Determining the quality of mass reared male codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), by assessing flight performance under laboratory, semi-field and field conditions.

Andri Visser

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Supervisor: Matthew Addison
Co-supervisor: Pia Addison

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Abstract

The codling moth, *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), is the insect pest that poses the greatest threat to the South African pome fruit industry. Traditionally, insecticides have been used to control this pest, but recently these chemicals have failed to adequately suppress codling moth due to the build-up of resistance in populations. Along with the added pressure from export markets to reduce insecticide residues, the need for feasible control alternatives has never been greater.

The Sterile Insect Technique (SIT) is an environmentally friendly control method that has had great success with the control of a number of different insect pests, including codling moth. The technique involves the mass production and release of sterile insects with the goal of over-flooding the natural population, but it is also crucial that the mass reared insects can successfully compete for mating opportunities. However, no comprehensive quality control assessment method currently exists for mass reared codling moth.

This study aimed to identify and develop laboratory, semi-field and field methods that can be used to assess the quality of mass reared codling moth. This would be accomplished by developing flight performance assessments that could be used to distinguish between mass reared moths of high and low quality.

The experiments conducted for each assessment method compared the flight performance of moths subjected to different treatments of radiation dose, ageing and handling during transport. For the laboratory quality control assessment, a matrix of nine flight cylinders with combinations of three heights (2.5, 15 and 26.5 cm) and three diameters (10, 14 and 16 cm) was developed which compared the escape rate from these cylinders of moths subjected to different treatments. The semi-field assessment (conducted in a horticultural tunnel) measured the arrival time of the moths subjected to different treatments at a trap 12 m from the point of release. Field release/recapture assessments were conducted at two apple orchards in Elgin (one covered with netting and one uncovered), as well as an uncovered pear orchard in Stellenbosch. The flight performance of mass reared moths was determined by evaluating their dispersal rate from a central point of release with a trapping grid of eight pheromone baited delta traps. The efficacy of the three assessment methods was also evaluated for mass reared false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), in separate experiments.

Results indicated that increasing radiation dose had a detrimental effect on moth quality, but no effect could be observed for the age treatments. The effect of bran handling on moth quality could be observed in the field only, suggesting that it is dependent on the time period the moths are transported in the bran. The flight cylinder method was able to accurately indicate of the quality of moths as it relates to flight performance, but that the field assessments were the best indication the overall competitiveness in the field. This was true for both codling moth and false codling moth. The semi-field assessment method was not successful at distinguishing between moths of different quality; the development of an effective semi-field quality test is therefore still on-going.

Opsomming

Die kodlingmot, *Cydia pomonella* (Linaeus) (Lepidoptera: Tortricidae), is die insekpes wat die grootste bedreiging inhou vir die Suid-Afrikaanse kernvrugbedryf. Dié pes is tradisioneel met behulp van insekdoders beheer, maar die toename in weerstandbiedendheid van die kodlingmotpopulasies het veroorsaak dat die chemikalieë hul doeltreffendheid verloor. Tesame met die druk van die uitvoermark om die residue van landbouchemikalieë op produkte te verminder beteken dit dat die behoefte aan n volhoubare beheer metode nog nooit groter was nie.

Die Steriele Insek Tegniek (SIT) is ‘n omgewingsvriendelike beheer strategie wat in die verlede suksesvol geïmplementeer is teen ‘n verskeidenheid van insekpeste, onder andere ook kodlingmot. Die tegniek behels die massaproduksie en –vrylating van steriele insekte met die doel om die wilde populasie se getalle te oortref, maar dit is van kardinale belang dat die steriele motte ook suksesvol kan kompeteer vir paringsgeleenthede. Ten spyte hiervan bestaan daar tans geen omvattende kwaliteitsbeheertoetse vir die kodlingmot nie.

Dit was die doel van die studie om verskillende kwaliteitsbeheertoetse te ontwikkel vir gebruik in die laboratorium, vir semi-veld omstandighede asook vir gebruik in die boord self. Hierdie doelwit sou bereik word deur toetse te ontwikkel wat die kwaliteit van die motte bepaal op grond van hul vlugvermoë.

Die eksperimente het die vlugvermoë van motte vergelyk wat aan verskillende behandelings van bestraling, veroudering en hantering tydens vervoer blootgestel is. Vir die laboartorium-kwaliteitstoets is ‘n vlugsilinder matriks saamgestel wat bestaan het uit nege silinders met kombinasies van drie hoogtes (2.5, 15 and 26.5 cm) en drie breedtes (10, 14 and 16 cm). Die semi-veld assesserings (wat plaasgevind het in ‘n hortologiese tonnel) het die aankomstyd van die verskeie behandelings by ‘n lokval wat 12 m vanaf die vrylatingspunt geplaas is, bepaal. Die vrylaat/terugvang metode is toegepas in ‘n bedekte en ‘n onbedekte appelboord in die Elgin-omgewing, asook in ‘n onbedekte peerboord in Stellenbosch. Die vlugvermoë van die motte is bepaal deur hul verspreidingstempo in ‘n lokvalrooster met agt feromoon-bevattende lokavalle te meet. Die effektiwiteit van die assesseringsmetodes vir die vals kodlingmot (*Thaumatotibia leucotreta*) is ook bepaal in aparte eksperimente.

Die resultate het aangedui dat bestraling mot kwaliteit nadelig beïnvloed, maar dat ouderdom geen effek op die vliegvermoë van motte gehad het nie. Daar kon slegs in die boord ‘n nadelige invloed opgemerk word wanneer motte in hawer vervoer is (en nie in die laboratorium toetse ook nie), wat daarop dui dat die mate van kwaliteit degradasie afhanklik is van die tyd en afstand wat motte in die hawer vervoer word. Die vlugsilindertoets kon die kwaliteit van die motte akkuraat bepaal deur dit te meet aan vlugvermoë, maar die kwaliteitstoetse in die boord het die beste aanduiding gelewer van die algehele kompeteringsvermoë van die massaproduksie-motte. Die semi-veld assesseringsmetode kon nie onderskeid tref tussen hoë- en lae kwaliteit motte nie; dus is die ontwikkeling van ‘n effektiewe semi-veld metode nog nie voltooi nie.

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CHAPTER 1 : RATIONALE AND LITERATURE STUDY

Problem statement

The South African pome fruit industry is well established and generates considerable income from trade in foreign and local markets. The pest of greatest economic importance for the local pome fruit industry is the codling moth (*Cydia pomonella*). The use of insecticides has been the traditional approach to codling moth control, but chemical control has failed to adequately suppress this pest in recent years. A feasible control alternative is the use of the sterile insect technique (SIT) in an area-wide integrated pest management (AW-IPM) programme. The success of SIT depends on the production of high quality sterile insects. However, there is currently no comprehensive quality control (QC) assessment method for mass-reared codling moth.

Literature review

Pome fruit industry in South Africa

Introduction to the deciduous fruit industry

With a turnover of more than R10 billion per annum, and representing 12% of the total income generated by South African agricultural exports, the deciduous fruit industry is well established in South Africa (DAFF 2013; HortGro 2014). It is estimated that the total area under deciduous fruit production is 77 805 ha, located mostly in the Western and Northern Cape provinces, and includes apple, pear, table grape, peach, nectarine, apricot and plum orchards (DAFF 2014a; HortGro 2014). Pome fruit (apples and pears) form a significant part of the deciduous fruit industry, contributing 33% and 16%, respectively of the total gross value in 2010/2011, a total value of R4.6 billion (DAFF 2012a, 2012b). The bulk of the pome fruit harvest is produced in the areas of Groenland, Ceres, Villiersdorp and Tulbagh in the Western Cape and the Langkloof area in the Eastern Cape (DAFF 2012a, 2012b; HortGro 2014).

Value of pome fruit as export commodity

Pome fruit are among the most consumed fruit types in the world. From 2008 to 2013, global pome production increased by 12.6% to peak at 7.5 billion metric tonnes in the 2012/2013 season (DAFF 2014b). With nearly half of its pome harvest destined for the foreign market (mostly to other African nations and European countries during their winter months), it is clear that the South African pome fruit industry is export oriented. South Africa may not be able to compete with the biggest pome fruit producing regions such as China, European Union (EU) and United States of America (USA) (DAFF 2014b) in terms of hectares, but South Africa plays a significant part in global exports (DAFF 2012a, 2012b). South Africa is ranked seventh in global pome fruit exports, signifying 4.14% of apple and 6.18% of pear exports worldwide. Produce achieves higher unit prices in foreign markets compared with local markets, which means that it contributes a substantial amount of revenue towards the South African economy (DAFF 2013, 2014a, 2014b). According to the South African Customs Union, the value of pome fruit exports reached R3.9 billion for the 2011/2012 season (DAFF 2013).

Future of the pome fruit industry in South Africa

The steady increase in the production of pome fruit over time indicates that, should this trend continue, the pome fruit industry will cement its position as a significant industry in South African agriculture (DAFF 2012a, 2012b, 2014; HortGro 2014). The industry's key strength is its excellent reputation for delivering high quality produce and the well established export relations and operations with foreign customers. There are, however, weaknesses within and external threats to the industry, a few of which have been identified in DAFF (2012a, 2012b). A prominent threat is the competition from other southern hemisphere pome producing countries in the export market, e.g. Chile, Brazil and Argentina, as well as the effect of climate change on environmental conditions in the pome producing regions in South Africa. Vreysen et al. (2010) mentioned the threat of decreased availability of effective agricultural chemicals to control the numerous pests and diseases that attack pome fruit, e.g. *C. pomonella*, as well as the growing dissatisfaction of especially the European market with the residues of these chemicals on the produce (Simmons et al. 2010; Vreysen et al. 2010; EFSA 2015). Apples are currently the crop with the highest pesticide residue load, with reportedly as many as 47 types of chemicals found on some samples (USDA 2014). As the maximum residue loads of the most commonly used insecticides are continually adjusted to lower levels (Table 1), the major challenge in the future will be to continue the production of high quality crops that can compete on a global stage, while utilising sustainable agricultural practices.

Table 1: Maximum residue levels as prescribed by the European Food Safety Authority of four insecticides commonly used on apples.

Insecticide	Maximum residue levels (mg/kg)
Chlorpyrifos (F)	0.5
Methoxyfenozide	2.0
Pirimicarb (including desmethyl pirimicarb)	2.0
Chlorantraniliprole	0.5

Note 1: (http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/)

Codling moth

Origin and distribution

The codling moth, *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), is the primary arthropod pest in most pome fruit producing regions around the world. The first verifiable description of the species was made in 1635, where it was simply called the ‘pear eater’. The common name ‘codling moth’ was derived from the collective name used for a number of English cooking apples and was assigned to *C. pomonella* in the mid-1700s (Barnes 1991). It is accepted that the species originated in Eurasia, as it is closely associated with pome fruit and walnuts (which also originated in that region) and due to studies conducted on germplasm stocks from around the world (Mills 2005; Wahner 2008).

As mentioned, the codling moth has spread to nearly all apple and pear producing regions in the world, including South Africa (Figure 1). The earliest sighting of codling moth in South Africa was recorded in 1892 (Annecke and Moran 1982) and by 1918 the species had spread to every pome fruit producing area in the country (Lounsbury 1918; Annecke and Moran 1982).

Biological characteristics

Codling moth is closely associated with pome fruit, but will also attack stone fruit (apricots, peaches and plums) and walnuts under certain conditions (Petty 1916; Myburg et al. 1973; Annecke and Moran 1982; Mills 2005). In colder climates codling moth populations only achieve one or two generations per season, but due to the higher temperatures, three to four generations are common in South Africa (Giliomee and Riedl 1998; Pringle et al. 2003; Wahner 2008). Codling moth in South Africa has exceptionally high biotic potential owing to a high level of fecundity in spring (Myburg 1980; Blomefield 2003; Timm et al. 2006) and because moth activity can stretch over eight months (August to April) (Myburg 1980; Barnes 1991).

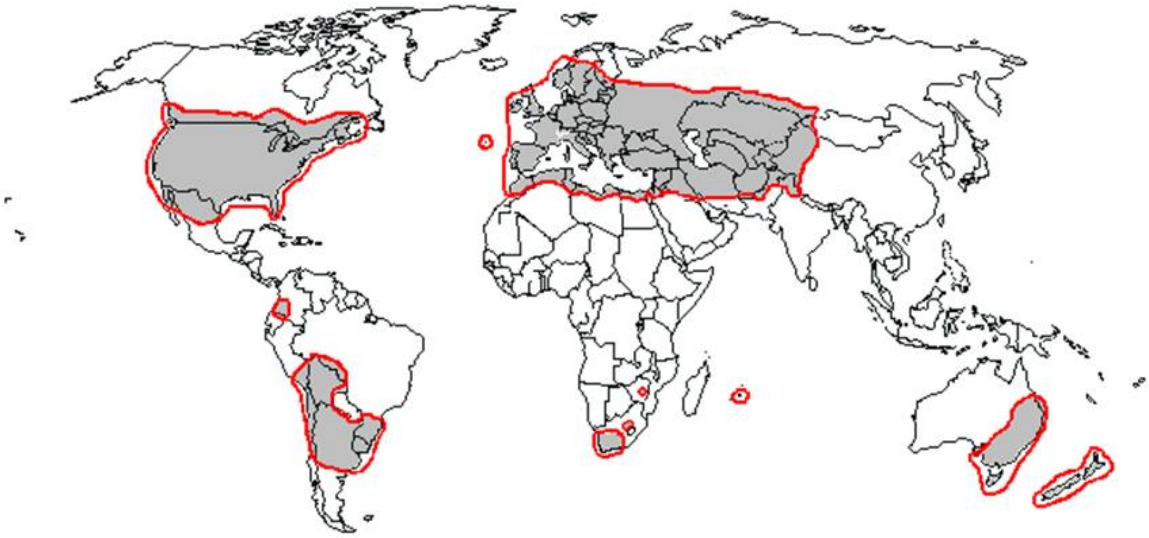

Figure 1: Worldwide distribution of the codling moth (*Cydia pomonella*) indicated by areas ringed by red lines. (http://ipmnet.org/codlingmoth/bionomics/distrib4.html) (Copyright: W.I. Bajwa & M. Kogan)

An important part of the life cycle of codling moth is the overwintering of final instar larvae in a cocoon in the bark of the host tree, in the leaf litter surrounding the tree or in the ground. In every generation a certain percentage of larvae enters diapause, irrespective of how many generations usually occur (Audemard 1991). The larvae in diapause are able to remain dormant for up to two years, ensuring the survival of the population in the absence of available fruit for extended periods of time (Yothers and Carlson 1941; Audemard 1991).

Typically, mating of adult codling moth occurs around sunset (Borden 1931; Cutright 1937). Mating can take place on the first day after eclosion, even though sex pheromone production of calling females only reaches its peak on day three (Geier 1963; Audemard 1991; Chidawanyika and Terblanche 2011a). Oviposition begins one day after mating and a mated female lays an average of 50-100 eggs (Geier 1963; Wearing and Ferguson 1971; Blomefield 2003; Chidawanyika 2010). As females possess mature oocytes, all the eggs are fertilised and can be laid after a single mating event (Howell 1991; Knight 2008). Eggs are laid individually, commonly on the leaves close to the fruit or on the fruit itself, which minimises the risk of parasitism and predation and alleviates larval competition for resources (Myburg 1980; Knight 2008). Larvae feed exclusively on the interior of the fruit (especially in the seed endocarp) and progress through five instar larval stages (Putman 1963; Falcon and Huber 1991; Wearing et al. 2001). The delay of feeding by the neonate larvae until inside the fruit significantly complicates the control of the species, as many pesticides require ingestion to be effective (Croft and Riedl 1991; Knight 2008).

These characteristics collude to increase the efficacy of codling moth as a pest on apples and pears. A fruit is rendered completely unmarketable by the presence of just one larva and there are many quarantine and phytosanitary requirements for produce on the international market (Hansen and Johnson 2007). No more than 1-2% damage is allowed for a fruit consignment to be classified as high quality (Varela et al. 1993; Azizyan and Asya 2005) and incidence of codling moth in shipments may lead to costly rejections of consignments and eventually to the deterioration of trading relations (Knight 2008). With infestation rates as high as 60% and 80% in neglected pear and apple orchards, respectively (Pringle et al. 2003; Vreysen et al. 2010), it is apparent that, although difficult, the control of this pest species is of utmost importance.

Management of codling moth

Three strategies are used to control codling moth: 1) increasing the mortality at certain periods of the life cycle, 2) increasing the efficiency of a natural limiting factor and 3) disrupting development, behaviour or relationships with host plants (Audemard 1991, as cited in Vreysen et al. 2010). With broad-spectrum insecticides providing successful control of codling moth and other pests, the first strategy was favoured until the early 1990s (Giliomee and Riedl 1998; Blomefield 2003).

Chemical control of codling moth

Because of the importance of codling moth as a pest and the heavy reliance on insecticides for control, the topic of chemical control for this species is extensively covered in literature. Croft and Riedl (1991), referring to work done by Madsen and Morgan (1970) and Croft and Hoyt (1983), stated the importance of codling moth control as follows:

‘*Generally, if codling moth is not adequately controlled, then most other associated systems of pest control for mites, aphids, leafhoppers, leafrollers, scale insects etc. are of little value, since production of a high-quality crop is impossible. In many cases, it is the chemical control regime for codling moth that sets the chemical environment around which pest control or IPM systems for other key and secondary pests are organized.*’

Throughout history a trend can be observed where a specific pesticide enjoys preference above others, but after a period of time a new pesticide replaces it and becomes more widely used for control. In the first half of the 20th century lead arsenate (Hough 1929) and dichlorodiphenyltrichloroethane (DDT) (Cutright 1954) dominated the market, but from the 1950s organophosphates grew in popularity until the control of codling moth was achieved almost exclusively with one or more of these broad-spectrum insecticides (Croft and Riedl 1991). Synthetic pyrethroids gained some ground during the 1980s, but proved to have too big of an impact on non-target organisms in the field and their use declined

(Blomefield 2003). Organophosphates, especially azinphos-methyl, and some carbamates, reigned supreme until codling moth populations (along with many other pest species) developed widespread resistance to these compounds in the 1990s (Varela et al. 1993). By this time up to 14 azinphos-methyl sprays a year were being applied in apple orchards (Blomefield 2003).

This resulted in a surge of interest in benzoylphenylureas (Badowska-Czubik et al. 1991), insect growth regulators and granulosis virus (Knight 2008), all of which have less adverse effects on non-target and beneficial insects than organophosphates (Blomefield 2003). Codling moth has, however, developed resistance to all these insecticides and cross-resistance among the different chemical classes is already reducing the efficacy of new insecticides before these have been introduced on the market (Dunley and Wleter 2000).

Chemical control: benefits and disadvantages

Chemical control has contributed significantly to the production of high quality produce, as it is fast-acting and broad-spectrum insecticides allow for the control of many pest species with a single chemical (Croft and Riedl 1991; Knight 2008). In addition, the relative low cost to producers and easy application (Way and Van Emden 2000) makes the complete abandonment of all chemical control highly unlikely.

