ± 0.01	0.00	0.00	0.00

3.4 Discussion

Results of this study showed that residues of chlorpyrifos and tau-fluvalinate are effective in killing adult male FCM on contact regardless of irradiation. However, residues of these particular insecticides generally only have a significant effect for a period of seven days after application on a leaf substrate and a considerably reduced effect as fresh residues on a soil substrate. These results present a worst case scenario due to the possibility of fumigation inside Munger cells, despite the air-flow system that prevents chemical build-up inside cells, the forced continuous contact with the residues inside the Munger cells, and the high estimated amount of chemical determined as run-off in the soil. Whether these insecticides will have a positive or negative effect on an SIT programme for suppression of FCM is important for integration of various control methods in an AW-IPM programme on citrus.

SIT is not a stand-alone technology, and as a result it should be integrated into a programme with supplementary control tactics (Bloem *et al.* 2005, Mangan *et al*. 2005). Chemical insecticides have often been used in conjunction with AW-IPM that includes SIT. For the correct implementation of AW-IPM programmes, information on the toxicity of pesticides to sterile male FCM is imperative. In the eradication of New World screwworm (NWS), *Cohliomyia hominovorax* (Coquerel) (Diptera: Calliphoridae), in North America, integration with methods such as treatment of infested wounds with larvicides, as well as quarantine actions were implemented (Klassen and Curtis 2005). In comparison, various fruit fly programmes in which sterile flies are released for suppression or eradication of the pest, SIT is integrated with chemical baits, lures and cultural practices (Enkerlin 2005, Hendrichs *et al*. 2005, Mangan 2005). The SIT programme for sustained suppression of codling moth (CM), *Cydia pomonella,* in British Columbia, Canada, required the direct use of insecticides to reduce the adult pest population (Bloem & Bloem 2000a). Twice-weekly sterile moth releases were supplemented with multiple chemical sprays coinciding with critical life-cycle stages, sex pheromone traps for monitoring and mating disruption with synthetic sex pheromones. Pre-release suppression of wild populations remains a prerequisite for many AW-IPM programmes. Where a high proportion of the wild population is present as eggs or larvae when the releases of sterile insects begins, the wild population will increase for a time in spite of the presence of sterile insects (Klassen 2005). To reduce the lag period between initiation of releases and a noticeable effect on the density of the pest, an insecticide could be applied to kill females previously mated to wild males.

This study proved that chlorpyrifos and tau-fluvalinate are harmful to sterile and wild male FCM to varying degrees. If an insecticide is applied simultaneously to sterile insect releases, as in the case of chlorpyrifos and tau-fluvalinate, this study has shown that there will be mortality of a proportion of

the sterile insects. Providing that the sterile : wild ratio remains the same, this would not weaken the efficacy of SIT (Mangan 2005). Remarkably, the level of toxicity for both chemicals diminished to such an extent that seven day-old leaf-residues were harmless. Susceptibility of irradiated and non-irradiated male FCM proved almost identical over the period of assessment. This indicates that the irradiation process does not adversely affect the resistance of FCM to pesticides of this nature, strengthening the argument for possible integration with SIT. In addition the lack of significant toxicity of tau-fluvalinate residues in soil showed that spray run-off will not kill a large amount of irradiated male FCM that land on the ground after release. Chlorpyrifos residues in the soil should not have an adverse effect on SIT, particularly if releases occur 12 hours after application of this pesticide. Both chlorpyrifos (48 g/100 L)and tau-fluvalinate (30 ml/100 L) were tested at the recommended concentrations for control of three important citrus pests in South Africa, African bollworm, citrus thrips and mealybugs, reflecting commercial application of these pesticides. Importantly, sub-lethal effects of these chemicals could cause changes in physiology and behaviour of irradiated male FCM, compromising their longevity, flight ability and realized fecundity. Further field research should be conducted on these sub-lethal effects of chlorpyrifos and tau-fluvalinate on sterile male FCM to determine the compatibility with an SIT programme.

In citrus producing areas such as the Sunday's River Valley (SRV) in the Eastern Cape, where African bollworm and citrus thrips are particularly prevalent and these pests exceed their respective economic thresholds for restricted periods of the season, control measures are applied on a regular basis from petal fall in early October. The impact of broad spectrum insecticides on natural enemies of renders the use of these products undesirable in an IPM programme (Moore *et al.* 2004b). The SIT programme for suppression of FCM in South Africa requires the use of insecticides as supplementary control for FCM mostly due to its phytosanitary status in export markets. However, a microbial insecticide such as the *Cryptophlebia leucotreta* granulovirus is recommended for control of the larval stage after a flight peak is observed in pheromone traps. This approach encourages development of natural enemies such as the wasp *Trichogrammatoidea cryptophlebiae* (Nagaraja) (Hymenoptera: Trichogrammatidae). As an egg parasitoid of FCM, *T. cryptophlebia* is the most effective biological suppressant of FCM. It occurs naturally in all citrus- producing areas and is especially abundant in the SRV. Spray programmes should be structured to minimise non-target effects on these parisitoids as they can attack more than 80% of FCM eggs after summer, if undisrupted throughout the season (Moore 2011a). The use of chlorpyrifos and tau-fluvalinate in an SIT programme for control of FCM is extremely limited due to their short period of residual action and their undesirable effect on non-target organisms, particularly beneficial predators and parasites. However, due to the extent of the target pest problem and restriction of application to early spring,

their use should not affect the SIT programme. Timing of irradiated moth releases should not be altered in an effort to circumvent these sprays. Application of chlorpyrifos and tau-fluvalinate are not governed as area wide application, but instead applied by individual growers when the specific threshold is breached. Due to the short-lived and comparable effect of these chemicals on irradiated and non-irradiated male FCM, ratios should be maintained regardless of whether sprays are applied or not.

4

RELEASE TECHNOLOGY IN THE SIT PROGRAMME FOR CONTROL OF FALSE CODLING MOTH

4.1 Introduction

A vital requirement for SIT is an effective procedure for release of sterile insects, the end result being economical distribution, without injury, at controllable rates (Tan & Tan 2013, McMechan & Proverbs 1971). Three methods exist by which sterile insects are released into the environment: static release, ground release and aerial release. Each programme is unique in terms of the target area and size, geographical situation, pest situation, release density, swath as well as the accessibility to affordable labour (Tan &Tan 2013, Dowell *et al.* 2005). A number of factors determine the efficacy of the specific release method for a particular SIT programme in a given area, and are outlined below.

Static releases are used in small sized, accessible areas mainly for release of sterile Mediterranean fruit flies (medfly), *Cerratitis capitata* (Wiedemann) (Diptera: Tephritidae), as pupae or adults placed in fixed containers distributed uniformly throughout the release area (Dowell *et al.* 2005). Studies in dispersion of medfly after ground (Meats *et al.* 2006) and static (Plant & Cunningham 1991) methods have motivated development of modern release technology for these particular SIT programmes. Meats *et al.* (2006) also applied his dispersion theory for SIT on the Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) following static releases to determine the ideal release density. He proposed a model by which mean weekly recaptures of sterile flies could be related to the percentage of traps in which a certain number of flies were caught. He concluded that dispersion is of great significance to SIT because patterns of abundance of sterile and wild insects will influence its efficiency. Koyama *et al.* (2004) investigated static release of sterile melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae), in Japan but has subsequently developed a method for aerial release of chilled adults. When sterile fly pupae are placed into release containers, emergence of adults is synchronised with local diurnal and temperature cycles (Dowell *et al.* 2005, Meats *et al.* 2006). This method reduces labour and eliminates the need for satellite radiation facilities making it affordable and effective for small, accessible regions (Dowell *et al.* 2005). However, provision and distribution of release receptacles is labour intensive, limiting size and

location of release sites to areas that are quickly and easily accessible. Depending on the insect's natural ability to disperse, distribution of sterile insects from a point source often causes areas of high density separated by areas of low density (Plant & Cunningham 1991, Dowell *et al.* 2005, Meats *et al.* 2006). Susceptibility of emerged flies to bad weather and predators might also influence the outcome of an SIT operation that uses the static release method (Liu & Yeh 1982, Koyama *et al*. 2004).