There are, however, many disadvantages of relying too heavily on this technology. As already mentioned, the development of resistance by pest populations to these insecticides is one of the major drawbacks (Varela et al. 1993; Giliomee and Riedl 1998; Makee 2005) and lepidopterans are especially likely to do so (Simmons et al. 2010). Codling moth is no exception; the resistance mechanisms of this species include various physiological pathways, e.g. altered target sites and detoxification enzymes (Fuentes-Contreras et al. 2007). Many broad-spectrum insecticides also have detrimental effects on beneficial arthropod species, natural enemies and parasitoids. This leads to secondary pest outbreaks, often forcing the producer into using more chemicals to control the upsurge in new pest populations. Secondary pests following insecticidal control of codling moth include pear psylla, *Cacopsylla pyricola* (Foerster) (Hemiptera: Psyllidae) and tetranychid mites. These secondary pests can also develop resistance to insecticidal sprays (Croft 1979; Van de Baan and Croft 1991; Knight 2008), which inevitably forces producers into a ‘pesticide treadmill’. Van den Bosch (1989) described the pesticide treadmill as the situation where continually increasing amounts of insecticide are required to achieve the same level of control.

The excessive use of insecticides has dire consequences for environmental and human health. Even though the fruit treated with insecticides may look innocuous and of high quality externally, it contains chemical residues possibly harmful to the consumer (Croft and Riedl 1991). Unfortunately, insecticidal compounds that are more environmentally friendly are not as fast-acting and effective as more commonly used insecticides and are frequently considerably more expensive. This is often the reason why producers do not incorporate these chemicals into their control programmes (Blomefield 2003).

The risk of adverse impacts on the health of consumers and the environment is prompting regulatory bodies to reduce the acceptable residue levels of selected insecticides. Some insecticides have been removed from the market altogether (Simmons et al. 2010; Vreysen et al. 2010). Producers, therefore, have access to fewer effective insecticides to control pests in their orchards and this affects their ability to produce harvests with high enough quality to be marketed overseas (Vreysen et al. 2010).

Apart from the risks associated with the insecticides themselves, there are also many practical problems with implementing a strictly chemical control programme. The window period for the application of insecticides can be very narrow – the ideal time depending on many factors, such as the season and weather conditions, development of the fruit and the life cycle stage of the pest (Croft and Riedl 1991). For the chemicals to be effective, spraying needs to be done thoroughly. Achieving adequate insecticide coverage in large tree canopies requires the use of the right type of nozzle, water volume and air velocity to spray the chemicals and the tractor applying the chemicals needs to maintain the correct speed uniformly to ensure good spray cover of all the trees in the row (Howell and Maitlen 1987). Chemical control is also hindered by certain operational factors on farms: overhead sprinklers (Howell and Maitlen 1987; Knight 2008), and evaporative cooling for the reduction of sunburn damage to the fruit (Williams 1993) will remove the insecticidal residues from the plants. Contaminated fruit bins can also reintroduce pests to the already treated orchards (Proverbs and Newton 1975).

IPM and non-chemical control of codling moth

The problems associated with an overemphasis on insecticidal control of pests increased the demand for effective but more environmentally friendly control strategies and required that researchers and agrochemical companies find more sustainable control alternatives (Wahner 2008). Although the challenges brought on by insecticidal control are not unique to codling moth, the term and concept of 'integrated control' was coined by researchers working on codling moth (Michelbacher and Bacon 1952; Kogan 1998). IPM is an approach to pest management that aims to integrate multiple control methods into a single control strategy, as no single tactic would be able to protect crops sufficiently (Kogan 1998).

There are numerous non-chemical control tactics employed against codling moth, as seen in the list compiled by Vreysen et al. (2010): insect growth regulators, mating disruption, cultural practices (e.g.

tree banding and removal of wild host plants in the vicinity), attract-and-kill strategies and microbial control agents. Blomefield (2003) also adds the use of oils, mass trapping, biopesticides and SIT. The identification of codling moth pheromone components has made many of these technologies possible (Vickers and Rothschild 1991). An example of this is pheromone-mediated mating disruption, which is one of the most widely used non-chemical control strategies (Vreysen et al. 2010). The technique works on the premise that the occurrence of successful mating will decrease if the orchard is sufficiently flooded with pheromone, as males will have difficulty finding the females. Generally, 500 to 1000 pheromone dispensers are distributed per hectare to achieve effective control (Giliomee and Riedl 1998; Pringle et al. 2003).

The pome fruit producing regions of South Africa, especially those in the Western Cape, have great potential for the use of biological control strategies that involve the large scale release of biological agents. These areas are mostly located in valleys that are well separated and isolated, making it possible to employ control on an area-by-area basis (Addison 2005). This led to the investigation of using the SIT as a control method for codling moth in the Western Cape.

Sterile insect technique

The principle of SIT is essentially a form of ‘birth control’ (Knipling 1955) where a dominant lethal mutation is introduced into a wild population with the aim of reducing the number of offspring in the next generation. To achieve this, the insect pest species is mass reared and treated with radiation before being released into the target area. The treatment with radiation renders the insects sterile, but does not affect their ability to find and mate with the opposite sex. Thus, if enough sterile insects are released into the wild population, the majority of wild fertile insects will mate with mass-reared sterile insects and the eggs produced will be unviable, causing a significant decrease in the size of the following generation (Addison 2005; Dyck et al. 2005) (Figure 2). SIT is most effective when applied on an area-wide scale in conjunction with other suitable control strategies, i.e. area-wide integrated pest management (AW-IPM). AW-IPM recognises that effective management of pests entails the cooperation and coordination of a large area of producers, as pests do not keep to the boundaries of individual orchards or fields (Knipling 1979; Knight 2008).

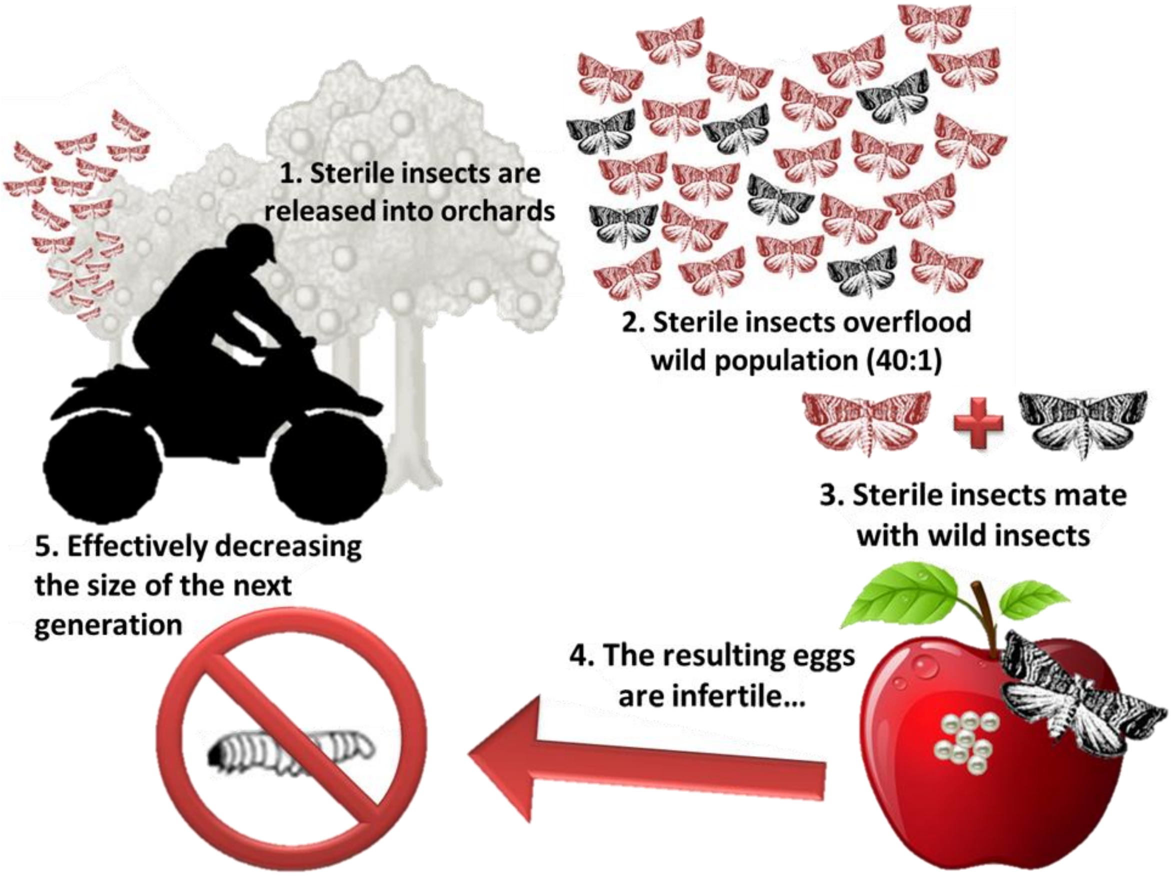

Figure 2: The basic concept of the sterile insect technique for codling moth (*Cydia pomonella*). The red coloured moths represents the sterile insects released into the orchard, whereas the black moths represent the naturally occurring wild population. (Adapted from www.oksir.org/results.asp).

History of SIT

The concept of SIT (or autocidal pest control) was proposed by Knipling in 1955 (Knipling 1955; Butt 1991). The technology was first employed against the screwworm, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) (Baumhover et al. 1955; Bushland 1960; Butt 1991), which was successfully eradicated from the USA and Mexico (Baumhover et al. 1955; Graham 1985). Since then SIT has been successfully employed against many agricultural, veterinary and medical pests, e.g. Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Vreysen et al. 2006), the tsetse fly, *Glossina sp*. Wiedemann (Diptera: Glossinidae) (Vreysen et al. 2000) and the malaria-carrying mosquito, *Anopheles arabiensis* (Patton) (Diptera: Culcidae) (Townson 2009).

SIT was also investigated for use against lepidopteran pests, and it was found that many of the species required a much higher radiation dose to achieve sterility (LaChance 1985; Bakri et al. 2005; Vreysen et al. 2010). This high dose adversely affected the competitiveness and field performance of the sterile individuals. To counter this problem, Proverbs (1962) investigated the effect of sub-sterilising radiation doses. What followed from this work is a derivate of SIT known as inherited sterility (IS) or F1 sterility (Proverbs 1962; LaChance 1985; Carpenter et al. 2005; Simmons et al. 2010), where a lower dose of radiation is applied that renders female moths completely sterile but only partially sterilises males. The offspring of a partially sterile male and a wild female will be viable, but completely sterile (hence the name 'F1 sterility') (Simmons et al. 2010).

Since this discovery, the interest in employing SIT against lepidopteran pests has increased dramatically. Indeed, more lepidopteran pests have been investigated in laboratory and field-cage studies compared to any other group of insect pests (Bloem et al. 2005). Simmons et al. (2010) compiled a list of the operational programmes that currently exist for Lepidoptera, namely pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), codling moth and false codling moth, as well as the several pilot studies that showed promise for future use, namely the gypsy moth, *Lymantria dispar* (Linnaeus) (Lepidoptera: Erebidae), the tobacco budworm, *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae), the corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), the oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), the carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae), and the Asian corn borer, *Ostrinia furnicalis* (Guenée) (Lepidoptera: Crambidae).

SIT to manage codling moth

After the initial investigations and pilot programme by Proverbs (1982), an AW-IPM programme (SIT with the addition of mating disruption, tree banding and insecticidal sprays) was initiated in an attempt to control codling moth in the pome fruit producing areas of southern British Columbia, Canada (Proverbs et al. 1982; Bloem et al. 2005). The Okanagan-Kootenay Sterile Insect Release (OKSIR) programme aimed to eventually protect 8000 ha of pome fruit orchards, starting with an initial 3200 ha (Zone 1) in 1992, with Zone 2 and 3 added to the programme in 1998 and 1999 (Dyck et al. 1993). The programme initially experienced a number of problems (e.g. an underestimation of the costs involved and the size of the wild population) (Bloem and Fielding 1996) and the first two years had disappointing results (Bloem et al. 2005). However, in 1995 the wild moth catches in Zone 1 were reduced from 13 moths per trap per week for the first generation to 2.5 for the second generation. By 2000, both the first and second generation wild moth catches were reduced to 0.08 moths per trap per week. Furthermore, between 1995 and 2000 the percentage of orchards with no codling moth damage increased from 42% to 95% and the sales of organophosphate pesticides in the area declined by 90% (Bloem et al. 2005, 2007). This trend has continued and it is estimated that the number of wild codling moth caught per trap per week in 2014 showed a 98% decline from 1995 in Zone 1 and a decline of 95% and 91% for Zone 2 and 3 respectively was recorded between 1999 and 2010 (www.oksir.org/results.asp) (Figure 3). The success of the OKSIR programme has inspired various pilot studies for codling moth SIT in other countries, including Brazil, Argentina and South Africa (Bloem et al. 2007).

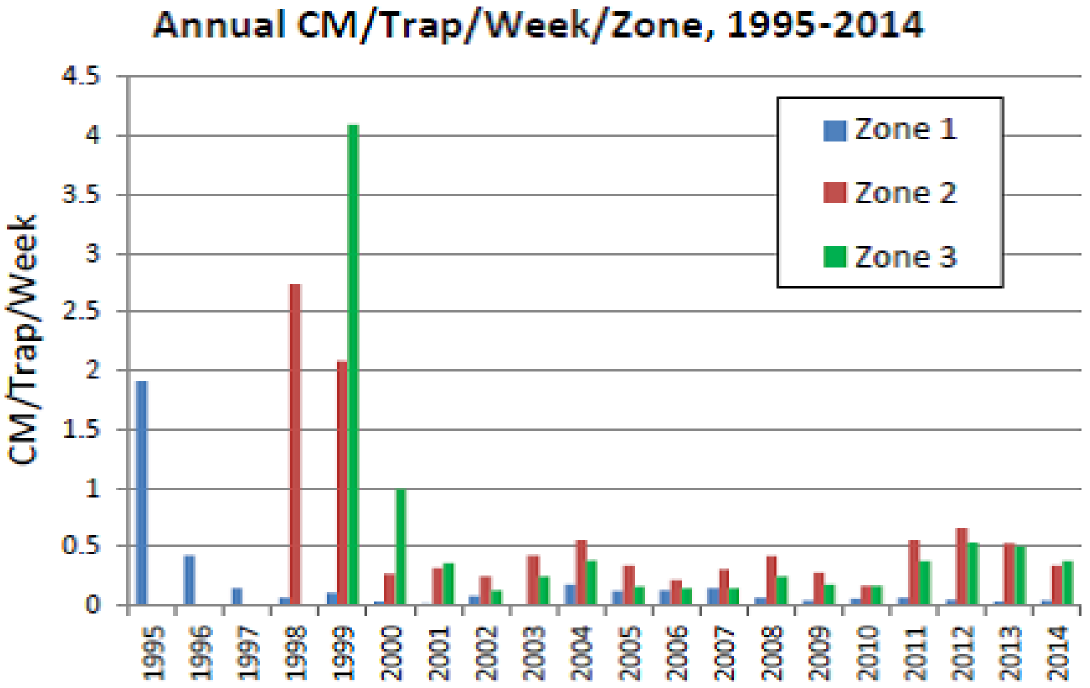

Figure 3: The results for the average number of codling moth (CM) caught per trap each week in each of the three treatment zones at the Okanagan-Kootenay Sterile Insect Release (OKSIR) programme in British Columbia, Canada (www.oksir.org/results.asp).

Prerequisites for SIT

The biggest constraint to the adaptation of SIT is commonly the large financial input required to initiate and run an SIT program, e.g. the building of a mass-rearing facility for the insects, acquiring a radiation source and the costs of mass rearing and transporting the insects (Dyck et al. 2005; Wahner 2008). There are also other concerns, for example the outbreak of other pest species once the application of insecticides are reduced (Bloem et al. 2007).

These challenges are, however, outnumbered by the numerous advantages associated with SIT: the technique is species specific and, therefore, does not affect non-target organisms, is inversely dependent to wild population size (a major advantage as it allows for use in an eradication programme) and can easily be integrated with other biological control methods (Knipling 1992; Vreysen et al. 2010). Indirect advantages that are difficult to quantify include access to new markets (e.g. organic markets), fewer health and safety risks for farm workers and lower commodity production costs (Carpenter et al. 2005).

Unfortunately, SIT cannot be applied against all pest species. There are certain prerequisites that need to be met. These are listed by Vreysen et al. (2010) as: 1) rearing of the target insect in large numbers should be feasible at reasonable cost, 2) the target population can be reduced by other control means to such levels where the release of sterile males becomes economic and effective, 3) the sperm of the sterile males should be as competitive as the sperm of the wild males and 4) the released sterile males should be of a high quality.

Quality control (QC) in SIT programmes

The mass rearing of the target insect species is central to SIT. Previously, much emphasis was placed on producing an ever increasing number of insects, which resulted in the quality of these insects to be overlooked or marginalised (Singh 1977; Calkins and Parker 2005). In recent years, however, many researchers have recognised that the success of an SIT program depends greatly on the quality of the final product (sterile insects) being released in the field (Bloem et al. 1998, 1999; Calkins and Parker 2005; Vreysen et al. 2006; Vreysen et al. 2007; Simmons et al. 2010). Huettel (1976) defined the quality of a mass-reared population as the measure of how well the mass-reared insect population functions in its intended role. The quality of insects produced for use in an SIT programme will, therefore, be evaluated on the basis of the capability of sterile insects to compete with wild insects for mating with other wild insects (especially the ability of sterile males to compete for wild females) (Vreysen et al. 2007).

Generally QC is divided into three categories: production, process and product QC (Leppla and Ashley 1989; Leppla and Fisher 1989; Calkins and Parker 2005; Dyck 2010). Production QC involves the inputs into rearing (e.g. diet ingredients and environmental conditions), process QC involves the actual procedures involved (diet preparation, insect collection and holding) and product QC focuses solely on the produced adult insects and their ability to complete the purpose for which they were reared (Dyck 2010).

Product QC in SIT

Although the implementation of QC will differ somewhat for each of the categories (production QC, process QC and product QC), the underlying concept remains the same. For product QC, the first step is the identification of biologically meaningful traits. Acceptable quality standards must then be developed by measuring and relating these traits (or parameters) to the performance of the insect in the field (Webb et al. 1981; Simmons et al. 2010). If it is found that a parameter is not up to satisfactory standards, the cause of the lack in quality needs to be identified and eliminated. This can only happen if a sensitive, reliable QC test is in place to detect deviations from the set standards for the given parameter (Cohen 2004).

QC tests that are conducted on a large scale in field conditions can only be conducted periodically as they require specific environmental conditions (no wind, ideal temperatures etc.), are expensive and/or time consuming and are by nature more difficult to reproduce. These assessments are referred to as periodic (QC) tests, and although they are very important in the QC process, tests that can be conducted on a routine basis are crucial for effective quality management. Routine QC assessments must be simple, affordable, reproducible and sensitive to changes in the quality parameter tested (Huettel 1976; Stewart 1984; Simmons et al. 2010).

The Arava/Araba programme in Israel serves as an example of the importance of routine QC in SIT programmes. Initially this programme required the long-distance shipment of mass-reared Mediterranean fruit flies from Guatemala (Cayol et al. 2004). Due to the routine QC assessments that were set in place, a decrease in the flight ability of the emerged male insects could be identified after the transit time of the pupae was greatly extended as a result of increased bureaucratic regulations (Vreysen et al. 2007). Currently, the insects for the Arava/Araba SIT programme are produced locally, which eliminates the need for long-distance shipment.