Ground release methods include release of sterile insects from slow moving vehicles such as tractors, trucks or ATVs (All Terrain Vehicles). Insects are released directly into the environment by hand or machine, or in open containers from which they could escape (Koyama *et al*., 2004, Bloem & Bloem 2000b, McMechan & Proverbs 1971). Development of a chilled, immobilized adult release method simplified handling and preserved insect quality during shipment and loading (Simmons *et al.* 2010, Judd & Gardiner 2006, Koyama *et al.* 2004, McMechan & Proverbs 1972). However, keeping insects at ambient temperature during release procedure maximises dispersal from the release site (Anonymous 2010, Dowell *et al*. 2005). Using an adaptable release route, the ground release method covers a large area, rapidly distributing sterile insects more efficiently than static release methods (Dowell 2005, McMechan & Proverbs 1971). Release of insects directly into the environment reduces the negative impact of predators and provides a uniform distribution that facilitates dispersal. Safety of slow moving vehicles on open roads and inaccessibility into areas of dense vegetation are the main constraints for ground release methods.

Aerial release involves discharge of sterile insects from helicopters or fixed-wing aircraft in protected containers such as paper bags and cardboard containers (Baumhover *et al.* 1959, Nadel *et al.* 1967, Boving *et al.* 1969, Rhode& Calderon 1971), or else directly into the environment (Higgins 1970, Rhode & Calderon 1971). In this case immobilized insects are fed from a hopper on a modified aeroplane through a release system in flight. Aerial releases cover wide areas rapidly and effectively, irrespective of the terrain, particularly if a navigational programme controlled by a Global Positioning System (GPS) is calibrated to distribute sterile insects at a variable flow rate over the target area. By varying the flow rate more insects are delivered to areas with a high native population (Briasco 2011). Release rate and aircraft size determine amount of insects that can be released per flight, although insect tolerance will generally determine the length of the flight (Judd & Gardiner 2006). Some insects have a limited tolerance of extreme temperatures and prolonged exposure could negatively affect their performance in the field (Chidawanyika & Terblanche 2011). Aerial releases are more weather sensitive and ideally require an emergence facility nearby (Dowell

et al. 2005). Additionally, availability and cost of specially equipped aircraft could be restricting factors for small SIT operations.

The aim of this chapter was to compare efficacy of two main methods used to release insects directly into the environment *viz.* ground and aerial. The rate of pest suppression in a particular SIT programme is directly proportional to the ratio of sterile to wild insects maintained over time (McMechan & Proverbs 1971, Meats *et al.* 2006). Consequently, population distribution of sterile male FCM after release in citrus orchards was evaluated to conclude which method optimally accelerates dispersal of individuals and general field performance.

4.2 Materials and Methods

4.2.1 Release sites

Recently irradiated FCM were delivered daily from Citrusdal in the Western Cape province to Addo, Eastern Cape province, in cooler boxes as immobilized adults (Chapter 2). Cooler boxes were packed directly into a cold room, maintained at 4-5°C, until collection for release. The goal was to release a total of 2000 moths per ha once per week from 10 September to 11 November 2012, and 1000 moths per ha from 12 November 2012 to 31 March 2013, over two trial sites in the Addo region of the Sunday's River Valley. However, it was occasionally unavoidable that the release rate would decrease due to a reduced supply of irradiated moths from Citrusdal as well as mechanical problems with the aircraft (Table 4.1). Aerial releases were conducted over a 65 ha trial site at Bernol Farm (-33.475848° 25.611687°), Addo (Figure 4.1), whereas ground releases were conducted on a 74 ha trial site at Penhill Estates (-33.579619° 25.693488°), Addo (Figure 4.2).

The insecticidal spray programme differed at the two trial sites during the 28 week trial period of the 2012/13 citrus producing season. Application of abamectin EC (19 g/l) (Agrimek, Syngenta) (21 to 27 October, 6 to 12 January, 27 January to 2 February), spinetoram WG (250 g/kg) (Delegate, Dow AgroSciences) (11 to 17 November, 9 to 15 December), and *Cryptophlebia leucotreta* SC ($5x10^{10}$ ob's/ml) (Cryptogran, River BioScience) (2 to 8 December, 27 January to 2 February) were applied on the ground release orchards whereas profenofos EC (500 g/l) (Curacron, Syngenta) (23 to 29 September 2012), tau-fluvalinate EW (240g/l) (Klartan, Makhteshim Agan) (7 to 13 October), abamectin EC (19 g/l) (Agrimek, Syngenta) (4 to 10 October, 16 to 22 December, 6 to 12 January), and spinetoram WG (250 g/kg) (Delagate, Dow AgroSciences) (18 to 24 November) were applied at the aerial release site. A particular difference is likely to be the inclusion of profenofos (organophosphate) and tau-fluvalinate (pyrethroid) at the aerial release site. However, as these products were applied very early in the season for control of citrus thrips *Scirtothrips aurantii* (Faure)

(Thysenoptera: Thripidae), citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) and African bollworm ,*Helicoverpa armigera,* (Hübner) (Lepidoptera: Noctuidae), their effect on recaptures of released irradiated FCM would be limited to seven days after application (see Chapter 3). Both release sites consists of several citrus types including cultivars of Navels, Valencias and Lemons (Appendix A). The ground release site was adjacent to a 50 ha farm included in the commercial SIT programme, although at least 100 m separated the two farms on either side of a tar road. The aerial release site was completely surrounded by bush with the closest farm on the SIT programme more than a kilometre away.

Table 4.1 Number of irradiated male and female FCM released per hectare (ha) per week by ground and aerial methods from 10 September 2012 to 25 March 2013.

Method	10-Sep	17-Sep	24-Sep	01-Oct	08-Oct	15-Oct	22-Oct	29-Oct	05-Nov	12-Nov	19-Nov	26-Nov	03-Dec
Aerial	0	2000	1500	0	2000	2000	0	2000	2000	0	2000	1000	2000
Ground	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

Method	10-Dec	17-Dec	07-Jan	14-Jan	21-Jan	28-Jan	04-Feb	11-Feb	18-Feb	25-Feb	04-Mar	11-Mar	18-Mar	25-Mar
Aerial	2000	1000	2000	1000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000
Ground	3000	1000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

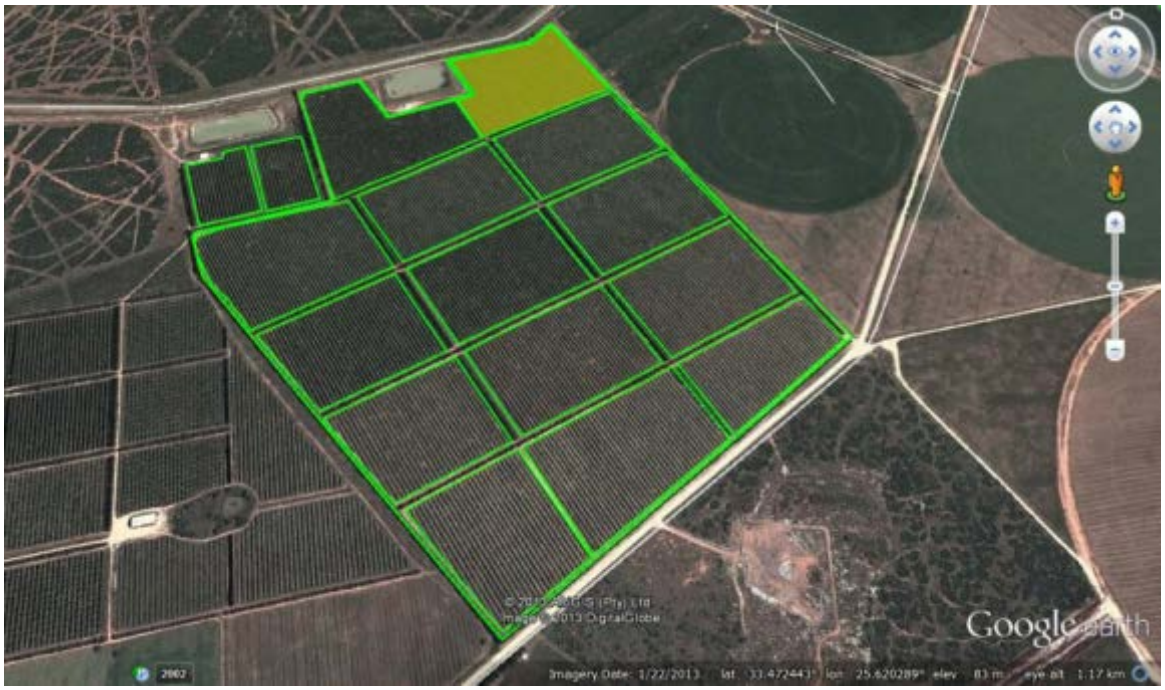

Figure 4.1 Bernol Farm, Addo, where aerial releases were conducted. Blocks with green boundaries form part of release area while yellow shaded (lemon orchard) block does not.

Figure 4.2 Penhill Estates, Addo, where ground releases were conducted. Blocks with green boundaries form part of release area while yellow shaded (lemons) and green shaded (fallow land) blocks do not.

Microclimate and particularly night temperature, between 19:00 and 02:00, was considered a critical factor that influences activity and consequently distribution of sterile and wild FCM (Anonymous 2010). Electronic data loggers (HOBO UA001) were placed inside a tree in a centrally located orchard on both release sites to state variation in temperature.

4.2.2 Release methods

Moth boxes, containing an estimated 8000 irradiated male and female FCM (measured volumetrically in the production facility prior to irradiation) (estimated 1:1 male : female), that were designated for ground release were separated into smaller cooler boxes, holding 16 moth boxes each, and packed with Poly-ice® packs to maintain moths in an immobilized state until arrival at the release site. After arrival, a cooler box was opened to remove two moth boxes for acclimatisation before loading into the automated ground release system. Moth temperature was gauged with a normal glass-stem thermometer, to standardise the ideal level of moth activity for loading at approximately 15°C. Ground releases were done by one person on an ATV, equipped with an automated release system (Figure 4.3).

Figure 4.3 A specially equipped ATV used in the SIT programme for ground release of irradiated FCM in citrus orchards.

This system consists of a small plastic hopper, a steel auger for feeding moths that drop from the hopper and a fan that blows at a constant speed to shoot moths through a slightly angled release pipe into the trees. The flow rate was calibrated weekly to ensure the auger completes 36 revolutions per minute at a motor speed of 20 kilometres per hour (kph) for delivery of 1000 moths per ha. The ATV was driven through the first and last row of every block and after that every eighth row, releasing moths constantly from the first to the last tree. The variable speed drive enables release of 500, 1000, 1500 or 2000 moths per ha.

Aerial releases were conducted from a specially equipped gyroplane (Figure 4.4). The pilot was assisted by ground crew for loading and fastening of a large aluminium moth hopper. A full load consisted of approximately 1 million moths released at a pre-determined density over citrus orchards. The automated release system was tailored to fit on the rear seat of the gyroplane, feeding moths into a release pipe attached to the undercarriage of the aircraft and blown out by the natural flow of air directly into the environment.

Figure 4.4 Specially equipped gyroplanes used in an SIT programme for aerial release of irradiated FCM on citrus orchards.

Once airborne, the pilot controlled the moth release system by switching between on and off, at a set rate over designated citrus orchards. A camera attachment on the tail of the aircraft allowed the pilot to observe the release of moths on a small screen on the instrument panel. Flow rate was calibrated according to the ground speed determined by the GPS on the aircraft, programmed with an ideal speed of 120 kph, displayed as a row of lights indicating the ideal range of 110 to 130 kph. Moths were released in 100 m transects from 20 to 30 m above ground. A normal handheld GPS (Garmin Montana 600 ®) was uploaded with a flight plan for navigation to the release orchards.

4.2.3 Monitoring moth populations

Delta FCM traps (Chempac, South Africa) loaded with Lorelei sex pheromone dispensers were positioned in a uniform grid, approximately 4-6 ha apart, at both trial sites (Moore 2011a). Traps were placed on the southern side of the orchard, in the fifth row from the perimeter and on the eastern side of the tree in order for the prevailing south-easterly wind to facilitate pheromone distribution through the orchard (Hofmeyr 2003). Traps were suspended at two metres from the ground, in partial shade in the outer foliage of the tree. Twigs and leaves around traps were removed to ensure moths had free and unhindered access. Weekly trap catches were assessed by removing the sticky floor from the trap and counting the number of sterile and wild male moths. Sticky floors were replaced at irregular intervals, approximately every 2 to three weeks differing from orchard to orchard and month to month, when the sticky substance was considered dry or insufficient to trap moths. A sterile male moth was identified by the lack of dorsal scales (due to handling and packaging) or if the moth was lying on its back on the adhesive agent. If any uncertainty remained, a moth was squashed and identified by the colour of its intestines.

4.2.3 Statistical analyses

General linear models and post-hoc Tukey tests, where appropriate, were used to compare recaptures of irradiated male FCM after aerial and ground release methods. Data were analysed using Statistica version 11 (StatSoft, Inc. 1984-2012). The effects of release farm, release method, release rate, release date, and temperature on the number of recaptured released male FCM cause data in this study to be confounded to a degree. Limitation of experimental design could affect statistical analysis and possibly the outcome of this study.

4.4 Results

4.4.1 Release methods

Although initially more sterile moths were recorded from traps in aerial release compared to ground release orchards, recaptures were very low during spring at both trial sites. Sterile male activity in ground release orchards increased in subsequent weeks up to 9 December 2012, compared to the decreased activity in aerial release orchards (Figure 4.5, Appendix 1). A notable increase in trap catches from both release sites was recorded after 10 December 2012, reaching an overall peak between 17 and 23 December. Although numbers subsided once again during the following weeks, recaptures in ground release orchards increased steadily up to 24 February 2013 compared to the erratic trend of moth activity in aerial release orchards. In the two weeks preceding the end of the trial slightly more sterile moths were trapped in ground release orchards, while the last recording on March 25 2013 was exactly the opposite.