In the Food and Agriculture Organisation/International Atomic Energy Agency (FAO/IAEA) handbook on mass rearing codling moth, Dyck (2010) named the following parameters for product QC: 1) mating ability, 2) mating compatibility, 3) female calling behaviour and production of sex pheromone, 4) response of males to calling females, 5) mobility, flight ability and dispersal, 6) male competitiveness, 7) adult longevity and 8) fecundity.

Flight ability as QC parameter for codling moth

Although there are many valuable traits that can be used to determine the quality of insects, flight ability is widely considered to be a key characteristic, especially for lepidopterans such as the codling moth (Carpenter et al. 2013). Many of the other important traits depend on the male moth’s ability to disperse in the field to locate wild females, e.g. mating ability and compatibility, response of males to calling females and male competitiveness. If a moth is unable to fly, if it cannot disperse adequately in the orchard, then it will not be able to complete its intended purpose (Calkins and Parker 2005; Vreysen 2005; Simmons et al. 2010; Carpenter et al. 2013). It is, therefore, vital that routine assessments of male moth mobility, flight propensity and flight ability is incorporated into the QC protocol at codling moth mass-rearing facilities. Commonly used methods to determine mobility, flight propensity and flight ability includes the use of actographs (Chambers 1975; Keil et al. 2001;

Bloem et al. 2006a), flight mills (Chambers 1975; Huettel 1976; Schumacher and Weber 1997), flight tunnels (Suckling et al. 2007), flight cylinders (Simmons et al. 2010; Carpenter et al. 2012; Carpenter et al. 2013) and, on a larger scale, the use of field-cage assessments (Miller et al. 1994; Hofmeyr et al. 2005; Simmons et al. 2010) and the release/recapture test with marked adults in the field (Butt et al. 1970; Bloem et al. 1998, 2004; Carpenter et al. 2013).

Aims and objectives of the study

Aim

To identify and develop laboratory, semi-field and field methods that can be used to assess the quality of mass-reared codling moth.

Objectives

1. Develop flight performance assessments that can be used to determine the quality of mass-reared codling moth.
2. Evaluate the effect of factors such as age, radiation dose and handling on moth quality.
3. Determine whether the methods developed for codling moth can be used on false codling moth.

Limitations of research

The flight performance of males was prioritised above that of females.

Genetic sexing strains are used in SIT programmes for a number of other insect species, e.g. the Mediterranean fruit fly. Currently, no such sexing mechanism exists for codling moth (or any other Lepidoptera) and, therefore, these SIT programmes rely on the simultaneous release of both sexes. Bloem & Carpenter (2001) found that the release of both sexes did not impact negatively on the efficacy of the releases. In fact, they found that the release of females could prove beneficial to overall control. Even so, models depicting the genetic control of codling moth predict that better control would be achieved by releasing males only (Anisimov and Shvedov 1996). Apart from increasing control in the field, there are many other advantages related to exclusively rearing and releasing males, namely a decrease in the percentage males that have already mated prior to release in the field, an increase in overall productivity because males are smaller and more can be reared from a diet tray and, a speedier achievement of the over-flooding ratios (Vreysen et al. 2010). This proves that the efficacy of an SIT programme is determined more by the quality and performance of male moths than of females and that the development of a genetic sexing strain for codling moth may be imminent. Because of this, it was decided to use only male moths to develop the flight performance assessments for codling moth.

Flight performance and ability (as opposed to flight propensity) was investigated.

The flight assessment methods used or developed in this study aimed to evaluate the ability of the moths to fly and not their propensity to do so. UV-light (ultraviolet light) was used in the flight cylinder assessments (Chapter 2) with the clear goal of increasing the leaving rate (i.e. the propensity to fly from the cylinders). This must be kept in mind when considering the quality of mass-reared codling moth, as it has been shown that the process of mass rearing selects for moths that are less irritable and, therefore, less inclined to attempt multiple flights (Huettel 1976). Thus, these quality assessments will not give an indication if this type of selection is prevalent in a mass-rearing colony and should be tested for separately, as this could hamper the overall efficacy in the field.

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CHAPTER 2 : FLIGHT CYLINDER METHOD

Introduction

The quality of mass-reared insects is determined by how successful the insects fulfil their intended purpose (Huettel 1976; Bloem et al. 1998, 2004). For area-wide sterile insect technique programmes (AW-SIT) this role entails the ability of mass-reared males to successfully compete with wild males to mate with wild females (Vreysen et al. 2007; Vreysen et al. 2010).

The strategies and processes involved in the production of mass-reared insects can have a significant impact on their behaviour and competitiveness in the field (Calkins and Ashley 1989; Calkins and Parker 2005; Simmons et al. 2010; Vreysen et al. 2010). Calkins and Parker (2005) identified a number of operational attributes of SIT that likely affect insect quality. Namely methods of diet production, constant temperature and environmental conditions, sanitation procedures, methods of insect collection, irradiation procedures and general handling of insects (including storage, packaging, transport and release techniques). Mass rearing a colony of insects for an extended period of time will also cause genetic drift towards sedentary characteristics, which will negatively impact the competitiveness of mass-reared moths in the field (Singh 1977; Proverbs 1982; Barclay 2005; Calkins and Parker 2005; Lance and McInnis 2005; Simmons et al. 2010; Vreysen et al. 2010). It is, therefore, crucial that wild individuals are periodically introduced into the mass-reared colony to increase genetic diversity (Fisher and Caceres 1998; Krafsur 1998).

Although many factors of the SIT process are known to affect the quality of mass-reared moths, the extent to which each factor reduces the competitiveness and performance of the moths in the field is not well understood (Mackauer 1980; Dyck et al. 2005; Sørensen et al. 2012). However, the effect of the radiation dose is better understood, as various studies have investigated and proven the influence of radiation on moth quality (Robinson and Hendrichs 2005). Many species of Lepidoptera exhibit high resistance to radiation (LaChance 1985; Bakri et al. 2005; Vreysen et al. 2010). For some species radiation doses as high as 400-500 Gy are needed to achieve complete sterility, but high doses of radiation have a severely detrimental effect on moth performance in the field (LaChance et al. 1967; LaChance 1985; Bakri et al. 2005; Vreysen et al. 2010). The development of Inherited Sterility (IS) (which involves treating moths with low, sub-sterilising doses of radiation that induce partial sterility in the treated moths and complete sterility of the F1 and F2 progeny) has provided a solution to the problem of high doses of radiation, allowing the release of vigorous and competitive mass-reared moths (Proverbs 1962; North 1975; IAEA 1993, 2002; Vreysen et al. 2010). It is crucial that similar solutions are found for other factors that affect mass-reared moth quality, as the improvement of the SIT process in current programmes will lead to the implementation of SIT against more lepidopteran species (Robinson and Hendrichs 2005; Simmons et al. 2010). More than 30 moth species that exhibit inherited sterility have been identified and are, therefore, possible candidates for SIT control, However, thus far the only species to have dedicated SIT-programmes are codling moth, pink bollworm, false codling moth, cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) and painted apple moth, *Teia anartoides* (Walker) (Lepidoptera: Lymantriidae) (Simmons et al. (2010) and references therein). Establishing a mass-rearing facility and initiating sterile releases are costly (Lance and McInnis 2005; Vreysen et al. 2010; Vreysen and Robinson 2011) and potential stakeholders would be more willing to invest in the programme if it can be shown that other existing programmes are growing and improving continuously (Enkerlin and Quinlan 2004; Robinson and Hendrichs 2005) and that these improvements can be integrated early in the launch of a new programme.

The establishment of quality standards and reliable methods to determine sterile moth quality is of utmost importance if solutions are to be found for problematic factors in SIT (Huettel 1976; Singh and Ashby 1985; Calkins and Parker 2005; Carpenter et al. 2013). There is a shortage of methods that measure parameters concerning the in-field performance of moths, e.g. mating ability and compatibility, flight ability and dispersal and the increase of sterility in the wild population (Stewart 1984; Dyck 2010; Simmons et al. 2010; Carpenter et al. 2013). Methods to evaluate life history parameters of codling moth (e.g. egg hatch, larval developmental time, pupal weight and adult emergence) are well established, as these characteristics are easily measured and can be used to indicate the efficiency of the mass-rearing process (Dyck 2010; Carpenter et al. 2013).

However, these life history parameters are not reliable indicators of the performance and efficacy of moths in the field (Huettel 1976; Dyck 2010). Flight performance is a more appropriate parameter to indicate moth quality, as flight ability is a prerequisite for the execution of other crucial behaviours, such as responding to calling females, successful mating and the infusion of sterility into the target population (Calkins and Parker 2005; Vreysen 2005; Simmons et al. 2010; Carpenter et al. 2013). Thus, the development of reliable methods to determine flight performance would contribute substantially to the improvement of QC of codling moth (Carpenter et al. 2012; J. Carpenter et al. 2013).

Various methods have already been developed and used to successfully measure the flight performance of moths (Carpenter et al. 2013), e.g. actographs (Keil et al. 2001; Bloem et al. 2006b; Gu et al. 2006), flight mills (Huettel 1976; Schumacher and Weber 1997), wind tunnels (Suckling et al. 2011) and flight cylinders (Carpenter et al. 2012). Many of these methods are not ideal for routine

QC assessments, as they require a certain level of expertise and specialised equipment, have a low throughput, which severely limits sample size, and are not readily transportable (Carpenter et al. 2013). The flight cylinder method is a simple, inexpensive method and has been used effectively in QC protocols for Tephritid flies (FAO/IAEA/USDA 2003; Carpenter et al. 2013). It involves the use of vertical cylinders of various dimensions to evaluate the ability of moths to escape using flight: tall, narrow cylinders will be more difficult to escape from than shorter, wider cylinders. The initial investigation to determine the suitability of the method for codling moth was conducted by Carpenter et al. (2012) and was used as the groundwork for this study.

Objectives for this chapter

1. To develop a laboratory-based flight ability assessment method that is able to detect differences in moth quality.
2. To evaluate the effects of factors such as age, radiation dose and handling on the flight performance of moths.
3. To determine whether the flight cylinder assessment method developed for codling moth can be used for false codling moth.

Material and methods

Experimental logistics

Insect material

Codling moth were obtained from Entomon, the insect rearing facility of Stellenbosch University in Stellenbosch, Western Cape, South Africa. The colony was established in 2002 from larvae collected from infested apples in the Elgin area, Western Cape. The codling moth were reared on an agar-based diet according to the methods of Stenekamp (2011) and collected using similar methods to those described by Dyck (2010), i.e. moths were attracted to a UV-light placed in the opening of a large tube (diameter 20 cm) through which they were transported with airflow to a plastic collection tray (60 cm x 40 cm x 20 cm) in a cold room (2.5 m x 3 m, 3-5°C).

False codling moth were obtained from X-SIT (Pty) Ltd., a mass-rearing facility situated in Citrusdal, Western Cape, that has been in operation since 2007. The larvae were reared on an artificial diet described in Moore et al. (2014), at a temperature of 25°C with 50% relative humidity and a photoperiod of 12 h:12 h (L:D). At both Entomon and X-SIT, wild individuals (overwintering pupae) were introduced into the existing colonies to avoid inbreeding depression and to diversify the genetic make-up of the colony.

Location of experiments

The flight cylinder trials were conducted in a controlled environment room (3 m x 3 m) in the insectary at the Department of Conservation Ecology and Entomology of Stellenbosch University. For the duration of the experiments, the room was darkened and the temperature was set at 25°C. The relative humidity in the room was not controlled. Two temperature loggers (Model DS1921G iButton, ColdChain Thermodynamics, Fairbridge Technologies, South Africa) recorded the temperature in the room throughout all the trials. The loggers were fixed to the frames of the experimental stations close to the flight cylinders.

Handling and transportation of the insects

A ‘cold-chain’ series of handling activities is standard practice at most SIT facilities, as it ensures that the quality of the insects is not diminished during preparation and transportation (Dyck 2010). Moths were kept chilled throughout the processes of collection, storage and sorting at the mass-rearing facilities. During transit and radiation, moth-filled Petri dishes (plastic, 150 x 10 mm) were placed in polystyrene containers (400 x 300 x 300 mm) with icepacks. The Petri dishes were isolated from the icepacks with pieces of cardboard to prevent the direct exposure of the moths to the freezing temperature of the icepacks. The cardboard sections also prevented the excessive movement of the Petri dishes inside the container during transportation.

Radiation procedure

Moths were radiated at Fruit Fly Africa in Stellenbosch. The polystyrene container holding the Petri dishes with the moths was placed on one of the smaller turntables (diameter 20 cm) imbedded in a larger turntable (diameter 1.2 m). The larger turntable revolved around the panoramic Cobalt^{60} point source that delivered a radiation rate of 7.67 Gy/min. The smaller turntable counter-rotated to ensure a 360° treatment of the Petri dishes.

Developing the flight cylinder method

Flight cylinder matrix

In preliminary trials conducted with non-radiated, high quality mass-reared codling moth, cylinders of various dimensions were evaluated for their level of escape-difficulty (indicated by the percentage escape that the cylinder allows for 24-hour-old male moths). The cylinder dimensions used by

Carpenter et al. (2012) (diameters 11 and 16 cm, and heights 4, 6, 8, 16 cm) were used as a starting point in initial trials and subsequently a larger array of dimensions were tested. Three heights (2.5, 15, and 26.5 cm) and three diameters (10, 14 and 16 cm) were identified that produced cylinders that allowed for approximately 20%, 50% and 80% moth escape (thus, producing cylinders with a wide range of escape-difficulty). A matrix of nine flight cylinders was made using all combinations of the three heights and diameters (Figure 4). The flight cylinders were made from laminated black cardboard and dusted with talc powder (10 µm, Sigma Aldrich, Kempton Park) to ensure that moths could only escape cylinders via flight and did not crawl out. During the preliminary trials various numbers of moths per cylinder were tested. Escape rate increased with an increase in the number of moths, but it was established that 20 to 30 moths per cylinder was ideal, as higher numbers of moths did not lead to any significant increase in the leaving rate.

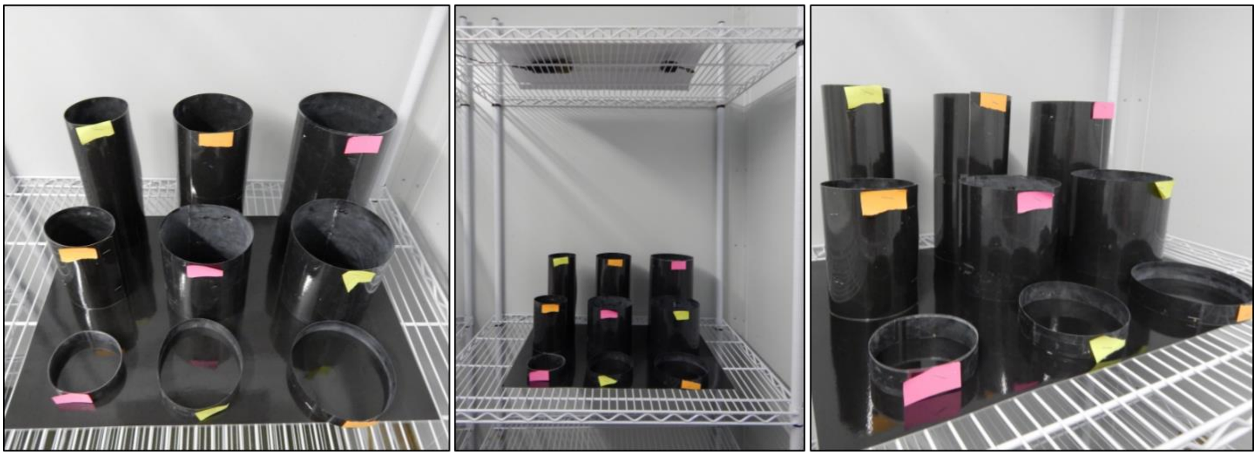


Figure 4: The experimental setup of the flight cylinder matrix assessment, with matrix of nine flight cylinders using all combinations of cylinder heights (2.5, 15, and 26.5 cm) and diameters (10, 14 and 16 cm). The cylinder placement was randomized during experiments, with the coloured pieces of paper indicating the treatment that was placed in the specific cylinder.

UV-light as lure

Carpenter et al. (2012) observed that flight cylinders were more suitable for indicating flight propensity than flight ability. To counter this, UV-light was added as a lure in an attempt to increase the escape rate of the moths. A 220V/8W black light (TL 4W BLB 1FM, Philips, South Africa) with a white cardboard backing (420 x 594 mm) was mounted 1.2 m above the base of the cylinders, directly above the cylinders. The escape rate of moths (24-hour-old, male) was evaluated in a preliminary trial using four cylinders (heights 15 and 26.5 cm combined with diameters 14 and 16 cm). A Petri dish with 20 moths (24-hour-old, male, still in a chilled state) was emptied into each of the cylinders and the number of moths remaining in each cylinder was recorded every two hours for a duration of 12 hours. This experiment was conducted in June 2014 and was repeated three times.

Control cylinders

In preliminary trials, empty control cylinders (height 15 cm, diameter 12 cm) were interspersed in the flight cylinder matrix to determine whether the moths were re-entering other cylinders in the matrix. Prior to the addition of UV-light, moths were occasionally found to have entered the control cylinders. With the UV-light and white cardboard in place, no moths were recorded to have entered the control cylinders as all the escaped moths congregated on the white cardboard. The marking of different treatments also made it possible to identify moths that re-entered another cylinder and the control cylinders were subsequently removed from the setup.

Experiments and treatments

Four experiments were conducted using the flight cylinder matrix method. Codling moth were used for experiments 1, 2 and 3 and false codling moth for the fourth experiment. These experiments were conducted to assess the effect of radiation dose, age (days after eclosion) and handling during transport on the flight ability of the mass reared moths. The treatment specifications of each experiment were as follows (summary in Table 2):

Experiment 1: No radiation and standard radiation

For the first experiment, 32 g (approximately 1000 individuals) of chilled 24-hour-old male moths were collected in a Petri dish (150 x 10 mm) for each treatment. The non-radiated (NoRad) treatment did not receive any radiation, whereas the standard treatment was treated with 150 Gy (standard radiation dose of mass-reared codling and false codling moth (LaChance 1985; Bloem et al. 1999)).

Experiment 2: Radiation dose and bran-handling

For each of the three treatments, 32 g (approximately 1000 individuals) of chilled 24-hour-old male moths were collected in a Petri dish (150 x 10 mm). The standard and bran treatments received the standard radiation dose (150 Gy) and the high radiation (HighRad) treatment received double the standard radiation dose (300 Gy). The bran treatment moths were then mixed (by hand) with 1ℓ of bran (food grade wheat bran) until an even distribution of moths inside the bran was achieved (after about 1 minute of mixing).

Experiment 3: Ages

For the third experiment, 32 g (approximately 1000 individuals) of chilled 24-hour-old male moths were collected in a Petri dish (150 x 10 mm) and stored in a cold room (< 6°C). This was repeated every day for four consecutive days. After four days, the 24 h (1DayOld), 48 h (2DayOld) and 96 h (4DayOld) moths received the standard radiation dose (150 Gy).