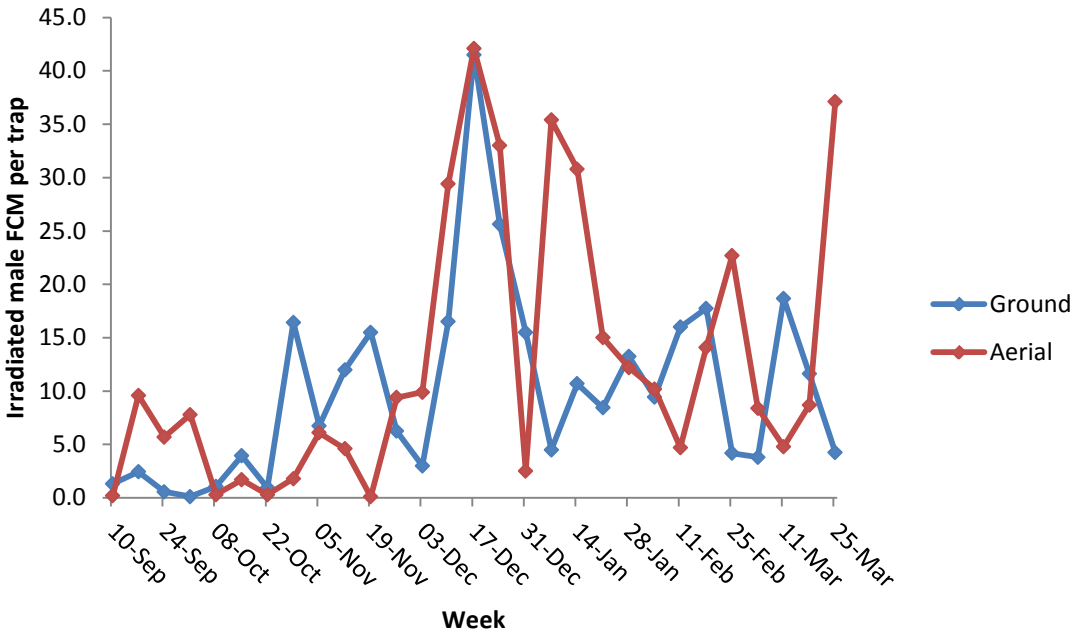

Figure 4.5 Mean weekly recaptures of irradiated male FCM per trap from orchards where releases were conducted by ground and aerial methods from 10 September 2012 to 25 March 2013. Standard Error (SE) bars have been omitted for clarity (Appendix 1).

Over the 29 week period from week 37 in September 2012 to week 13 in 2013, there was no significant difference in mean recaptures of irradiated male FCM in orchards released from the ground compared to orchards where aerial releases occurred ($F = 0.4370$; $P = 0.508798$). However, due to a shortage in supply of sterile FCM and the commercial nature of the trial, variation in the

rate of release was inevitable. Consequently, when the mean rate of release (1509 sterile FCM per ha was incorporated as a covariate, the outcome was significantly in favour of the ground release method (F = 12.021; P = 0.0056) (Figure 4.6, Appendix 1). Additionally, when mean minimum night temperature (16.65°C) was incorporated into the evaluation of release methods as a covariate, recaptures in ground release orchards were significantly better than those in aerial release orchards (F = 20.238; P = 0.00001).

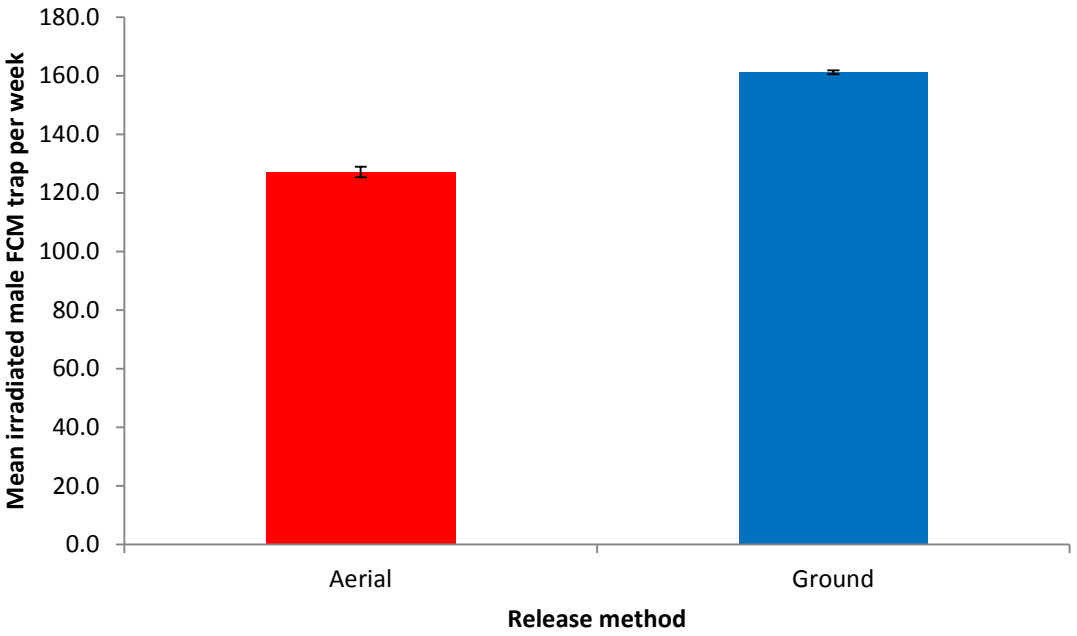

Figure 4.6 Mean irradiated male FCM per trap, with significantly higher recaptures from orchards where releases were conducted by ground compared to aerial method (F = 12.021; P = 0.0056).

The number of irradiated male FCM trapped for various release rates at both trial sites were considered, and was found to have a significant effect on recaptures of sterile male FCM, regardless of the mode of release (F = 10.587; P < 0.05) (Figure 4.7). After aerial release of 1500 irradiated FCM per ha on 24 September and zero irradiated FCM per ha on 10 September, 1 and 22 October and 12 November, no significant difference in recaptures was found. This is probably an experimental artefact due to the proximity of commercial SIT orchards to the ground release site. Non-irradiated (lab-reared) male FCM have been trapped up to 1.5 km from the point of release (Stotter 2009), thus assuming irradiation and transport did not affect its ability to disperse. It could be concluded that sterile moths flew from the adjacent farm, where commercial releases took place during the week where a release rate of 0 was assigned (Table 4.1), into traps on the trial site. In effect, this also means that the adjacent farm with commercial releases could have influenced the number of irradiated male FCM captured in the aerial release farm for any and every capture date. Importantly,

no significant difference in recaptures of male FCM existed after release of 1000, 1500 or 2000 irradiated male and female moths. However, the relationship of release volume to number of recaptures was shown due to a significant increase in sterile male FCM trapped at a release rate of 3000 moths per ha, for the ground release method.

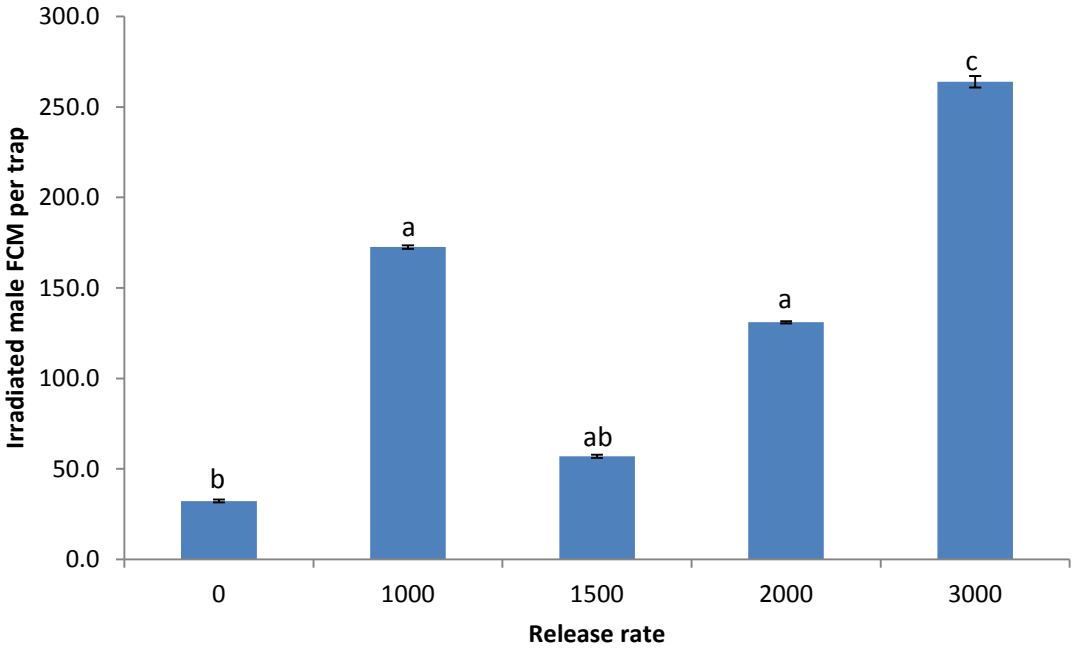

Figure 4.7 Mean irradiated male FCM per trap from orchards where releases were conducted at different release rates, regardless of mode of release. Means not followed by the same letter differ significantly ($P < 0.05$).