Experiment 4: Radiation dose (false codling moth)

For the false codling moth experiment, 19 g (approximately 1000 individuals) of chilled 24-hour-old male moths were collected in a Petri dish (150 x 10 mm). The non-radiated (NoRad) treatment did not receive any radiation, the standard treatment was treated with 150 Gy and the high radiation (HighRad) treatment received double the standard radiation dose (300 Gy).

After radiation, the moths of each treatment were sorted according to sex until at least 360 males were identified. The males of each treatment were separated into 18 Petri dishes (100 x 10 mm with 20 moths per dish). The Petri dishes containing the bran treatment moths were filled with bran before transportation. In each experiment, three colours of fluorescent powder (pink, green and blue) were used to mark the different treatments before their release into the cylinders. The moths were marked by placing 1 mg of coloured powder into the Petri dish of the specific treatment, and then slowly and gently rotating the Petri dish until the powder has dispersed evenly within the Petri dish.

Experiment 1 had six replications, experiment 2 had nine and experiment 3 and 4 both had seven replications. The codling moth experiments (experiments 1, 2 and 3) were conducted in October, November and December 2014, which coincided with the codling moth semi-field and field trials (Chapters 3 and 4). The false codling moth experiments (experiment 4) were conducted in June and July 2015.

Table 2**:** Summary of the treatments used in the flight cylinder assessment. The moths were exposed to various treatments of age (days after eclosion), radiation dose and handling to produce moths of different quality. Male codling moths were used in experiments 1–3, and male false codling moths were used in experiment 4.

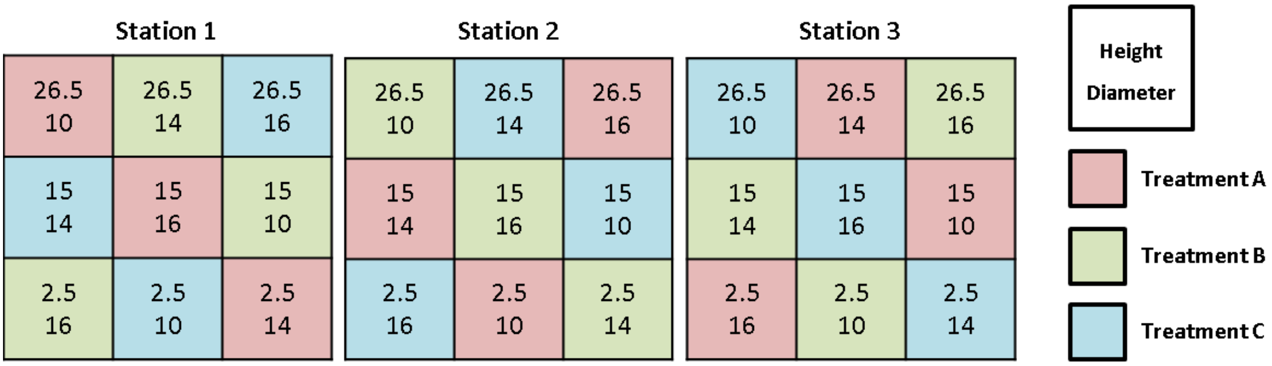
Experiment 1	Age of moths	Radiation dose	Transportation of moths
NoRad	< 24 h	0 Gy	Standard
Standard	< 24 h	150 Gy	Standard
Experiment 2	**Age of moths**	**Radiation dose**	**Transportation of moths**
Standard	< 24 h	150 Gy	Standard
HighRad	< 24 h	300 Gy	Standard
Bran	< 24 h	150 Gy	In bran mix
Experiment 3	**Age of moths**	**Radiation dose**	**Transportation of moths**
1DayOld	< 24 h	150 Gy	Standard
2DayOld	24 - 48 h	150 Gy	Standard
4DayOld	72 - 96 h	150 Gy	Standard

Experiment 4	Age of moths	Radiation dose	Transportation of moths
NoRad	< 24 h	0 Gy	Standard
Standard	< 24 h	150 Gy	Standard
HighRad	< 24 h	300 Gy	Standard

Laboratory flight cylinder assessment

For all the experiments, a 220V/8W black light (TL 4W BLB 1FM, Philips, South Africa) with a white cardboard backing (420 x 594 mm) was mounted 1.2 m above the base of the matrix of nine cylinders, directly above the cylinders. This represents one station (Figure 4). Three of these stations were set up in the controlled environment room and separated by cardboard partitions. The cardboard partitions completely isolated the stations from one another to prevent the moths from crossing over between stations.

At the start of the assessment, a Petri dish with 20 chilled male moths of a specific treatment was emptied into each cylinder. A randomised complete block design was used to determine the distribution of the treatments within the cylinder matrices of the three stations (Figure 5). The experiments lasted eight hours. At the end of the eight hours, the remaining moths in the cylinders were startled by disturbing the surface of the station (the observer tapped the surface a number of times until the moths remaining in the cylinders started to move around and flutter their wings). This caused moths left in the cylinders that had the ability (but not the propensity) to escape to fly from the cylinders. This would ensure that only the moths that were unable to escape remained in the cylinders. After the moths had settled again, the moths remaining in each cylinder were counted.



Station 1

🟥 26.5 10	🟩 26.5 14	🟦 26.5 16
🟦 15 14	🟥 15 16	🟩 15 10
🟩 2.5 16	🟦 2.5 10	🟥 2.5 14

Station 2

🟩 26.5 10	🟦 26.5 14	🟥 26.5 16
🟥 15 14	🟩 15 16	🟦 15 10
🟦 2.5 16	🟥 2.5 10	🟩 2.5 14

Station 3

🟦 26.5 10	🟥 26.5 14	🟩 26.5 16
🟩 15 14	🟦 15 16	🟥 15 10
🟥 2.5 16	🟩 2.5 10	🟦 2.5 14

Height
Diameter

🟥 Treatment A
🟩 Treatment B
🟦 Treatment C

Figure 5: The randomised complete block design used for the experimental setup of three stations and the distribution of the treatments. Each square resembles a cylinder, with the height and diameter given as the top and bottom number respectively.

Statistical analysis

The raw data for the cylinders were recorded as the number of moths remaining in the cylinder at the time of recording. The number of moths escaping from the cylinder (the variable used in all of the analyses of the flight cylinder assessments) was calculated by subtracting the number of moths remaining in the cylinder from the initial number of moths in the cylinder (20 moths).

Analysis of variance

The data from the experiment that assessed the effect of the addition of UV-light on the moth escape rate were analysed using a mixed model repeated measure analysis of variance (ANOVA). The dependent variable was the number of moths escaping from the cylinders, with cylinder height, cylinder diameter and time (with all possible interactions) included as the independent variables. The results of the flight cylinder method using the matrix of nine cylinders were analysed with a mixed model ANOVA. The dependent variable was the number of moths escaping from the cylinders and the independent variables were treatment, height and diameter (with all possible interactions). The effect of replicate date (and station number for the flight cylinder matrix analyses) on the number of moths escaping was also tested for each experiment using factorial ANOVA. The normality of the data was confirmed with normal probability plots and Fisher's LSD post hoc tests were used to separate means at $p = 0.05$. The data analysis software used was STATISTICA 12 (StatSoft, USA).

Multiple regression

Multiple regression analyses were used to test whether cylinder height, diameter and/or the treatments were able to significantly predict moth escape from cylinders. Dummy variables (DVs) were used to incorporate the treatments into the regression analyses. The predictor variables included in the full model were height, diameter, DV1, DV2, as well as interactions of DV1 and DV2 with both height and diameter. By using backwards variable selection methods, reduced models were identified for each analysis. In each case the predictors of height, diameter and the interaction of height with both DV1 and DV2 produced the multiple regression models with the best fit, i.e. with highest adjusted R^2 and F-values. The reduced models were compared to the full models to ensure that there was no significant difference between the two models. The data analysis software used was STATISTICA 12 (StatSoft, USA).

Results

Escape rate experiment

A significant effect was found for ‘time’ (Figure 6), as well as for the interactions for ‘time x height’ (Figure 7) and ‘time x diameter’ (Figure 8). The number of moths escaping from cylinders increased significantly between the periods 2, 4, 6 and 8 hours, after which the leaving rate slowed down so that were no differences in leaving rate for the time periods 8, 10 and 12 hours (Figure 6). This trend was observed for the escape rate from both of the heights (Figure 7) and both of the diameters (Figure 8), and, therefore, indicates that the number of moths escaping from cylinders peaked at 8 hours. The main effects ‘height’ and ‘diameter’ did not significantly influence moth escape, neither did the interaction for factors ‘height x diameter’, nor the three-way interaction between the factors ‘height x diameter x time’. Although the difference in escape rate between the two heights and two diameters were not significant, the trend suggests that more moths are able to escape from shorter, wider cylinders than from taller, narrower cylinders. See Table 3 for F-statistics and significance values of effects.

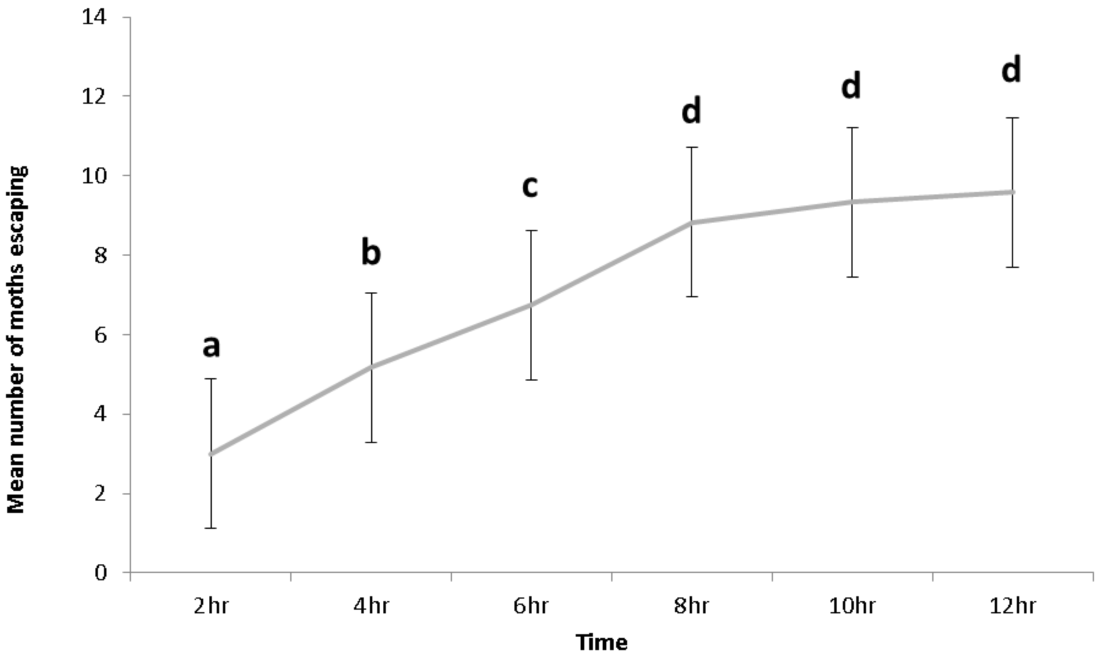


Figure 6: The mean accumulating number (±SE) of codling moth males (initial n = 25) escaping from four cylinders (heights 15 and 26.5 cm combined with diameters 14 and 16 cm) over a period of 12 hours. The mean numbers at each time period with the same letter are not significantly different ($F_{5,40} = 26.05$, $p < 0.001$). SE: standard error.

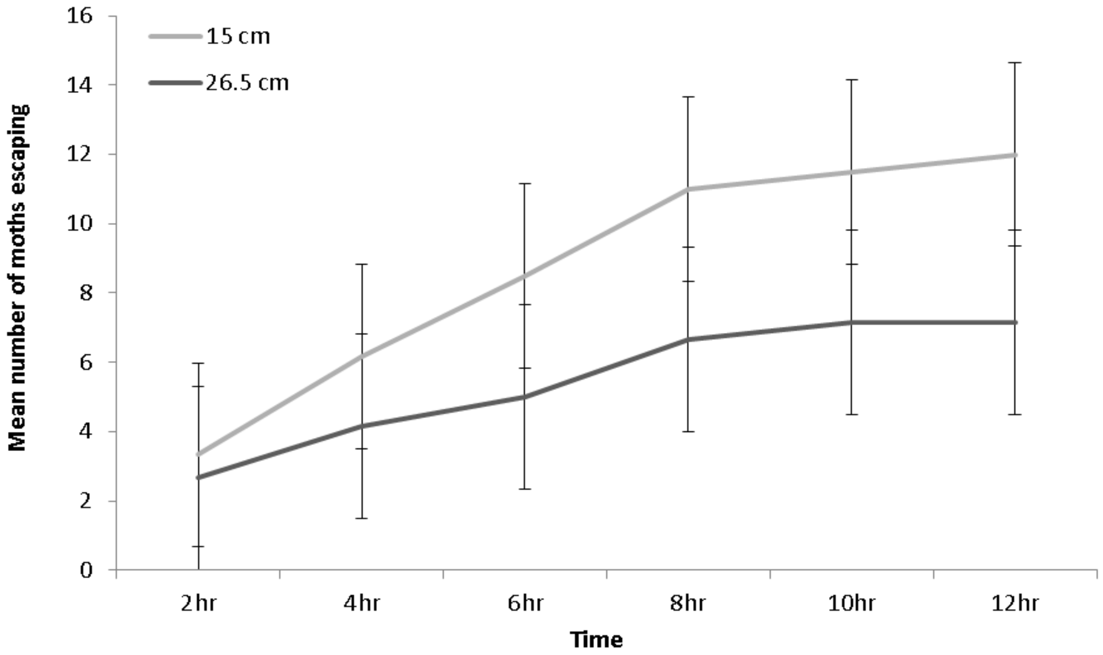


Figure 7: The mean accumulating number (±SE) of codling moth males (initial n = 25) escaping from each cylinder height (15 cm and 26.5 cm) over a period of 12 hours (data from both diameters 14 cm and 16 cm combined). ($F5,40 = 2.458$, $p = 0.049$). SE: standard error.

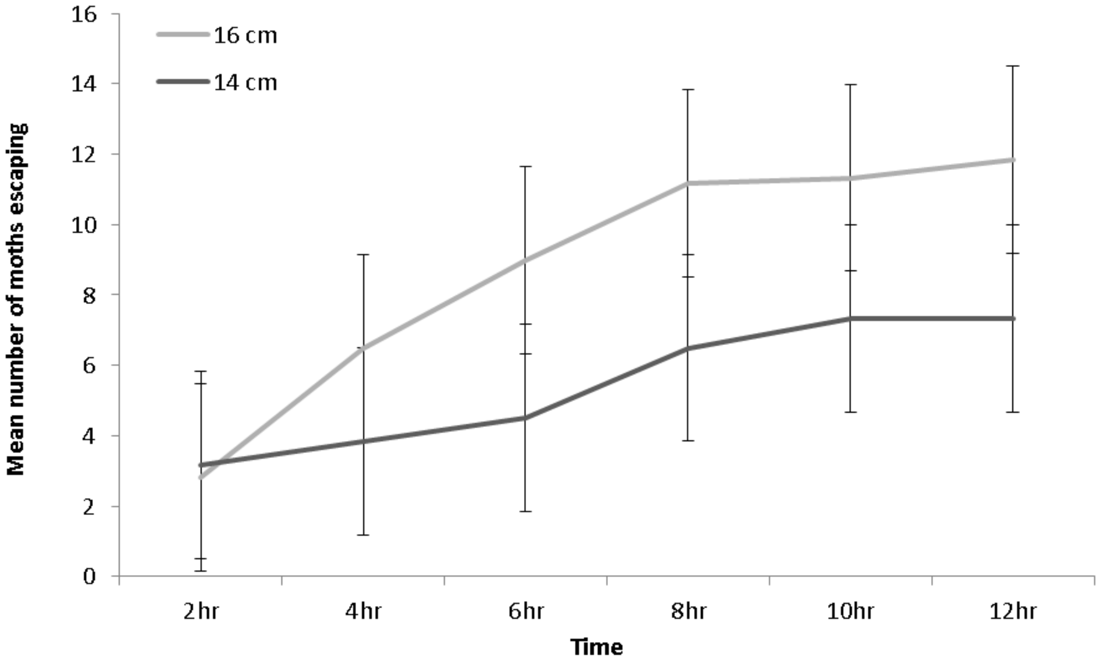


Figure 8: The mean accumulating number (±SE) of codling moth males (initial n = 25) escaping from each cylinder diameter (14 cm and 16 cm) over a period of 12 hours (data from both heights 15 cm and 26.5 cm combined) ($F_{5,40} = 3.522$, $p < 0.009$). SE: standard error.

Table 3: The repeated measures factorial analysis of variance results for the number of codling moth males (initial n = 25) escaping from four cylinders over a period of 12 hours. The cylinders consisted of combinations of the heights 26.5 cm and 15 cm with the diameters 14 cm and 16 cm. Num. d.f.: degrees of freedom of the numerator; Den. d.f.: degrees of freedom of the denominator.

Effect	**Num. d.f.**	**Den. d.f.**	**F**	**p**
Height	1	8	0.804	0.396
Diameter	1	8	0.831	0.388
Time	5	40	26.053	0.000
Height x Diameter	1	8	0.011	0.917
Height x Time	5	40	2.458	0.049
Diameter x Time	5	40	3.522	0.009
Height x Diameter x Time	5	40	1.191	0.330

Flight cylinder assessment: ANOVA analyses

Experiment 1: No radiation and standard radiation

The main effect of ‘treatment’ was found, with moth escape being significantly lower for the standard treatment compared to the NoRad treatment (Figure 9). ‘Height’ was also found to influence moth escape rate, as all three heights differed significantly in the number of moths escaping from each (26.5 cm height displayed the lowest escape rate, and 2.5 cm height the highest). The factor ‘diameter’ had no significant effect on moth escape, nor did the interaction between the factors ‘height x diameter’. The three-way interaction for ‘treatment x height x diameter’ was found to be statistically significant, with a lower escape rate for the standard treatment and the differences between moth escape of the two treatments being most pronounced at height 26.5 cm and diameter 10 cm diameter (Figure 10). See Table 4 for F-statistics and significance values of effects.

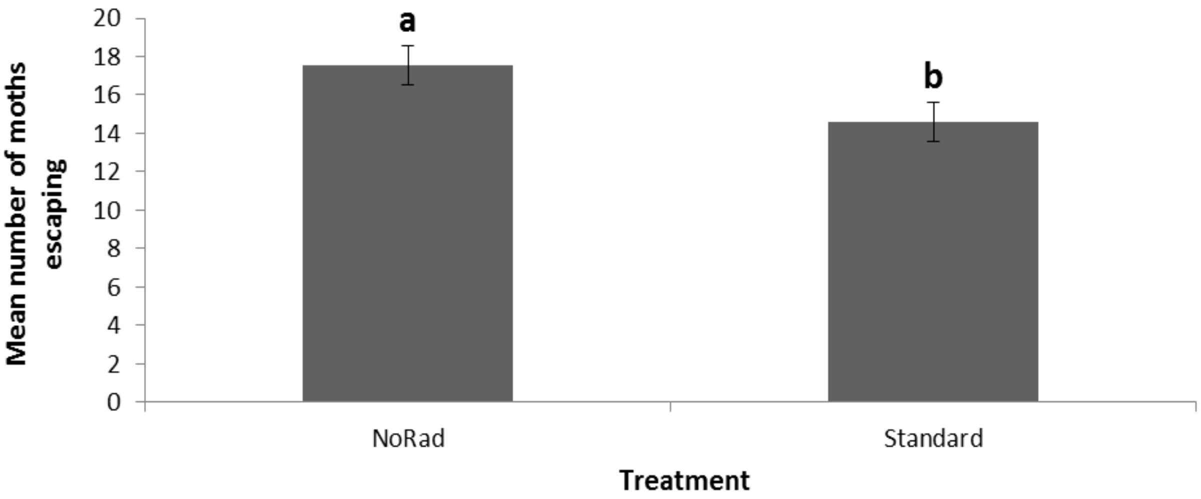


Figure 9: The mean number (±SE) of non-radiated (NoRad) and standard radiated (Standard) codling moth males (initial n = 20) escaping from a nine-cylinder matrix after eight hours. The mean number of moths escaping for each treatment differed significantly, as indicated by the different lowercase letters above the histogram ($F_{1,90}$ = 129.21, $p < 0.001$). SE: standard error.

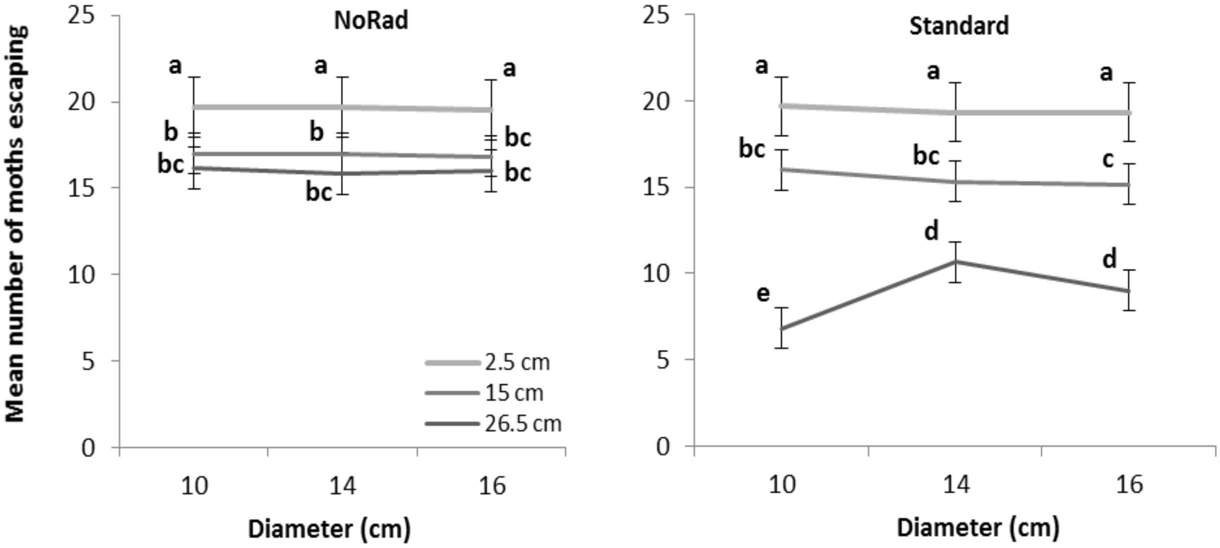


Figure 10: The mean number (±SE) of non-radiated (NoRad) and standard radiated (Standard) codling moth males (initial n = 20) escaping from a nine-cylinder matrix after eight hours. The cylinder matrix consisted of the combinations of three heights (26.5 cm, 15 cm and 2.5 cm) and three diameters (10 cm, 14 cm and 16 cm). The mean numbers with the same lowercase letter are not significantly different across both graphs ($F_{4,90}$) = 3.10, $p = 0.019$). SE: standard error.

Table 4: The factorial analysis of variance results (with means and standard deviation values) for the number of non-radiated (NoRad) and standard radiated (Standard) codling moth males (initial n = 20) escaping from a nine-cylinder matrix after eight hours. The cylinder matrix consisted of the combinations of three heights (26.5 cm, 15 cm and 2.5 cm) and three diameters (10 cm, 14 cm and 16 cm). SS: sum of squares; d.f.: degrees of freedom; MS: mean squares.

Effect	SS	d.f.	MS	F	p
Treatment	231.15	1	231.15	129.21	0.000
Height	911.72	2	455.86	254.83	0.000
Diameter	3.50	2	1.75	0.98	0.379
Treatment x Height	250.13	2	125.06	69.91	0.000
Treatment x Diameter	5.02	2	2.51	1.40	0.251
Height x Diameter	16.94	4	4.24	2.37	0.058
Treatment x Height x Diameter	22.20	4	5.55	3.10	0.019
Error	161.00	90	1.79		

Experiment 2: Radiation dose and bran-handling

The number of moths escaping from the cylinders in the nine cylinder matrix was significantly influenced by ‘treatment’, ‘height’ and ‘diameter’. Moths of the HighRad treatment were statistically less likely to escape compared to moths of the standard and bran treatments (Figure 11). Moth escape decreased significantly with an increase in cylinder height, as well as with a decrease in cylinder diameter. The interaction factor of ‘height x diameter’ also produced significant differences in moth escape, with the most acute effect (i.e. the lowest escape rate) observed for the combination of height 26.5 cm and diameter 10 cm (Figure 12). The interactions of ‘treatment’ with either ‘height’ or ‘diameter’ had no significant influence on moth escape, nor did the three-way interaction between ‘treatment’, ‘height’ and ‘diameter’. However, the predominant trend indicated that the effect of decreased cylinder diameter on moth escape became more pronounced as cylinder height increased. This was especially true for HighRad treatment moths (Figure 13). See Table 5 for F-statistics and significance values of effects.

Table 5: The factorial analysis of variance results for codling moth males (initial n = 20) of three different radiation and handling treatments escaping from a nine-cylinder matrix after eight hours. The codling moth received either the standard radiation dose (Standard), a high radiation dose (HighRad) or the standard radiation dose with bran-handling during transit (Bran). The cylinder matrix consisted of the combinations of three heights (26.5 cm, 15 cm and 2.5 cm) and three diameters (10 cm, 14 cm and 16 cm). SS: sum of squares; d.f.: degrees of freedom; MS: mean squares.

Effect	SS	d.f.	MS	F	p
Treatment	126.10	2	63.05	9.202	0.000
Height	3756.22	2	1878.11	274.103	0.000
Diameter	523.14	2	261.57	38.175	0.000
Treatment x Height	43.90	4	10.98	1.602	0.174
Treatment x Diameter	25.88	4	6.47	0.944	0.439
Height x Diameter	287.31	4	71.83	10.483	0.000
Treatment x Height x Diameter	2.42	8	0.30	0.044	0.999
Error	1480.00	216	6.85		

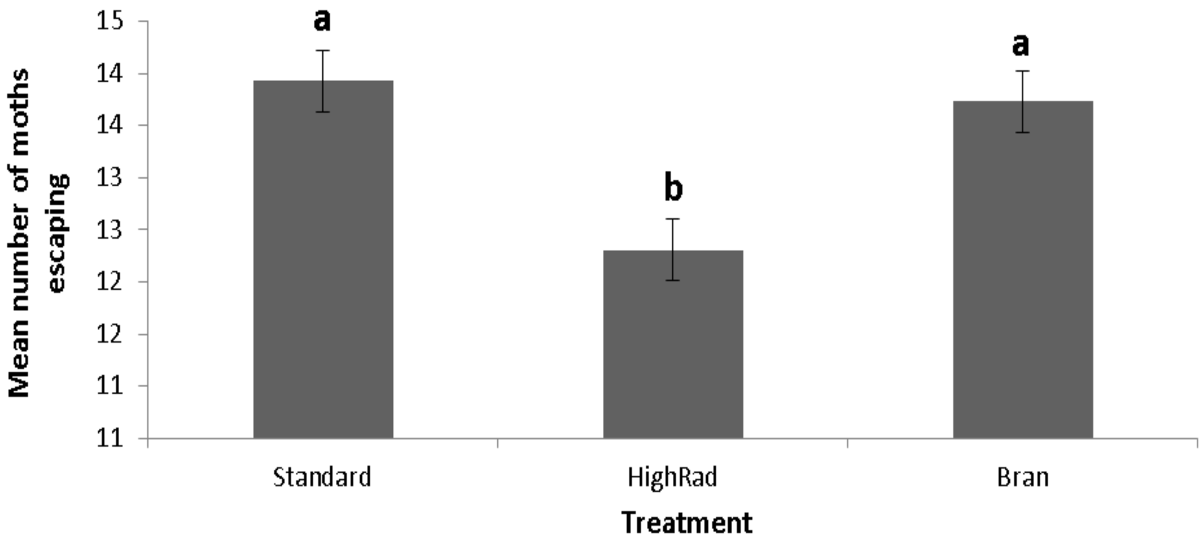


Figure 11**:** The mean number (±SE) of codling moth males (initial n = 20) of three different radiation and handling treatments escaping from a nine-cylinder matrix over an eight-hour period. The codling moth received either the standard radiation dose (Standard), a high radiation dose (HighRad) or the standard radiation dose with bran-handling during transit (Bran). The mean numbers of each treatment with the same lowercase letter above the histogram are not significantly different ($F_{2,216} = 9.202$, $p < 0.001$). SE: standard error.

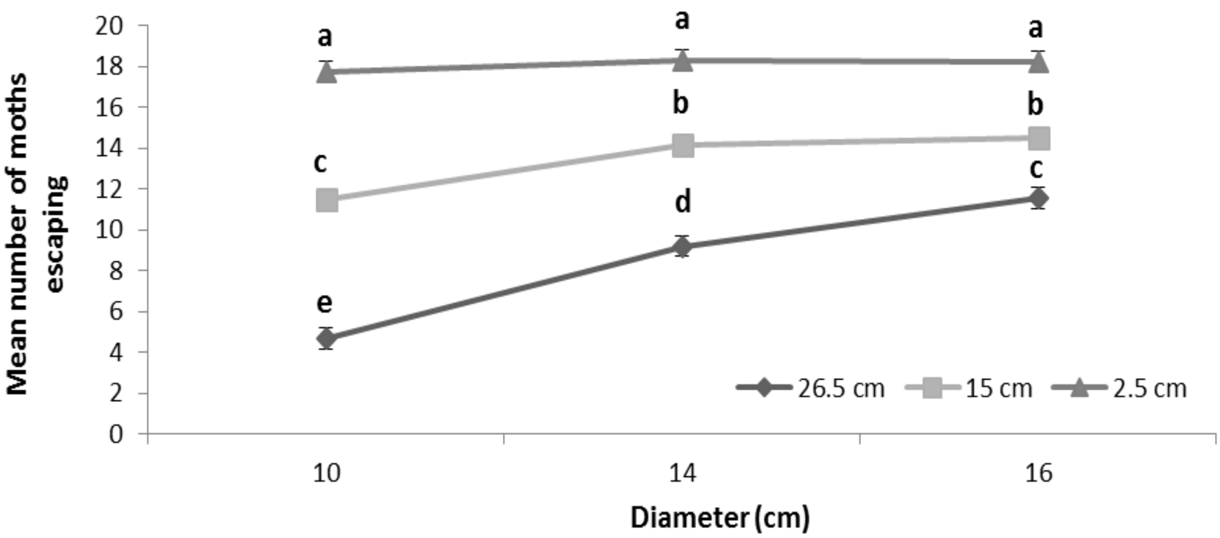


Figure 12: The mean number (±SE) of codling moth males (initial n = 20) of three different radiation and handling treatments escaping from a nine-cylinder matrix over an eight-hour period. The cylinder matrix consisted of the combinations of three heights (26.5 cm, 15 cm and 2.5 cm) and three diameters (10 cm, 14 cm and 16 cm). For each cylinder height, the mean numbers with the same lowercase letter at each cylinder diameter are not significantly different ($F_{4,216} = 10.483$, $p < 0.001$). SE: standard error.

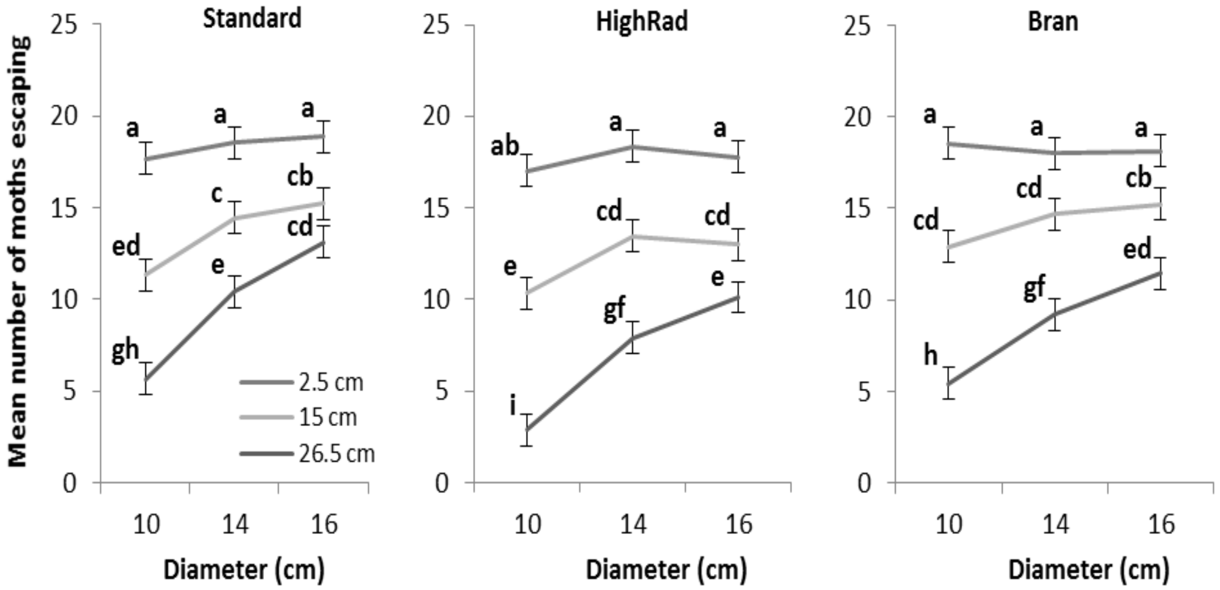


Figure 13: The mean number (±SE) of codling moth males (initial n = 20) of three different radiation and handling treatments escaping from a nine-cylinder matrix over an eight-hour period. The codling moth received either the standard radiation dose (Standard), a high radiation dose (HighRad) or the standard radiation dose with bran-handling during transit (Bran). The cylinder matrix consisted of the combinations of three heights (26.5 cm, 15 cm and 2.5 cm) and three diameters (10 cm, 14 cm and 16 cm). The mean numbers with the same lowercase letter are not significantly different across all three graphs ($F_{8,216} = 0.044$, $p < 0.999$). SE: standard error.

Experiment 3: Ages

Significant main effects were found for the factors ‘height’ and ‘diameter’. Moth escape differed significantly for all three heights (decreasing as the cylinder height increased), but only the 10 cm cylinder diameter caused a significant decrease in moth escape. The number of moths escaping from the cylinders were not influenced by ‘treatment’, nor was there a significant interaction between ‘treatment’ and ‘height’ or ‘treatment’ and ‘diameter’. A significant interaction was found for ‘height x diameter’, with moth escape being lowest for the combination of height 26.5 cm and diameter 10 cm (Figure 14). The three-way interaction of ‘treatment x height x diameter’ was not significant, mainly due to the lack of difference between moth escape of the three treatments. See Table 6 for F-statistics and significance values of effects.

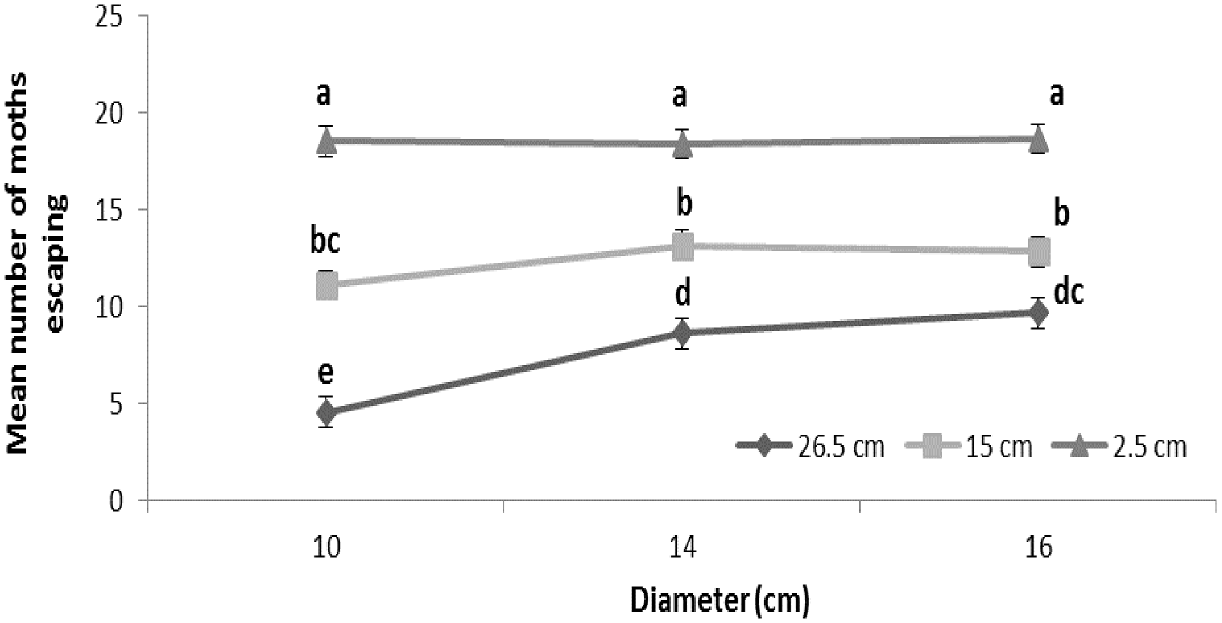


Figure 14: The mean number (±SE) of codling moth males (initial n = 20) of three different age treatments escaping from a nine-cylinder matrix after eight hours. The cylinder matrix consisted of the combinations of three heights (26.5 cm, 15 cm and 2.5 cm) and three diameters (10 cm, 14 cm and 16 cm). For each cylinder height, the mean numbers with the same lowercase letter at each cylinder diameter are not significantly different ($F_{4,135} = 3.167$, $p = 0.015$). SE: standard error.

Table 6: The factorial ANOVA results for codling moth males (initial n = 20) of three different age treatments escaping from a nine-cylinder matrix after eight hours. The codling moth were either aged 24 hours (1DayOld), 48 hours (2DayOld) or 96 hours (4DayOld). The cylinder matrix consisted of the combinations of three heights (26.5 cm, 15 cm and 2.5 cm) and three diameters (10 cm, 14 cm and 16 cm). SS: sum of squares; d.f.: degrees of freedom; MS: mean squares.