The effect of night temperature (19:00 and 02:00) on activity of irradiated male FCM released over citrus orchards was evaluated. Linear regression of mean minimum night temperature and the amount of irradiated male FCM trapped showed a positive correlation ($R^2 = 0.4677$) (Figure 4.8) regardless of release method. The data showed mean minimum night temperature during the 29 week period never dropped below 10°C or rose above 20°C. The lowest number of moths was trapped at a mean of 12.5°C while the highest number was trapped at a mean of 18.1°C. A large percentage of total recaptures (72%) was trapped at a minimum night temperature above 16°C from week 49, December 2012, to week 13 in March 2013.

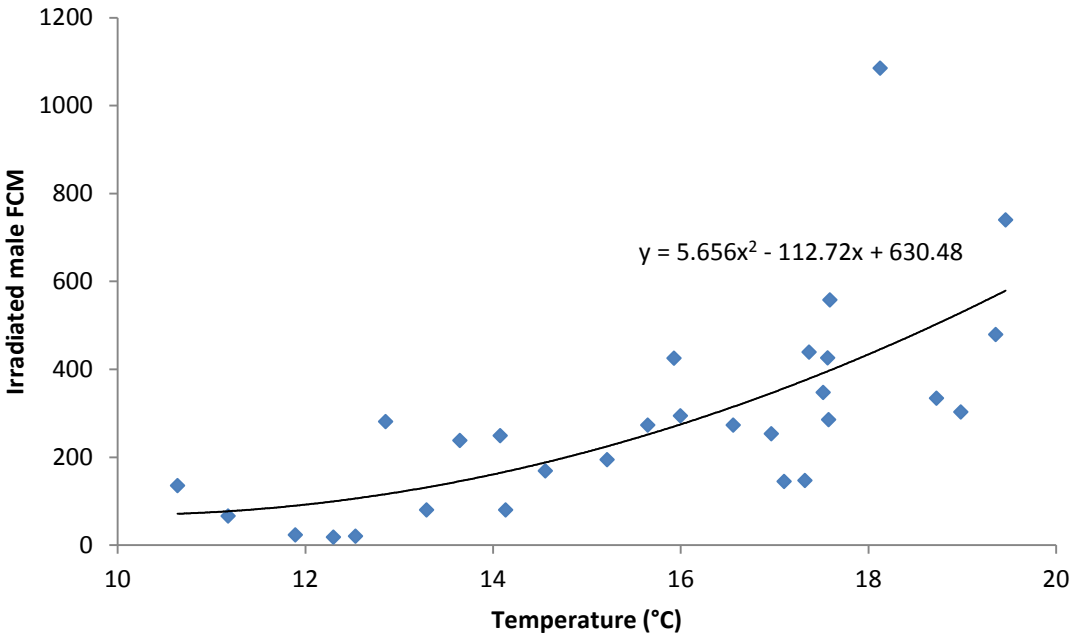

Figure 4.8 Total amount of irradiated male FCM per trap from orchards where releases were conducted by ground and aerial methods combined at mean minimum night temperatures (19:00 to 02:00).

4.3 Discussion

Most area-wide scale pest population management programmes including an SIT component utilise aircraft for release of sterile insects directly into the environment (Dowell *et al.* 2005, Tan & Tan 2011). However, results of this study show ground release to be more effective than aerial release measured by recovery of irradiated FCM. Aerial release is faster, more cost-effective (see below) and provides a uniform distribution of sterile insects over a target area and is consequently preferred to ground releases over a certain operational scale (Bjeliš *et al.* 2013, Tan & Tan 2013, Tween & Rendon 2007).

Comparison of release methods can be done in terms of fixed and operational cost requirements for an SIT operation. Aircraft used in this study fall in the microlight class and cost on average ZAR 500 000 per aircraft compared to an ATV at ZAR 50 000. Advantages of a gyroplane used for release of sterile FCM are its low cost and relatively low technical requirement. These aircraft function on the principle of autorotation, relying on wind from forward motion to create lift and a propeller engine for forward propulsion. However, consistency is integral to the success of an SIT programme and relies on a comprehensive service schedule to sustain its usefulness over an entire season. A gyroplane can release at a rate of 350 ha/h on average while an ATV can only manage 10 ha/h.

Operating costs include fuel consumption, labour requirements, general maintenance and insurance. Gyroplanes use approximately 20 litres of fuel per hour, which is reasonably low compared to other aircraft (Tan & Tan 2013), although relatively high compared to an ATV using only one litre per hour. Cost of labour for an SIT operation using gyroplanes is low, requiring only one pilot and one ground crew during take-off and landing. SIT operations using ATVs are more labour intensive, due to the number of vehicles needed to cover the same area. General maintenance of an ATV is considerably less compared to an aircraft as a result of regular inspections required to maintain the level of safety, although the low level of regulation and mandatory inspections as well as affordable hardware means a gyroplane is less costly to maintain compared to fixed-wing aircraft (Tan & Tan 2013). Total operational cost for a single release of irradiated FCM amounts to ZAR 2.72 per ha for release by gyroplane, compared to ZAR 4.68 per ha for an ATV. Ultimately, release rate is the deciding factor, where large contiguous areas favour the high productivity of the aerial release method.

The reliability of equipment for release of sterile FCM males and females directly into the environment was considered satisfactory. The automated systems developed for ground and aerial release methods did not cause significant mortality or adversely affect male response to pheromone baited traps, provided that sterile moths were loaded in an inactive state to prevent excessive scale accumulation, causing blockage at release rates exceeding 2000 moths per ha in the auger part of the release system. The problem was overcome by improved handling procedures and temperature control prior to loading. Examination of recaptures of irradiated, mass-reared moths in the Sundays River Valley SIT programme has improved the understanding of moth dispersal after release. Additionally, the only influential difference in insecticidal applications between the two release sites was the inclusion of a pyrethroid (tau-fluvalinate) and an organophosphate (profenofos) at the aerial release site (not used at the ground release site). Applications of both these chemicals were limited to early in the season, 23 to 29 September 2012 (profenofos) and 7 to 13 October (tau-fluvalinate), in order to minimise the non-target effect on beneficial organisms. Results from Chapter 3 show these products would have had no effect on recaptures of irradiated male FCM beyond the first week after application and consequently results from this study could be considered valid and reliable.