Effect	**SS**	**d.f.**	**MS**	**F**	**p**
Treatment	17.94	2	8.97	0.826	0.439
Height	3240.98	2	1620.49	149.294	0.000
Diameter	169.12	2	84.56	7.791	0.000
Treatment x Height	31.36	4	7.84	0.722	0.578
Treatment x Diameter	15.88	4	3.97	0.366	0.832
Height x Diameter	137.51	4	34.38	3.167	0.015
Treatment x Height x Diameter	15.72	8	1.96	0.181	0.993
Error	1465.33	135	10.85		

Experiment 4: Radiation dose (false codling moth)

Moth escape from the cylinders in the nine cylinder matrix was significantly influenced by ‘treatment’, with moths of the standard and HighRad treatments shown to be less likely to escape from the cylinders than the moths of the NoRad treatment (Figure 15). ‘Height’ was also found to be a significant main effect, as moth escape decreased significantly with an increase in cylinder height. There was no evidence for an effect of ‘diameter’ on moth escape. The lack of an effect of ‘diameter’ on moth escape also affected the interaction between the factors ‘height’ and ‘diameter’, which did significantly influence moth escape. The three-way interaction for ‘treatment x height x diameter’ was not significant either, although a critical effect (i.e. lower moth escape rate) was observed for the combination of height 26.5 cm and diameter 10 cm (Figure 16). See Table 7 for F-statistics and significance values of effects.

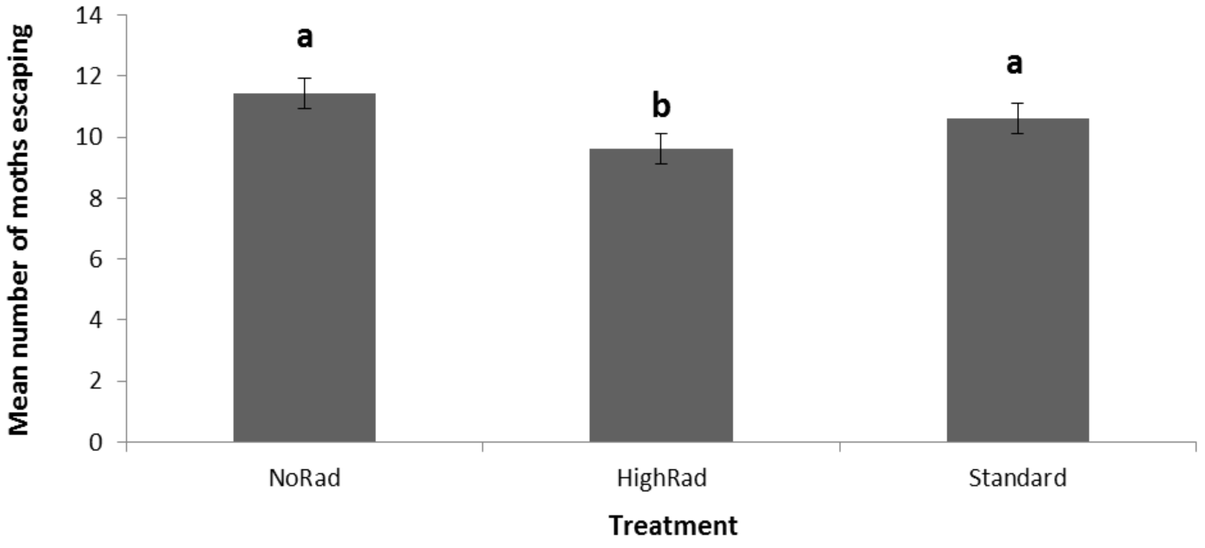


Figure 15: The mean number (±SE) of false codling moth males (initial n = 20) of three different radiation treatments escaping from a nine-cylinder matrix after eight hours. The false codling moth received either no radiation (NoRad), the standard radiation dose (Standard) or a high radiation dose (HighRad). The mean numbers of each treatment with the same lowercase letter above the histogram are not significantly different ($F_{2,162} = 3.321$, p = 0.038). SE: standard error.

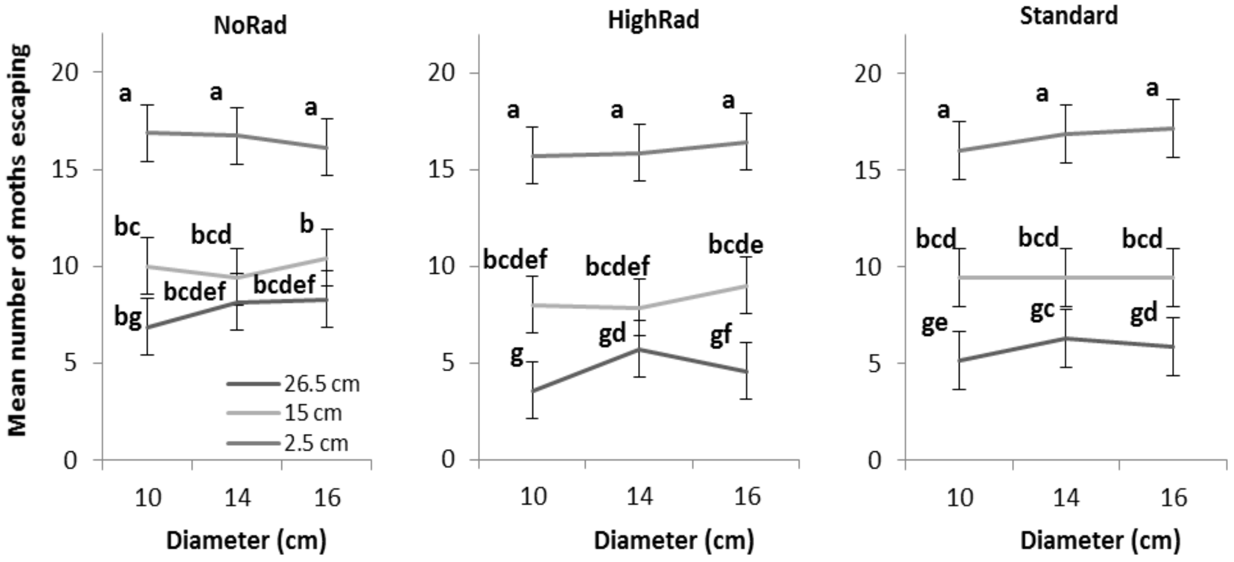


Figure 16: The mean number (±SE) of false codling moth males (initial n = 20) of three different radiation treatments escaping from a nine-cylinder matrix after eight hours. The false codling moth received either no radiation (NoRad), the standard radiation dose (Standard) or a high radiation dose (HighRad). The cylinder matrix consisted of the combinations of three heights (26.5 cm, 15 cm and 2.5 cm) and three diameters (10 cm, 14 cm and 16 cm). The mean numbers with the same lowercase letter are not significantly different ($F_{8,162} = 0.1$, p = 0.999). SE: standard error.

Table 7: The factorial analysis of variance results for false codling moth males (initial n = 20) of three different radiation treatments escaping from a nine cylinder matrix after eight hours. The false codling moth received either no radiation (NoRad), the standard radiation dose (Standard) or a high radiation dose (HighRad). The cylinder matrix consisted of the combinations of three heights (26.5 cm, 15 cm and 2.5 cm) and three diameters (10 cm, 14 cm and 16 cm). SS: sum of squares; d.f.: degrees of freedom; MS: mean squares.

Effect	**SS**	**d.f.**	**MS**	**F**	**p**
Treatment	101.66	2	50.83	3.321	0.038
Height	3553.53	2	1776.77	116.090	0.000
Diameter	14.49	2	7.24	0.473	0.623
Treatment x Height	40.59	4	10.15	0.663	0.618
Treatment x Diameter	2.40	4	0.60	0.039	0.997
Height x Diameter	18.24	4	4.56	0.298	0.878
Treatment x Height x Diameter	12.20	8	1.53	0.100	0.999
Error	2479.43	162	15.31		

Flight cylinder assessment: multiple regression analyses

Multiple regression analysis was used to test whether cylinder height, diameter and/or the treatments were able to significantly predict moth escape from cylinders. Significant F-tests were obtained for each regression analysis and all the adjusted R^2 values were above 0.66 (with the exception of experiment 4, where $R^2 = 0.56$), which indicated that the variables included accounted for at least two thirds of the variation in the data. The significance of each of the variables in the different analyses can be seen in Table 8. A regression equation was constructed for each treatment, which can be used to substitute untested values for the variables in order to predict outcomes (Table 8).

Table 8: Results of the multiple regression analyses to assess the ability of cylinder height, cylinder diameter and treatments of radiation, age and handling to predict the number of moths (initial n = 20) that would be able to escape from a cylinder. Backwards variable selection methods were to produce reduced models with the best fit. DV: dummy variable.

Experiment 1	Adjusted $R^2 = 0.841$		$F(4,103) = 143.53$	$p < 0.001$
Predictor	Coefficient (b)	Standard error	t(103)	p-value
Intercept	20.037	0.834	24.005	0.000
Height (X_1)	-0.151	0.020	-7.200	0.000
Diameter (X_2)	-0.022	0.062	-0.368	0.713
(DV1)(Height)	-0.289	0.029	-9.946	0.000
(DV1)(Diameter)	0.099	0.037	2.626	0.009
NoRad:	$E(y) = 20.037 - 0.151X_1 - 0.023X_2$			
Standard	$E(y) = 20.037 - 0.44X_1 + 0.076X_2$			

Experiment 2	Adjusted $R^2 = 0.703$	$F(4,238) = 144.217$		$p < 0.001$
Predictor	Coefficient (b)	Standard Error	t(238)	p-value
Intercept	11.387	1.001	11.368	0.000
Height (X_1)	-0.359	0.023	-15.608	0.000
Diameter (X_2)	0.585	0.071	8.230	0.000
(DV1)(Height)	-0.103	0.024	-4.187	0.000
(DV2)(Height)	-0.020	0.024	-0.823	0.410
Standard:	$E(y) = 11.387 - 0.359X_1 + 0.585X_2$			
HighRad:	$E(y) = 11.387 - 0.462X_1 + 0.585X_2$			
Bran:	$E(y) = 11.387 - 0.383X_1 + 0.585X_2$			
Experiment 3	Adjusted $R^2 = 0.664$	$F(4,157) = 80.669$		$p < 0.001$
Predictor	Coefficient (b)	Standard Error	t(238)	p-value
Intercept	14.171	1.444	9.814	0.000
Height (X_1)	-0.420	0.033	-12.669	0.000
Diameter (X_2)	0.401	0.102	3.911	0.000
(DV1)(Height)	-0.047	0.035	-1.349	0.179
(DV2)(Height)	-0.056	0.035	-1.595	0.112
1DayOld:	$E(y) = 14.171 - 0.420X_1 + 0.401X_2$			
2DayOld:	$E(y) = 14.171 - 0.467X_1 + 0.401X_2$			
4DayOld:	$E(y) = 14.171 - 0.476X_1 + 0.401X_2$			
Experiment 4	Adjusted $R^2 = 0.564$	$F(4,184) = 61.857$		$p < 0.001$
Predictor	Coefficient (b)	Standard Error	t(184)	p-value
Intercept	15.466	1.558	9.926	0.000
Height (X_1)	-0.373	0.035	-10.409	0.000
Diameter (X_2)	0.109	0.110	0.987	0.324
(DV1)(Height)	-0.117	0.038	-3.063	0.002
(DV2)(Height)	-0.064	0.038	-1.692	0.092
NoRad:	$E(y) = 15.466 - 0.373X_1 + 0.109X_2$			
HighRad:	$E(y) = 15.466 - 0.49X_1 + 0.109X_2$			
Standard:	$E(y) = 15.466 - 0.437X_1 + 0.109X_2$			

Note 2: The treatments of each experiment are as follows: Experiment 1 had codling moth receive either no radiation (NoRad) or standard radiation (Standard), experiment 2 had the codling moth receive either standard radiation (Standard), a high radiation dose (HighRad) or standard radiation with bran-handling during transit (Bran), experiment 3 used codling moth of different ages, namely 24 hours (1DayOld), 48 hours (2DayOld) and 94 hours (4DayOld), and experiment 4 had false codling moth receive either no radiation (NoRad), standard radiation (Standard) or a high radiation dose (HighRad).

Discussion

Objective 1: Develop a laboratory based flight ability assessment method that is able to detect differences in moth quality.

A weakness of the flight cylinder method, identified by Carpenter et al. (2012), was that some moths that were able to escape from the cylinders would remain sedentary inside the cylinder until purposely startled by an observer. It was, therefore, suggested that flight cylinders did not provide a measurement of moth flight ability, but rather of their flight propensity (Carpenter et al. 2012). This lethargic behaviour meant that the escape rate of the moths were very slow, which necessitated conducting observations over relatively long periods of time, i.e. 24 h, 48 h, 64 h and 88 h (although it was eventually shown that treatment differences could already be observed at 24 h) (Carpenter et al. 2012). In an attempt to improve the escape rate of the moths, it was decided to incorporate a lure into the experimental setup. In the current study, the use of pheromone as a lure was considered, but it was assumed that it would be difficult to determine whether the pheromone was equally present in each of the cylinders, or that the pheromone was uniformly present in the room and would consequently not provide the directionality needed to lure the moths from the cylinders. Codling moths are highly attracted to light in the UV-spectrum and UV-light has been used in mass-rearing facilities to attract moths to the opening of a vacuum collector (Bloem and Bloem 1995, 2000; Bloem et al. 1998; Dyck 2010). Using light as a lure would also bypass some of the problems predicted for pheromone use, e.g. the unequal distribution of the lure in the different cylinders. . With the addition of UV-light to the experimental setup, the escape rate of the moths from the cylinders increased markedly during the preliminary trials, peaking at 8 h (Figure 6, Figure 7 and Figure 8). This increase in escape rate meant that it was possible to determine treatment differences in a third of the original time (24 hours). The UV-light lure also addressed the problem of overcoming the flight propensity of the moths. By the end of the assessment period (12 h), the number of moths that were left in the cylinders that were able to escape after being startled by an observer were negligible.

It has been specified that the performance of wild moths in QC assessments should be the baseline for what is considered ‘good quality’ (Chambers 1975; Huettel 1976; Dyck 2010), as mass-reared moths must at least match the abilities and performances of wild moths to accomplish their purpose in the field. However, Huettel (1976) conceded that this standard is difficult to apply in many cases (e.g. collecting enough wild individuals to conduct a suitable number of replications could be problematic) and suggested that untreated moths from the laboratory colony could be used as an ‘internal standard’ until the mass-reared colony is directly compared to the wild population. During September 2014 overwintering larvae were collected from the Welgevallen orchard in Stellenbosch (methods described in Giliomee & Riedl 1998) with the intention of using the wild moths emerging from the collected larvae to establish the quality standard for the flight cylinder method. Unfortunately, the wild moths emerged sparsely and sporadically, making it impossible to evaluate their performance in the flight cylinder matrix. As a result, pristine (moths with little to no physical damage), untreated mass-reared codling moth were considered the internal standard and were used during the preliminary development of the flight cylinder matrix.

The quality of a mass-reared insect is not a binomial concept (i.e. categorically either high or low quality), but falls on a spectrum between the two extremes. The use of a matrix of cylinders with a relatively wide range of heights and diameters not only allows one to determine whether a moth has the ability to fly, but how well it is able to fly, i.e. where on the spectrum of quality the flight ability of the insect is. If only one cylinder is used, e.g. a very tall and narrow cylinder that would only allow the best flyers to escape, it would only be able to indicate what percentage of the sample of moths are of exceptional quality. It would not be known whether the moths remaining in the cylinders are of very poor quality and are essentially non-flyers or whether they are of a poorer quality and could not overcome the difficulty of the selected cylinder, but are still able to fly. The 2.5 cm cylinder height was included in the matrix to identify the percentage of absolute non-flyers, as this height would only just prevent the moths from crawling out but should pose no difficulty to fly out from. The 15 cm and 26 cm cylinder heights were used to distinguish the percentage of high quality moths with good flight performance from the moths of lesser quality. These specific heights were selected from preliminary trials that aimed to identify cylinder heights that allow approximately 80%, 50% and 20% of the internal control (mass reared, untreated) moths to escape. In subsequent chapters, the results obtained from the flight cylinder assessments are correlated to the performance of the moths in the field, demonstrating the in-field difference in flight performance exhibited by moths of good quality and moths of a lesser quality.

Once the three heights and three diameters for the flight cylinder matrix were identified from preliminary tests, the matrix was used to compare the flight ability of pristine, non-radiated moths to standard radiated moths. The escape percentage of the non-radiated moths was consistently above 80% for all cylinder heights, with cylinder diameters having no significant effect on the escape percentage (Figure 9 and Figure 10). This was also reflected in the regression analysis, which showed that the null hypothesis for the factor diameter could not be disproved (Table 8). The standard radiated moths displayed the same escape percentages for cylinder heights 2.5 cm and 15 cm, which coincided with the results obtained by Carpenter et al. (2012), who also found no difference in the ability of standard reared moths and untreated moths from Entomon to escape from a 16 cm cylinder height. At a height of 26.5 cm, however, the standard radiated moths could only achieve an escape rate of 53%.

When the 26.5 cm cylinder height was combined with the narrowest diameter (10cm), the escape percentage dropped to 34%, a difference of 45% from the untreated internal control moths (Figure 10). This proved that pristine, non-radiated mass-reared moths were superior in quality as indicated by flight ability and could be used as the baseline for good quality in the absence of wild moths.

Untreated moths were not included as a treatment in the subsequent experiments, as the flight cylinder, semi-field and field release/recapture assessments were run simultaneously to allow the results obtained in each trial to be correlated to the others. This required the standardisation of treatments used for all the assessments and as untreated codling moth could not be released in the orchards, the treatment was excluded from all assessments. The standard radiated treatment was, however, included in each of the experiments, which would allow the direct comparison between the results of different experiments.

Objective 2: Evaluate the effects of factors such as age, radiation dose and handling on the flight performance of moths.

Many attributes (e.g. radiation dose, storage and handling, rearing strategy, temperatures etc.) of the mass-rearing process have been shown to have a detrimental effect on moth quality (Bloem et al. 1998; Bloem et al. 2002; Calkins and Parker 2005; Dowell et al. 2005; Robinson and Hendrichs 2005; Whitten and Mahon 2005; Simmons et al. 2010). The effects of some of these factors are well documented, e.g. the effect of radiation doses on various quality parameters (e.g. flight ability, fecundity, mating success etc.) (Bloem et al. 1998, 1999; Robinson and Hendrichs 2005; Judd et al. 2006; Suckling et al. 2007; Carpenter et al. 2012). The inclusion of different treatments of radiation dose would indicate the sensitivity of the flight cylinders, as these effects are already well known (it would act as a ‘calibration’ of the flight cylinder matrix). As mentioned, significant differences in the flight performance of non-radiated and standard radiated moths were observed in the first experiment for the flight cylinder matrix (Figure 9, Figure 10 and Table 8). The results of experiment 2 revealed that moths treated with double the standard radiation dose were able to match the escape percentage achieved by the standard radiated moths in the 15 cm high cylinders, but that a mere 14% (2.88 ± 0.872) could escape from the 26.5 cm x 10 cm cylinder. This meant that twice as many (5.66 ± 0.872) standard radiated moths were able to escape from the 26.5 cm x 10 cm cylinder and significant differences were also observed for diameters 14 cm and 16 cm (Figure 11 and Figure 13). Similar results were obtained by Carpenter et al. (2012), who observed no difference in the escape percentage of moths that were mass reared at Entomon and treated with radiation doses of 150 Gy and 300 Gy from flight cylinders with a height of 16 cm. These results are indicative of the ability of the flight cylinder matrix to differentiate between good and poor quality moths on the grounds of flight performance and also underline the importance of the use of multiple heights and diameters, as treatment differences may only be observed in taller cylinders.

Studies on the effect of age and handling procedures on moth quality have been conducted (Armes and Cooter 1991; Bloem et al. 1998, 2006a; Hughes and Dorn 2002; Nepgen et al. 2015), but these factors are not as well understood as radiation dose. These factors were selected as they could have significant implications in the mass-rearing operations, but can also be easily addressed. Previous studies (Bloem and Bloem 1995, 2000; Bloem et al. 1998, 1999; K. Bloem et al. 2002; S. Bloem et al. 2002; Jallow and Judd 2007; Dyck 2010; Carpenter et al. 2012) have shown that the excessive handling of mass-reared moths severely affected their vigour and overall quality.

In this study, the hypothesis that transporting moths in bran would prevent or at least decrease quality degradation during handling was tested. In theory, the bran should discourage movement of moths that could lead to physical damage, and isolate the moths to prevent overheating. However, the process of mixing the moths into the bran could lead to an excessive loss of scales and damage to the wings and antennae, affecting their flight ability. The outcomes of experiment 2 did not report any discernible influence of transportation in bran (either positive or negative) on the flight performance of mass-reared codling moth, as there was no statistical difference between the escape percentages achieved under standard handling protocol and the bran-handling protocol (Figure 11, Figure 13 and Table 8)

By determining the influence that moth age and the length of time kept in cold storage has on moth flight performance, mass-rearing facilities can evaluate options such as the stockpiling moths over short periods until a postponed release date or transporting moths over great distances to other SIT programmes or release sites. Experiment 3 of the flight cylinder trials compared the flight performances of moths that were aged in cold storage for 24 h, 48 h and 96 h (1, 2 and 4 days), and found no significant differences between the three treatments (Table 6 and Table 8). This differed from the results observed by a number of other studies (Leopold 2000, 2007; Calkins and Parker 2005; Parker 2005; Jallow and Judd 2007; Dyck 2010), which all found that storing chilled insects for extended periods (>12 h) decreased the quality of the insects. Other studies (Bloem et al. 2006a; Blomefield et al. 2011; Chidawanyika and Terblanche 2011b) have reported observations similar to the results obtained in the flight cylinder matrix. All of the above mentioned studies did not use flight cylinders for their trials, but the effect of cold storage on the quality of the moths were still observed.

The results for the age treatments in this study appear to suggest that the handling procedures and radiation dose applied to moths chilled for extended periods were responsible for the decrease in quality, which was also observed in a number of other studies (Bloem and Bloem 1995, 2000; Bloem et al. 1998; K. Bloem et al. 2002; S. Bloem et al. 2002) and that moths that had minimal handling and were stored at constant low temperatures would not suffer a significant decrease in flight ability and quality. The height x diameter interaction in experiments 2 and 3 indicated that the combination of 26.5 cm height and 10 cm diameter is the best indicator of good moth quality (as indicated by flight ability), as it had the lowest number of moths escaping (Figure 12 and Figure 14). For experiments 1 and 4, height was the only determining factor for moth escape, and diameter had no noticeable effect (Figure 10).

Objective 3: Determine whether the flight cylinder assessment method developed for codling moth can be used for false codling moth.

Interest in adopting SIT for control of lepidopteran pests has increased decidedly over the last decade (Hofmeyr et al. 2005; Dyck et al. 2005; Vreysen et al. 2007; Vreysen et al. 2010), but this interest might not come to anything if the current programmes that exist for lepidopteran SIT are not streamlined nor their cost-effectiveness improved (Simmons et al. 2010). Consequently, it was decided to evaluate the efficacy of the flight cylinder method as a QC test for another mass-reared species, namely the false codling moth, to examine whether the method has wider application potential. Different doses of radiation were once again used to degrade the quality of the moths (Bloem et al. 2004; Vreysen et al. 2007; Carpenter et al. 2010).The flight cylinders proved able to differentiate between good quality false codling moths and those of degraded quality.

Experiment 4 revealed that untreated mass-reared moths outperformed the moths that received standard and high radiation doses, achieving an escape rate of 34% (6.85 ± 1.47) in the 26.5 x 10 cm cylinder, compared to the 18% (3.57 ± 1.47) and 25% (5.14 ± 1.47) of the high radiation and standard treatments respectively (Figure 15 and Figure 16). Interestingly, the untreated false codling moths had a substantially lower escape percentage (57%, 11.42 ± 0.49) compared to the untreated codling moth, which achieved a minimum escape percentage of 87% (17.52 ± 1.02) in all of the cylinders (Figure 11 and Figure 15). Also, no difference in quality could be observed between standard radiation and high radiation treatments (Figure 16), as was observed for codling moth. The reason for the apparent poor performance of false codling moth in the flight cylinder assessment is the high variation in the results obtained. The flight performance of the false codling moth fluctuated from one extreme to the next in consecutive replications (significantly more so than for the experiments with codling moth), which impacted the overall mean number of escaping moths. This can also be observed in the relative low adjusted R^2 value (0.56) obtained from the regression analysis, which suggested that only 56% of the variation in the data for the false codling moth experiment could be accounted for by the variables included in the model. The cause of the fluctuating performance (and, therefore, the variation that is unaccounted for in the model), is not known, but could be attributed to the long transportation period (two to three hours between Citrusdal and Stellenbosch), accompanied by excessive handling (Nepgen et al. 2015).

A note on the effect of replications on the results

During the analysis of the results, the effect of replication date was also investigated and a significant effect was found for each of the experiments. The regression analyses for experiments 1, 2 and 3 indicated that this variation did not impact the ability of the model to predict the number of moths escaping, as relatively high adjusted R^2 values were attained (0.84, 0.70 and 0.66 respectively), but might have affected the results for the false codling moth experiment, as mentioned earlier (Table 8). Replication effects have also been observed in other studies (Chidawanyika and Terblanche 2011a; J. Carpenter et al. 2013) and emphasise the importance to conduct QC tests on a regular basis, as the quality of moths differ from day to day. The trend of the performance of the different treatments remained the same (i.e. more high quality moths were able to escape compared to moths of poor quality) even though the overall escape percentages of moths for a particular day were higher or lower. An example of variation of moth escape can be viewed in Figure 17 (data from experiment 2). Even though there might have been significant differences between the numbers of moths escaping during some of the replications, there was never an interaction with the treatments applied in the experiment. Thus, the flight cylinder method was able to differentiate between the quality degradation caused by a specific treatment applied to the cohort of moths and the lack of quality inherent in that day's batch of moths.

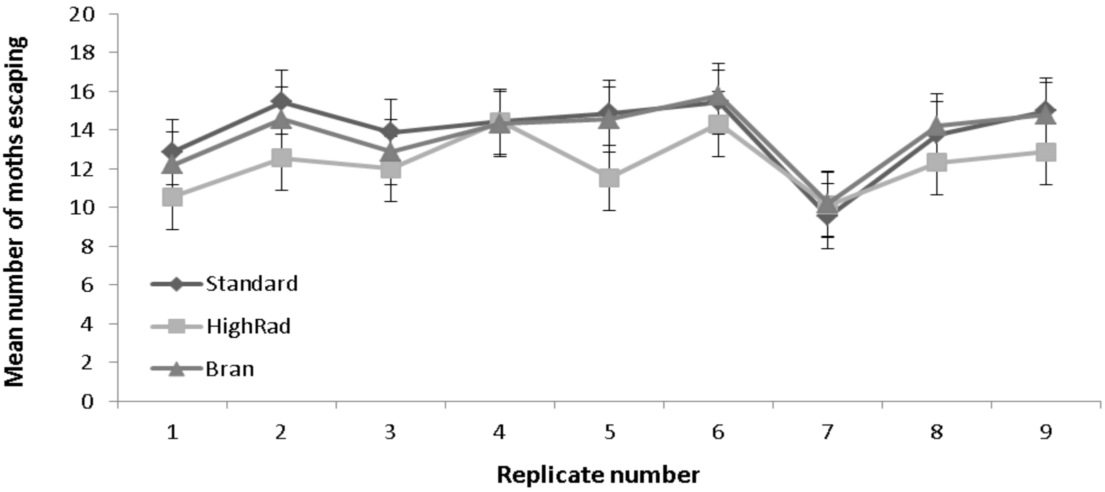


Figure 17: The mean number (±SE) of codling moth males (n = 20) escaping from a nine-cylinder matrix for each of nine replicates. Moths were treated with either one of three different radiation and handling treatments. The cylinder matrix consisted of the combinations of three heights (26.5 cm, 15 cm and 2.5 cm) and three diameters (10 cm, 14 cm and 16 cm) and the assessment lasted eight hours ($F_{16,216} = 0.173$, $p = 0.999$). SE: standard error.

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CHAPTER 3 : FLIGHT TUNNEL ASSESSMENT

Introduction

The success of a codling moth and false codling moth SIT programme depends on the release of high quality sterile mass-reared moths. The ultimate measure of quality is the ability of the sterile moths to compete with wild individuals for mating opportunities and the infusion of sterility into the wild population (Robinson and Hendrichs 2005; Carpenter et al. 2010). To achieve the constant production of high quality competitive insects, quality standards need to be established and applied for key parameters using reliable QC tests (Singh and Ashby 1985; Leppla and Ashley 1989; Leppla 1993; Boller 2002; Cohen 2004; Parker 2005; Simmons et al. 2010).

QC tests are divided into routine and periodic quality tests. Routine tests are relatively simple, small-scale methods that can be performed regularly at the mass-rearing facility to assess a specific quality parameter. Periodic tests are more complex as they are performed on a larger scale (usually in-field testing) (Stewart 1984; Dyck 2010). Traditionally, field assessments are considered to be the ultimate indicator of moth quality as they encompass the complete efficacy of the mass-reared moth in its intended role and are widely used by SIT programmes to evaluate moth performance (Bloem et al. 1994, 1998). However, these tests are expensive, time consuming, labour intensive and subject to environmental conditions (Stewart 1984; Dyck 2010). Laboratory assessments generally involve the isolation of a single parameter, evaluating the expression of this parameter by the mass-reared insect in an assay that controls all other factors and then extrapolating the quality of the insect from the results gained from the assays (Robinson and Hendrichs 2005).

Recently, the need for an intermediate assessment method that links laboratory and field tests has been identified (Vreysen et al. 2010; Carpenter et al. 2013). These ‘semi-field’ tests should ideally be conducted on a larger scale that incorporates some of the fluctuating environmental factors that would be faced in the field and allow the insect to express normal behaviour (Robinson and Hendrichs 2005; Vreysen et al. 2006), but it should also be robust enough in the evaluation of a specific trait that it can be used to determine quality and it should be more readily applicable than field-level assessments. If successful, semi-field tests would contribute enormously to the improvement of QC at mass rearing facilities and should be considered a priority when developing a QC protocol at an insect mass-rearing facility (Simmons et al. 2010). A number of semi-field assessments have been developed for other mass-reared insect species. Most notable is the field-cage method where a net cage is constructed in an orchard, in some cases including at least one tree, into which a number of mass-reared insects are released (FAO/IAEA/USDA 2003; Vreysen 2005; Vreysen et al. 2006; Carpenter et al. 2013).

Parameters such as mating competitiveness have been evaluated successfully for fruit and tsetse fly (Mutika et al. 2001; FAO/IAEA/USDA 2003; Robinson and Hendrichs 2005) and similar field-cage trials have been conducted for other mass reared lepidoptera (Miller et al. 1994; Hofmeyr et al. 2005; Simmons et al. 2010). There has, however, been a lack of flight performance assessments conducted using this method. Semi-field tests that focused on lepidopteran flight performance include wind tunnels, used by Suckling et al. (2011) to successfully distinguish between treatments applied to painted apple moth and a simple flight test for cactus moth that involved the release of moths in a way similar to that employed in the field and measuring the distance covered in a single flight (Lopez-Martinez et al. 2014). In the wind tunnel, the painted apple moth males had to fly towards a calling female in wind speeds of 25-30 cm/s, and the flight ability was expressed by the successful arrival of the male at the calling female.

This study aimed to develop a semi-field flight performance test in a horticultural type tunnel fitted with an extraction fan at one end. The premise of the test would be similar to that of the smaller wind tunnel (i.e. male moths would be released at one end and must fly up-wind to a sex pheromone source) (Suckling et al. 2011), but it would incorporate some characteristics of the field-cage method, such as an increase in scale of the experiment and the exposure to fluctuating conditions of ambient temperature and light intensity.

Objectives for this chapter

1. To develop a semi-field flight ability assessment method that is able to detect differences in moth quality.
2. To evaluate the effects of factors such as age, radiation dose and handling on the flight performance of moths.
3. To determine whether the semi-field assessment method developed for codling moth can be used for false codling moth.

Material and methods

Experimental logistics

Insect material

Codling moth were obtained from Entomon, the insect rearing facility of Stellenbosch University in Stellenbosch, Western Cape, South Africa. The colony was established in 2002 from larvae collected from infested apples in the Elgin area, Western Cape. The codling moth were reared on an agar-based diet according to the methods of Stenekamp (2011) and collected using similar methods to those described by Dyck (2010), i.e. moths were attracted to a UV-light placed in the opening of a large tube (diameter 20 cm) through which they were transported with airflow to a plastic collection tray (60 cm x 40 cm x 20 cm) in a cold room (2.5 m x 3 m, 3-5°C). wild individuals (overwintering pupae) were introduced into the existing colonies to avoid inbreeding depression and to diversify the genetic make-up of the colony.

Location of the experiments

These semi-field assessments were conducted in a horticultural type tunnel located at the Department of Horticulture at Stellenbosch University. The tunnel had a stainless steel tubing framework (15 m x 4 m, with the arch height at 2.4 m) and was covered in a UV-resistant, semi-transparent plastic (0.5 mm thick), which allowed for natural light and temperature conditions inside the tunnel. An electric extractor fan (diameter 52cm) was located at the back of the tunnel.

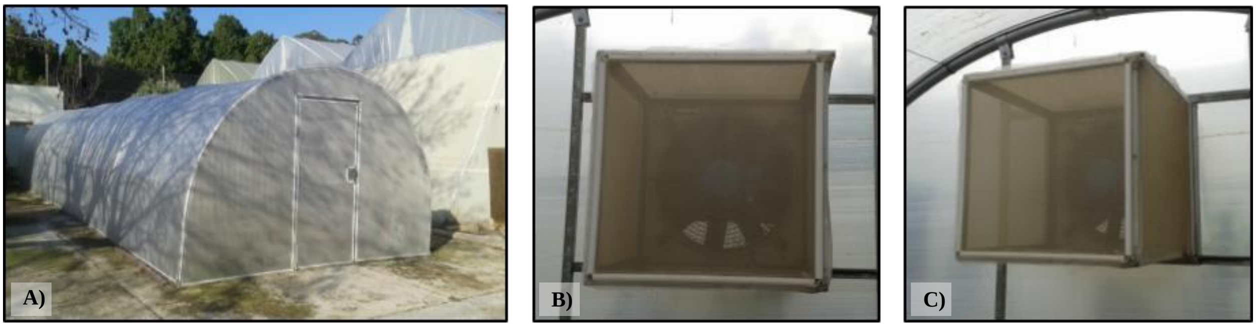

Figure 18: A) The horticultural type tunnel (15 m x 4 m, with the arch height at 2.4 m) at the Department of Horticulture at Stellenbosch University, which was used in the semi-field flight tunnel experiments. B & C) The extractor fan (diameter 52 cm), covered with a mesh box to prevent moths from escaping, mounted at the back of the tunnel.

Handling and transportation of the insects

A ‘cold-chain’ series of handling activities is standard practice at most SIT facilities, as it ensures that the quality of the insects is not diminished during preparation and transportation (Dyck 2010). Moths were kept chilled throughout the processes of collection, storage and sorting at the mass-rearing facilities. During transit and radiation, moth-filled Petri dishes (plastic, 150 x 10 mm) were placed in polystyrene containers (400 x 300 x 300 mm) with icepacks. The Petri dishes were isolated from the icepacks with pieces of cardboard to prevent the direct exposure of the moths to the freezing temperature of the icepacks. The cardboard sections also prevented the excessive movement of the Petri dishes inside the container during transportation.

Radiation procedure

Moths were radiated at Fruit Fly Africa in Stellenbosch. The polystyrene container holding the Petri dishes with the moths was placed on one of the smaller turntables (diameter 20 cm) imbedded in a larger turntable (diameter 1.2 m). The larger turntable revolved around the panoramic Cobalt^{60} point source that delivered a radiation rate of 7.67 Gy/min. The smaller turntable counter-rotated to ensure a 360° treatment of the Petri dishes.

Experiments and treatments

Two experiments were conducted using the flight tunnel assessment method. These experiments aimed to evaluate the effect of radiation dose, age and handling during transport on the flight ability of the mass reared moths. The treatment specifications of each experiment were as follows (see summary in Table 9):

Experiment 1: Radiation dose and bran-handling

For each of the three treatments, 32 g (approximately 1000 individuals) of chilled 24-hour-old male moths were collected in a Petri dish (150 x 10 mm). The standard and bran treatments received the standard radiation dose (150 Gy) and the high radiation (HighRad) treatment received double the standard radiation dose (300 Gy). The bran treatment moths were then mixed (by hand) with 1ℓ of bran (food grade wheat bran) until an even distribution of moths inside the bran was achieved (after about 1 minute of mixing).

Experiment 2: Age

For the third experiment, 32 g (approximately 1000 individuals) of chilled 24-hour-old male moths were collected in a Petri dish (150 x 10 mm) and stored in a cold room (< 6°C). This was repeated every day for four consecutive days. After four days, the 24 h (1DayOld), 48 h (2DayOld) and 96 h (4DayOld) moths received the standard radiation dose (150 Gy).

After radiation, the moths of each treatment were sorted according to sex until 150 males were identified and placed in a Petri dish (100 x 10 mm). The Petri dish containing the bran treatment moths were filled with bran before transportation. Three colours of fluorescent powder (pink, green and blue) were used to mark the different treatments before their release into the tunnel. Both experiments 1 and 2 had seven replications and were conducted in October and November 2014, which coincided with the codling moth laboratory and field trials (Chapters 2 and 4).

Table 9: Summary of the treatments used in the flight tunnel assessment. Male codling moths were exposed to various treatments of age (days after eclosion), radiation dose and handling to produce moths of different quality.