Various factors that impact the distribution of released moths interact across orchards and seasons in a complex operational programme (Judd & Gardiner 2006). Irradiation significantly reduced recaptures of wild and mass-reared codling moth, *Cydia pomonella* in field experiments, most likely by reducing general flight ability and dispersal as an indirect result of decreased pheromone

response (Judd & Gardiner 2006). The varied impact of irradiation on sterile male codling moth activity was also explained by lower quality of mass reared moths compared to wild moths (Judd *et al.* 2006a). Judd & Gardiner (2006) showed temperature had a significant effect on response of male codling moth to a pheromone lure, although mass-rearing had no effect on temperature thresholds for pheromone-mediated flight of wild (15.4°C) and mass-reared (14.7°C) male codling moth. Although no literature on minimum temperature threshold for flight activity of FCM has been published, Stotter & Terblanche (2009) accepted it to be in the region of 10-15°C, and speculated that it could be slightly higher for mass-reared moths due to temperature dependence of locomotor performance affected in the rearing process. In addition, moth temperature during loading and release, as well as ground temperature upon landing, will affect the moth's ability to escape predation, which could considerably reduce amount of recaptures (Judd & Gardiner 2006). Consequently, even though better distribution is achieved when chilled insects are released from an aerial compared to a ground release system (Bjeliš *et al.* 2013, Tan & Tan 2013), more insects will land on the ground in between rows, possibly increasing the percentage of released sterile moths subjected to predation (Judd & Gardiner 2006). Unpublished data from X Sterile Insect Technique (Pty) Ltd. (XSIT) shows a large portion of irradiated FCM released aerially will land on the ground between trees, only after which the moths will fly into the trees. This is particularly true during months where lower ambient temperature will sustain immobility of the irradiated FCM for an extended period.

Releases were postponed for urgent repair of aircraft on two occasions during the course of the trial, compromising quality of sterile insects as they were exposed to warm conditions only to be returned to the cold room for release the following day. Severe weather conditions in the Sundays River Valley from 7 to 28 October, 2013, also disrupted the release operation, reducing recaptures of sterile FCM in spring 2012. The effect of inclement weather conditions was far more exaggerated for aerial release compared to ground release operations. The ATVs were quick to respond in short patches of clear weather and managed to release their entire consignment of sterile moths while aerial releases failed to achieve this. Even though ground crew were permanently on standby, poor visibility and time constraints such as loading of release equipment and pre-flight preparation prevented the gyroplane from leaving the airfield with short notice. Flight conditions are important for a sterile insect release vehicle, particularly for aerial methods as insects will only be released when conditions are optimal for their survival and performance (Tan & Tan 2013), although McMechan & Proverbs (1972) showed wind speed during release does not affect released moth quality, although it could affect where they land.

In conclusion, both release methods have merit under specific conditions beyond a certain operational scale. Ground release from an ATV should be the chosen method in an SIT programme where the release area is small, accessible and contiguous. By shortening the transport distance between release areas, productivity and insect quality is increased (Chapter 2) while operational cost is lowered. Design of a holding container with a large capacity and temperature regulating technology for release of adult insects at the ideal temperature will minimise reloading time, while simultaneously optimising insect quality and reducing probability of predation. Vehicle routes can be easily adapted to meet fluctuating demands and where higher numbers of sterile insects are needed additional release trips can be made. Logistical challenges could be overcome by placing tracking units on the ATVs in order to pre-determine release routes for improved planning and record tracks for quality control and feedback. During periods of inclement weather, insects can be held at low temperature until favourable conditions allow releases to continue.

Aerial release should be the preferred method for release of sterile insects in an SIT programme over a large area with varied terrain, dense vegetation or a lack of roads. Installation of variable rate technology in aircraft will enable flexibility in the rate of release so that more insects can be delivered to those areas needing them while avoiding multiple flights over any given area. This technology coupled with a GPS aircraft guidance system might enable release of two or more different species of sterile insects at the same time, further optimising cost effectiveness. The high cost of an aircraft is economically justified for use in an SIT programme due to its ability to carry a large amount of sterile insects, thus covering a large area in a relatively short period. Severe weather conditions will delay the release operation and possibly affect the quality of insects to some degree. However, only one pilot and one skilled individual as ground crew is needed, making pro-active planning and quick reaction to favourable conditions possible. A uniform distribution over the target area is highly desirable, although the high percentage of sterile insects landing on the ground between rows of trees is not. Releasing sterile insects at ambient temperature could shorten their reaction time, thereby avoiding predation and consequently increasing the number of recaptures. A similar holding container with temperature regulating abilities is needed to optimise efficacy and increase productivity consequently making aerial releases less expensive and more effective than ground releases.

Appendix 1. Recaptures of irradiated male FCM (mean ± SE) per trap per week in orchards released by ground and aerial methods from 10 September 2012 to 25 March 2013.

	Number of Irradiated male FCM	
Date	Air	Ground
10-Sep	0.20 ± 0.20	1.31 ± 0.45
17-Sep	9.60 ± 2.46	2.43 ± 0.96
24-Sep	5.70 ± 0.88	0.56 ± 0.18
01-Oct	7.80 ± 1.56	0.12 ± 0.09
08-Oct	0.30 ± 0.15	1.06 ± 0.37
15-Oct	1.70 ± 0.47	3.93 ± 1.01
22-Oct	0.30 ± 0.21	0.93 ± 0.28
29-Oct	1.80 ± 0.68	16.43 ± 4.07
05-Nov	6.10 ± 1.15	6.75 ± 1.89
12-Nov	4.60 ± 1.51	12.00 ± 3.25
19-Nov	0.10 ± 0.10	15.50 ± 3.88
26-Nov	9.40 ± 2.78	6.25 ± 1.34
03-Dec	9.90 ± 1.85	3.00 ± 0.88
10-Dec	29.40 ± 5.67	16.50 ± 2.53
17-Dec	42.10 ± 8.39	41.50 ± 9.17
24-Dec	33.00 ± 7.39	25.62 ± 4.38
31-Dec	2.50 ± 0.50	15.50 ± 1.41
07-Jan	35.40 ± 6.77	4.50 ± 1.45
14-Jan	30.80 ± 7.79	10.68 ± 1.86
21-Jan	15.00 ± 3.57	8.43 ± 2.51
28-Jan	12.20 ± 3.68	13.25 ± 1.83
04-Feb	10.20 ± 3.15	9.43 ± 1.93
11-Feb	4.70 ± 1.73	16.00 ± 4.60
18-Feb	14.10 ± 3.24	17.75 ± 4.12
25-Feb	22.70 ± 5.69	4.18 ± 0.68
04-Mar	8.40 ± 1.80	3.81 ± 0.92
11-Mar	4.80 ± 0.90	18.68 ± 4.82
18-Mar	8.70 ± 1.92	11.62 ± 2.44
25-Mar	37.10 ± 6.55	4.25 ± 0.72

5

GENERAL DISCUSSION

5.1 Introduction

The goal of this study was to provide insight into application technology of the Sterile Insect Technique (SIT) with focus on false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), a key pest of citrus in South Africa. Introduction of an SIT programme in the Sunday's River Valley (SRV), Eastern Cape province, for suppression of FCM created an opportunity to investigate major questions such as the effect of long distance transportation on the fitness of irradiated FCM, the effect of broad spectrum insecticides and their role in an area-wide integrated pest management programme (AW-IPM) utilising SIT and the efficacy of two modes of release for application of SIT.

5.2 The importance of application technology in an SIT programme

A major challenge in the immediate future of commercial agriculture is to consolidate achievements in international trade of agricultural commodities and to reduce the number of people living in poverty and hunger while expanding environmentally-friendly practices such as SIT. Despite many successes achieved globally (Robinson & Hendrichs 2005, Vreysen *et al.* 2006, Hendrichs 2000), SIT may be a technology that is beyond plant protection infrastructures of less developed countries. Failure of SIT programmes is not due to the science but rather the lack of appropriate implementation of systemic large scale operations. Increased expertise in the science of application technology will lead to innovative development of such programmes and consequent growth of modern agriculture.

Strategies for commercialisation of an SIT programme can vary extensively. Options of separate processes that involve rearing, shipping, emerging and releasing of sterile insects could be the responsibility of a particular company or one or more of these processes could be outsourced to service providers in the industry. In addition, customers could be charged according to the area treated or the number of insects released, or else funded partly or entirely by the industry in question. For expansion of an SIT programme to remote areas, consideration should be given to

increasing the size of a centralized rearing facility that supplies sterile insects to satellite release programmes in geographically distinct areas. Due to the reduction in cost per insect produced with the increase in amount of insects produced at a mass-rearing facility, this remains the most cost effective strategy for expansion of an SIT programme (Dowell *et al.* 2005), particularly if this is an agricultural area growing mainly one crop (Robinson & Hendrichs 2005). Due to the sensitivity of irradiated insects and the rigorous quality parameters set for each procedure, definite advantages exist for a programme with exclusive application rights. Where application of each process is controlled by a central quality management outfit, different procedures could be linked to form a highly effective pest control programme, regardless of its strategy.

Robinson & Hendrichs (2005) showed application of SIT in an AW-IPM approach is more management intensive than traditional pest control tactics, requiring a large amount of model data to assess feasibility before introduction of the programme, while careful planning and stakeholder involvement ensures effective implementation. Hendrichs *et al.* (2005) stated that idealistic marketing of naïve goals create unrealistic expectations that are often unfulfilled and could be the cause of failure of SIT programmes. The greatest flaw in the strategy of failed programmes is overambitious attempts to eradicate a target insect with a lack of realistic planning, sufficient funding, adequate regulatory support or the lack of quarantine measures to protect an area from reinvasion, in order to achieve the objective (Myers *et al.* 1998). The option to change the strategy during implementation from eradication to suppression or containment remains less successful than setting the initial goal for suppression of the target pest, and eventually upgrading to eradication (Hendrichs *et al.*2005). Myers *et al.* (2000) explained that pest status, target markets as well as species biology, ecology and distribution must be considered in the evaluation of a specific programme on its potential to be successful. Given the fact that application procedures are logistically complex and management intensive, flexibility in the system that controls the organization is required thus giving individuals authority to take the necessary actions. In addition, research and development should be conducted independently to operations management, by separate individuals and preferably institutions. Due to a different skillset required, problem-solving and improvement of the technology could be outsourced to maintain operational stability, predictability and innovation (Hendrichs *et al.* 2005). Despite cost being an operational limitation, evolution of technology should be the leading principal if an SIT programme is likely to succeed in the long-term. Consequently, it remains of critical importance to obtain the support of experts to give a programme technical credibility in addition to constant research enabling improvement of technology. Failed programmes often showed lack of dynamic structures and dedicated leadership to respond to changing situations (Myers *et al.* 2000, Hendrichs *et al.* 2005).

5.3 Long distance transportation of irradiated insects for SIT

Robinson & Hendrichs (2005) showed that increases in the movement of agricultural products and changes in climatic conditions will inevitably lead to increased invasion and possibly establishment of exotic pests in new areas. Consequently, increased support will be seen for the use of SIT to prevent this from happening. When New World screwworm *Cochliomyia hominovorax* (Coquerel) (Diptera: Calliphoridae) was accidentally introduced into Libya in the early 1990s, this problem was addressed by application of SIT, fortunately available by importation of sterile pupae from Mexico. Successful eradication of this alien invasive was credited to systems that were already in place for monitoring, rearing and release as well as procedures that were implemented rather quickly upon detection of the pest (Carpenter *et al.* 2007, Lindquist *et al.* 1992). A highly effective preventative approach was followed in California where Mediterranean fruit fly (medfly), *Cerratitis capitata* (Wiedemann) (Diptera: Tephritidae), was eradicated in the Los Angeles Basin. Regular introductions from neighbouring states caused recurring outbreaks in the area and sterile fruit flies were continuously released in areas of high risk resulting in excellent suppression (Carey 1996, Enkerlin 2005). In Australia, comprehensive contingency plans were developed to be able to respond in case of an accidental introduction of Old World screwworm Chrysomya bezziana (Villenueve) (Diptera: Calliphoridae) (Vargás-Teran *et al.* 2005). The importance of mitigating the risk of exotic pest introductions has created opportunities to integrate SIT in the process of creating areas of low pest prevalence under a systems approach, either in the country from which agricultural products can be safely exported, or in the case where a pest was detected abroad (Robinson & Hendrichs 2005). Countries or regions should focus on providing remedial action by deciding on the most important exotic pest and developing specific technology as well as the required expertise in advance (Suckling 2003). The best practice would be to have an SIT programme in place prior to detection of the pest so that it could be implemented at a very early stage of invasion, rather than to react once the pest is well established and wide spread. Because of the impact of long distance transport on quality of the released insects as well as the efficacy of SIT, comprehensive protocols for this critical step in the process needs to be developed for each pestiferous insect with phytosanitary status. In the case of medfly, insects can be transported in the late pupal stage, with some form of cooling or anoxia such as dry-ice pellets, to prevent adult emergence in transit (Vreyen *et al.* 2006). Robinson & Hendrichs (2005) determined, although this will eliminate the operational cost of rearing, it may have an adverse effect on insect quality, particularly if the transport period is prolonged. Alternatively, fertile eggs can be transported from an egg production facility to a larval rearing facility, with subsequent measures for sterilizing and releasing of adult insects. This significantly simplifies operational protocols at the receiving programme and extends the quality of released insects compared to

transport during the pupal or adults stages. The Moscamed programme in Guatemala and Mexico successfully ships heat treated eggs from a temperature sensitive genetic sexing strain of medfly on a daily basis to a rearing facility in Tapachula, Mexico (Robinson & Hendrichs 2005, Caceres 2002).

Application of cold immobilization for transport and release of adult FCM in the SRV appeared to have a significant adverse effect on longevity, realized fecundity and flight ability of irradiated FCM (Chapter 2).Very little is known about which factors and parameters of transport have an effect on quality of released moths. Blomefield *et al.* (2011) investigated the effect transport has on long distance transportation of codling moth, *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), from Canada to South Africa, and showed no significant adverse consequence. Initial trials by Hofmeyr & Hofmeyr (2010) showed relatively little adverse effect of aerial transport by regular commercial airlines on sterile FCM quality from the Western Cape to the Eastern Cape, South Africa, thus motivating expansion of the commercial operation. However, inability of airline staff to adhere handling protocols and hand the unreliable nature of third party couriers during initial commercial consignments delivered to the SRV had a detrimental effect on sterile FCM quality. The importance of using trained, dependable personnel for handling and transport of live adult insects, adhering to strict quality control protocols, should be emphasized. In addition to control of temperature and humidity during transport, time spent under hypoxia should be highly predictable in order to use optimal cooling. Subsequently, transport by road freight using a company owned utility vehicle as well as trained employees was initiated which improved this situation to a large extent. More importantly, the significant adverse effect of transportation and cold immobilisation on sterilised insects shown in Chapter 2 is very disconcerting and needs to be addressed before expansion of this SIT programme to other citrus producing areas in South Africa.

Quality control protocols for the application process as a whole needs to be further developed, and preferably standardised. Such protocols have already been designed for sterile Mediteranean fruit fly, where an internationally agreed set of standards is used to monitor the quality of sterile insects (Robinson & Hendrichs 2005). Eventually transboundary trade in sterile insects should ensure that these harmonised standards are implemented globally (Enkerlin 2005).

5.3 Integration of insecticides in an SIT programme

If an insecticide is applied simultaneously to sterile insect releases, the chemical may kill some of the sterile insects; however, providing the ratio of sterile to wild insects remains the same, this would not impair the efficacy of SIT (Mangan 2005). By treating key pests with conventional insecticides, natural and augmentative biological control will be disrupted (Robinson & Hendrichs 2005) and

because of this only limited potential exists for natural enemies to deal with secondary pests (Moore *et al.* 2004a). Application of synthetic organic insecticides is compatible with SIT (Carpenter & Young 1991) and in many cases is considered to be IPM-compatible and therefore minimally disruptive of natural enemies (with a few exceptions). Suppression by an insect growth regulator (IGR) has been considered to be a more acceptable option if suppression of the wild pest population is required (Carpenter *et al.* 2005), although Alsystin has been shown to have a harmful effect on the *Trichogrammatoidea cryptophlebiae* (Nagaraja) (Hymenoptera: Trichogrammatidae) population (Hattingh & Tate 1997) and is no longer recommend for use in an IPM programme on citrus in South Africa. Other IGRs such as pyriproxifen have a much more devastating and long lasting effect on natural enemies, particularly beetle predators, therefore contrary to popular belief IGRs may not be IPM-compatible at all (Hattingh 1996).

SIT is not a stand-alone control practice, often requiring supplemental systems to reduce the native pest population to the point where sterile insects have an advantageous numerical ratio in order to induce sterility (Mangan 2005). Knipling (1979) classified different methods for population suppression by their degree of selectivity as well as their effectiveness at various pest densities which is of particular relevance to SIT. He determined conventional chemical insecticides were non-selective and equally effective at all pest densities, while highly selective biological methods such as pathogens, parasites and predators were most effective at high pest densities. Studies of SIT combined with simultaneous or sequential releases of natural enemies showed enormous potential for suppression of an insect population on the condition that these organisms could develop normally on the F1 sterile progeny (Carpenter *et al.* 2005). Consequently, SIT will be complemented by pest-specific biological tools used for control of key pests and will likely advance commercialisation of the technology (Robinson & Hendrichs). The egg parisitoid *T. cryptophlebiae* is a very effective natural suppressant of FCM eggs (Moore 2011a) and would be a positive addition to the efficacy of an AW-IPM programme for suppression of the pest (Carpenter *et al.* 2004). Integration of SIT with augmentative biological control is an extremely important opportunity that is often unexploited. In addition, a microbial insecticide, *Cryptophlebia leucotreta* granulovirus, forms an integral part of the AW-IPM programme in the SRV, whereby two mandatory applications, usually six weeks apart from December to January, are implemented to control the larval stage after flight peaks is observed.

5.4 Efficacy of different release methods for application of SIT

The area-wide approach to pest management requires uniform distribution of adult insects over a large area, usually by aircraft (Robinson & Hendrichs 2005). While some small programmes are able to use ground release methods, aerial release methods have been shown to be more cost-effective and enable a more uniform distribution of sterile insects above a specific operational scale, partly due to the altitude from which insects are released but also as a result of release paths not being dictated by existing roads (Tan & Tan 2011). Consequently, the accepted practice for release of sterile Mediteranean fruit fly is either by dropping paper bags within which adult insects emerge, opened upon release, or from a chilled container via an auger on an aircraft (Tan & Tan 2011, Vreysen *et al.* 2006). The advantage of using paper bags is low set-up costs although deficiencies such as less uniform distribution and higher predation rate of various predators on sterile medfly (Hendricjhs *et al.* 2007), especially the yellow jacket wasp *Vespula germanica* (Hymenoptera: Vespidae), outweigh the benefits of cost. The chilled adult release method eliminates the need to hold and release big biodegradable containers, thus optimising payload by reducing weight and flying time. For this process to be effective, adult insects must be collected after emergence from pupae and placed in a chilled container. Major concerns about the negative impact from stress of low temperature and high insect density on quality of sterilised insects inside the release machine remain (Tan & Tan 2011, Robinson & Hendrich 2005). To maintain insect quality at a high level is challenging, although the technology for holding insects immobilised for extended periods have improved greatly. Sophisticated technology utilising less-complicated mechanical components such as phase-change materials or frozen carbon dioxide can be used as a replacement for conventional cooling, with the additional benefit of being more reliable and cost effective (Tween & Rendon 2007), and as a result should be used in any alternative vehicle for the release of sterile insects. This should also simplify maintenance, reduce the load on aircraft battery power and enable greater control over temperature and humidity thereby optimizing quality and competitiveness of the insects (Robinson & Hendrichs 2005). Another indication of the future is computer software linked to a satellite guided aerial navigation system that can be programmed to release insects at variable rates over areas as needed and turning off when flying outside release blocks (Briasco *et al.* 2011). Better quality control of pilot, aircraft and machine can be achieved by monitoring performance and analysing post-release data (Dowell *et al.* 2005).

Although much research has been done in developing mass-rearing and sterilisation technologies for SIT, the application process is still lacking in cost efficiency. Cost saving that can be made in this area should have a positive impact on the total cost of an SIT programme. Modern aircraft are over-

specified in terms of capabilities needed for SIT programmes, while fuel consumption and take-off weight greatly reduces the payload. Aircraft such as gyroplanes used in a medium scale SIT programme for sterile FCM release in the SRV are better suited for the job, with a lower fixed cost and reduced capacity in relation to the insect's payload and the pilot's salary. Attempts to further increase cost effectiveness and reliability are made by contracting the aircraft, while operational costs consisting of fuel, pilot and ground crew salaries, hanger rental, runway charges and maintenance costs are not part of the agreement. Aircraft currently used in large SIT programmes for aerial release of sterile medfly are either fixed-wing versions such as the TurboLet-410 I Guatamala or the Cessna 401 and 402-twin engine in Mexico (Moscamed), or sometimes helicopters for their better manoeuvrability in arduous terrain (Tan & Tan 2011, Vreysen *et al.* 2006). Additional options such as custom-built unmanned vehicles or long-range radio-controlled aircraft for should be developed for major cost savings making aerial releases an even more attractive option in small-scale operations.

5.4 The way forward for SIT in South Africa

Efficacy of an SIT programme for control of any insect pest is affected by a combination of factors that are biological, financial, social or political in nature. Unpublished data from XSIT demonstrated that SIT is an effective means of FCM control in citrus producing areas of South Africa, confirmed by increased yield and export margins six years after inception of the programme in the Citrusdal region of the Western Cape Province. Promising results of overall population suppression three years after expansion to the Sundays River Valley of the Eastern Cape has motivated further development within South Africa. The success of this and other SIT programmes, both locally and abroad, can be attributed to fundamentally sound analysis of baseline data to determine programme feasibility, in combination with good management, adequate funding and political support subsequent to implementation. Although problems with application of the technology could delay the successful achievement of its objectives, this should not be the cause of programme failure. Technical constraints investigated in this study could help to establish realistic expectations while identifying shortfalls for application of SIT on FCM and other insect pests within southern Africa. Outcomes of the transportation study emphasized the importance of further research in methods of insect immobilization and actual modes of sterile insect transport due to the significant adverse effect this had on moth quality. The negative effect could be limited by reducing the duration of cold treatment while aiming to stabilise temperature fluctuation during transport of moths. Consideration should also be given to construction of a central irradiation facility, enabling transport of pupae for application of SIT in areas that are geographically distinct. SIT can be integrated in any pest control

programme within South Africa, while production areas that follow IPM guidelines should have an advantage due to abundant populations of natural enemies in the area. Chemical programmes can be modified for enhanced compatibility with SIT, reducing both total costs and the need for excessive insecticide application. In addition, larvacides should be more useful than adulticides for suppression of the target pest in an SIT programme, while products with high species specificity should further improve compatibility, consequently facilitating a particular programme to achieve its objectives. Further research for complimentary methods of suppression such as augmentative release of parasites and predators for control of a specific life-stage of the pest should be conducted in addition to integration of insecticidal control methods, especially at high pest densities. Efficacy of both aerial and ground release methods was proven in this study, providing opportunities for implementation of either method in areas where the geographical layout supports the technology. Although effective distribution and quality of released insects should remain priority, cost effectiveness should be considered for sustainability of a programme with long term goals. Continuous optimization of the release process is necessary for effective application of SIT. Research for improvement of satellite navigation systems and development of variable rate technology for aerial release of sterile FCM over a target area should be conducted for increased efficacy.

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