Experiment 1	Age of moths	Radiation dose	Transportation of moths
Standard	<24 h	150 Gy	Standard
HighRad	<24 h	300 Gy	Standard
Bran	<24 h	150 Gy	In bran mix
Experiment 2	**Age of moths**	**Radiation dose**	**Transportation of moths**
1DayOld	<24 h	150 Gy	Standard
2DayOld	24–48 h	150 Gy	Standard
4DayOld	72–96 h	150 Gy	Standard

Semi-field tunnel assessment

At the end of the tunnel opposite to the extraction fan, a 220V/8W black light (TL 4W BLB 1FM, Philips, South Africa) was mounted vertically above a flat surface (raised 60 cm off the ground). White cardboard (420 x 594 mm) provided a backing for the black light (placed perpendicular to the flat surface) and two sticky pads (20 cm x 20 cm, Chempac, South Africa) were placed at the base of the white cardboard beneath the black light. This setup was used as a light trap, with the moths being lured toward the black light and trapped on the sticky pad. A camera (model MBS-BC1-02, Moreton Bay Systems, Australia) was mounted 40 cm above the two sticky pads and programmed to take an image every 10 minutes, thereby capturing the arrival of the moths at the sticky pads of the light trap.

At the start of the assessment, 150 moths of each treatment in the set were released from a flat plastic surface (raised 1 m off ground) that was placed 12 m from the black light along the long axis of the tunnel. The moths were released 30 minutes before sunset and the assessment was conducted over 12 hours. Two temperature loggers (Model DS1921G iButton, ColdChain Thermodynamics, Fairbridge Technologies, South Africa), one placed at the release point of the moths and the other at the light trap,recorded the temperature in the tunnel throughout all the trials. During preliminary trials conducted in the tunnel, the extraction fan proved unable to produce uniform airflow of significant strength and was, therefore, not utilized in the assessments.

Moths caught on the sticky pads were visually assessed under a black light, which revealed the colour pigment the treatments were marked with. The number of moths of each treatment caught and the time of their arrival at the trap were determined by assessing the sticky pads and photographs together.

Statistical analysis

The raw data were recorded as the number of moths of each treatment arriving at the light trap every 10 minutes. Initial investigation of the data indicated that the number of moths arriving at the light trap after 180 minutes (three hours) were negligible. Consequently, it was decided to focus on the data of the first 180 minutes, grouping the data into six 30-minute intervals (therefore adding the number of moths of each treatment arriving at the light trap in each 30-minute slot). The data for each 30 min slot was calculated separately, and not cumulatively.

ANOVA

The derived data were analysed using a mixed model repeated measure ANOVA. The dependent variable was the number of moths arriving at the light trap, with ‘treatment’, ‘time’ and the interaction of ‘treatment x time’ as the independent variables. For each analysis the normality of data distributions was checked. The data for experiment 1 met the assumptions, but the data of experiment 2 required a log-transformation to achieve normality. The difference in the analysis results between transformed and untransformed data was negligible and, therefore, the results of the untransformed data of experiment 2 were reported. Fisher’s LSD post hoc tests were used to separate means at $p = 0.05$. The effect of replicate date on the number of moths caught was also tested for each experiment using factorial ANOVA. No replication effect was found. The data analysis software used was STATISTICA 12 (StatSoft, USA).

Results

Experiment 1: Radiation dose and bran-handling

The main effect ‘treatment’ was not significant, as the number of standard, HighRad and bran-treated moths that arrived at the light trap did not differ statistically. Moth arrival was significantly influenced by ‘time’. A significant increase in the number of moths arriving at the light trap for time periods 30, 60, 90 and 120 min were recorded, but moth arrival decreased markedly for periods 150 and 180 min and was statistically the same as for time periods 30 and 60 min (Figure 19). The interaction factor ‘treatment x time’ had no significant influence on moth arrival, which indicated that there was statistically no difference in the time it took the moths of each treatment to reach the light trap. See Table 10 for F-statistics and significance values of effects.

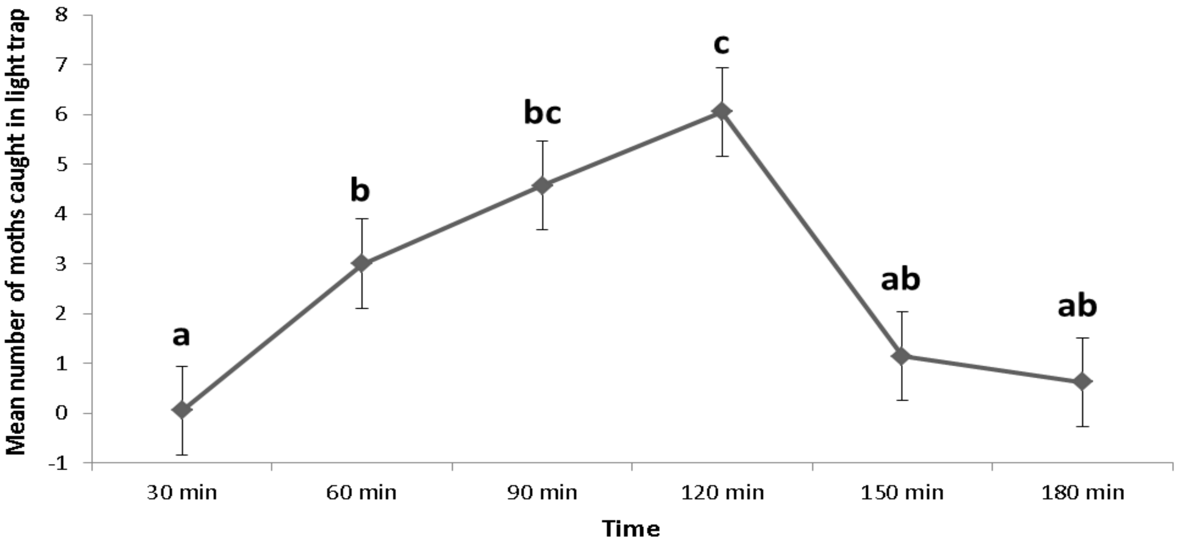

Figure 19: The mean number (±SE) of codling moth males (initial n = 150) of three different radiation and handling treatments that arrived at the light trap during each 30-minute time period (total time = 180 min). The flight assessment took place inside a horticultural type tunnel (dimensions 15 m x 4 m x 2.5 m), with the light trap spaced 12 m from the release point of the moths. The mean numbers at each time period with the same letters are not significantly different ($F_{5,90}$ = 7.133, $p < 0.001$). The experiment was replicated seven times in October and November 2014. SE: standard error.

Table 10: The repeated measure factorial analysis of variance results for the number of codling moth males (initial n = 150) of three different radiation and handling treatments to arrive at the light trap during each 30-minute time period (total time = 180 min). The flight assessment took place inside a horticultural type tunnel (dimensions 15 m x 4 m x 2.5 m), with the light trap spaced 12 m from the release point of the moths. Num. d.f.: degrees of freedom of the numerator; Den. d.f.: degrees of freedom of the denominator.

Effect	**Num. d.f.**	**Den. d.f.**	**F**	**p**
Treatment	2	18	0.988	0.391
Time	5	90	7.133	0.000
Treatment x Time	10	90	0.327	0.971

Experiment 2: Ages

There was statistically no difference in the number of 1DayOld, 2DayOld and 4DayOld treated moths that arrived at the light trap. Moth arrival at the light trap was again significantly affected by time, as for experiment 1. Unlike the increase in moth arrival over time seen in experiment 1, the number of moths arriving at the light trap during each time period was statistically similar except for 120 min, which yielded a significantly higher result (Figure 20). See Table 11 for F-statistics and significance values of effects.

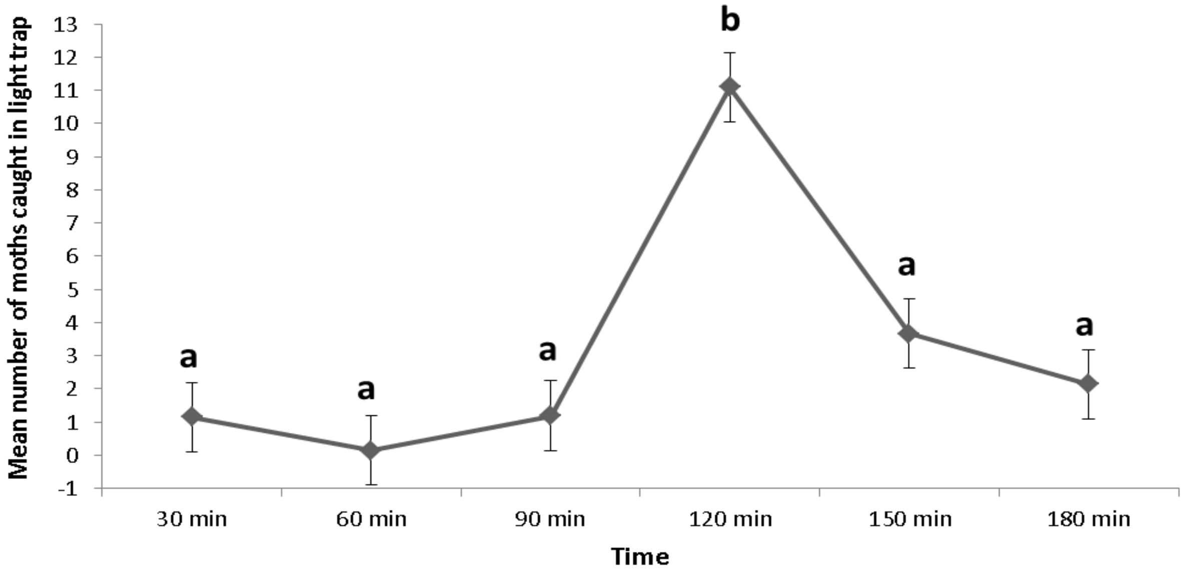

Figure 20: The mean number (±SE) of codling moth males (initial n = 150) of three different age treatments that arrived at the light trap during each 30-minute time period (total time = 180 min). The flight assessment took place inside a horticultural type tunnel (dimensions 15 m x 4 m x 2.5 m), with the light trap spaced 12 m from the release point of the moths. The mean numbers at each time period with the same letters are not significantly different ($F_{5,90}$ = 14.907, $p < 0.001$). SE: standard error.

Table 11: The repeated measure factorial ANOVA results (with means and standard deviation values) for the number of codling moth males (initial n = 150) of three age treatments to arrive at the light trap during each 30-minute time period (total time = 180 min). The flight assessment took place inside a horticultural type tunnel (dimensions 15 m x 4 m x 2.5 m), with the light trap spaced 12 m from the release point of the moths. Num. d.f.: degrees of freedom of the numerator; Den. d.f.: degrees of freedom of the denominator.

Effect	**Num. d.f.**	**Den. d.f.**	**F**	**p**
Treatment	2	18	0.271	0.765
Time	5	90	14.907	0.000
Treatment x Time	10	90	0.663	0.755

Discussion

Objective 1: Develop a semi-field ability assessment method that is able to detect differences in moth quality.

Semi-field tests are very valuable, as they act as a link between laboratory and field-level assessments (Simmons et al. 2010; Suckling et al. 2011). For this chapter, it was decided to investigate the potential of using a horticultural type tunnel (with a built-in extractor fan at one end) in a semi-field assessment that resembles a large scale wind tunnel. The tunnel provided an opportunity to evaluate the flight of the moths under environmental conditions closer to those found in-field, as temperature and light intensity would fluctuate naturally and the fan would create air flow that would simulate wind. The moths would also be evaluated on their ability to engage in normal behaviour (perceive a lure and undertake normal flight over a distance of 12 m), as opposed to, for example, a tethered flight mill, which severely limits the ability of the insect to express normal behaviour (Stewart 1984; Dyck 2010). The distance of 12 m was selected as moths would not need to cover greater distances in the field to achieve a relatively even distribution in the orchards (generally, the spacing between releases of sterile insect in orchards are no further than 30 m) (Bloem et al. 1998).

The possibility of using the fan installed at the back of the tunnel to create air flow in the tunnel was evaluated in preliminary trials. The air flow would encourage the directionality of moth flight and add to the difficulty of reaching the trap (Suckling et al. 2011). However, the fan proved too weak and the tunnel too large to attain uniform air flow of significant strength and, therefore, the fan was excluded from further trials. Consequently, it was decided to evaluate the time it took moths of different quality to reach a trap placed at a distance of 12 meters from the release point, without the presence of airflow.

In order to evaluate distance covered, the moths would need to be attracted to a specific point by using a type of lure. Several lures were tested in the preliminary trials, including synthetic pheromone lures, calling virgin female lures, host plants (small apple trees) and UV-light. The pheromone and calling female lures (with and without the addition of host plants) did not attract enough males to allow the comparison of flight ability and without air flow the pheromone was ineffective. The UV-light acted as a strong attractant and attracted many moths simultaneously. This produced results within 12 hours and provided the directionality of flight necessary for this assessment. The addition of the camera (which captured an image of the trap every 10 minutes) simplified the test considerably and negated the need for an observer to document the arrival times of the moths over several hours.

Objective 2: Evaluate the effects of factors such as age, radiation dose and handling on the flight performance of moths.

It was hypothesised that good quality moths would reach the trap sooner than moths of poor quality as they would recover faster and would be able to cross the 12 m distance more readily (Lopez-Martinez et al. 2014). However, it would seem that the abilities of the quality degraded moths were underestimated, as there were no significant differences between the arrival times of moths of different treatments. No differences were obtained between the age groups (which was not wholly unexpected, as this was observed in the results of the flight cylinder method as well (Table 6)), but the lack of difference obtained for the radiation treatments indicated that the tunnel was not robust enough to be used as a QC test, as the quality differences between moths of different radiation doses are well documented (Bloem et al. 1998, 1999; Robinson and Hendrichs 2005; Judd et al. 2006; Suckling et al. 2007; Carpenter et al. 2012) and were observed in the flight cylinder assessments. The cactus moth flight ability study of Lopez-Martinez et al. (2014) was conducted on a similar scale and in that study no difference could be detected between the flight distances of radiated and non-radiated cactus moths. It would appear, therefore, that distance alone does not provide enough of a challenge to the flight ability of the moths and that even moths of degraded quality are able to fly the necessary distance in ideal conditions.

Objective 3: Determine whether the semi-field assessment method developed for codling moth can be used for false codling moth.

As the method did not prove effective for codling moth, it was not conducted with false codling moth. The development of an effective semi-field flight assessment method is, therefore, still incomplete.

Concluding remarks

Although the tunnel flight assessment could not differentiate between the treatments, it did confirm that flight activity peaked at one to two hours after sunset (Figure 19 and Figure 20) and could possibly be used in future studies to evaluate the activity of moths in ambient temperature and light conditions. With further investigation and development, the flight tunnel assessment method could still prove to be an effective quality control test. Possible modifications to the experimental setup that might improve the robustness of the test is the inclusion of air flow and/or an increase in distance between the point of release and the trap. Including air flow would require using a smaller tunnel and stronger fan that could provide a uniform air flow that is strong enough to challenge the flight ability of the moths, as seen in smaller wind tunnel experiments (Kainoh 2011; Suckling et al. 2011).

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CHAPTER 4 : FIELD RELEASE/RECAPTURE METHOD

Introduction

The in-field recapture of released sterile insects is generally considered to be the definitive measure of moth competitiveness, as it requires a sterile male moth to perceive pheromone cues and to be able to follow these cues by directional flight to a pheromone-baited trap (Bloem et al. 1998, 1999, 2004; Bloem and Carpenter 2001; Keil et al. 2001; Robinson and Hendrichs 2005; Dyck 2010). Insect quality in the context of SIT programmes is defined as the ability of sterile insects to compete with the wild male population for mating opportunities (Huettel 1976; Bloem et al. 1998, 2004; Robinson and Hendrichs 2005; M. Vreysen et al. 2007) and the field release/recapture assessment provides a good approximation of quality. The use of mating tables instead of synthetic pheromone dispensers can provide an improved estimation of male competitiveness, as the pheromone released by the female is much less than the artificial lures and the mating success of the sterile male with the tethered female can be directly observed (Bloem et al. 2004). This method is, therefore, widely used to assess moth flight performance and quality (Bloem et al. 1994, 1998, 2004; Judd et al. 2006).

Large scale QC assessments (such as the release/recapture method) are known as periodic tests, as these tests are too expensive, time consuming and labour intensive to conduct on a regular basis (Stewart 1984; Dyck 2010). Consequently, it is of utmost importance that the results obtained from laboratory-based routine QC assessments are correlated to the performance of the mass-reared moths in the field (Leppla and Ashley 1989; Leppla 1993; Mutika et al. 2001; Calkins and Parker 2005; Dyck et al. 2005; Dyck 2010; Simmons et al. 2010).

Generally, field-based assessments of moths involves releasing a known number of marked males from a release point in an orchard and then assessing the number of marked males recaptured in a trapping grid of traps baited with a source of sex pheromone (i.e. either calling virgin females or synthetic pheromone lures) (Bloem et al. 1998, 1999, 2004; Bloem and Carpenter 2001; Keil et al. 2001; Dyck 2010). Passive interception, light traps or food-based lures can also be used (Weissling and Knight 1994), but as the success of SIT depends on the ability of sterile males to locate calling females, most studies are conducted with pheromone-baited traps. It is exceedingly beneficial that the mass-reared sterile moths can easily and unmistakably be distinguished from wild insects in traps, as data from traps are used to determine whether the over-flooding ratio (40:1 sterile male codling moth to wild male codling moth) is reached in the field and to directly monitor the size of the wild population (Robinson and Hendrichs 2005). To accomplish this, a dye is added to the diet of the larvae in the mass rearing facility. When trapped insects are brought in from the field, their origin is determined by examining them for the presence of the dye (Robinson and Hendrichs 2005; Parker 2005).

No standardised protocol has yet been developed for the release/recapture method, with each researcher adapting the design to the needs of their study. There are many factors that vary between the different studies that used the field release/recapture method, including size of the assessment area, type of trap and lure used, trap distance and density, the number of released moths and the release method, duration of the assessment and the intervals for traps servicing. As the design is determined by the aim of the experiment and the parameter it is evaluating, not one release/recapture assessment has identical protocols. It does, however, pose a serious challenge when comparing the results of two different trials. Environmental factors (e.g. wind speed and direction, temperature and presence of predators) and orchard characteristics (e.g. tree density, slope and pest control strategies applied) also affect the in-field performance of the released moths and can differ considerably from one orchard to the next (Khaliq et al. 2014). These external factors could, therefore, bias the outcomes of the assessments and give a false impression of the quality of the mass-reared insects. Developing a standardised method could contribute to the improvement of QC at all mass-rearing facilities for a specific species, as it would be possible to compare the results of different populations, shed light on which environmental factors have the greatest effect on moth performance in the field and it would enable the establishment of a quality standard for mass-reared moths that is crucial if international shipments are to be considered (Enkerlin and Quinlan 2004; Robinson and Hendrichs 2005).

Objectives for this chapter

1. To determine whether field release/recapture assessments are able to detect differences in moth quality.
2. To evaluate the effects of factors such as age, radiation dose and handling on moth performance in field.
3. To determine whether the release/recapture assessment developed for codling moth can be used for false codling moth.

Material and methods

Experimental logistics

Insect material

Codling moth were obtained from Entomon, the insect rearing facility of Stellenbosch University in Stellenbosch, Western Cape, South Africa. The colony was established in 2002 from larvae collected from infested apples in the Elgin area, Western Cape. The codling moth were reared on an agar-based diet according to the methods of Stenekamp (2011) and collected using similar methods to those described by Dyck (2010), i.e. moths were attracted to a UV-light placed in the opening of a large tube (diameter 20 cm) through which they were transported with airflow to a plastic collection tray (60 cm x 40 cm x 20 cm) in a cold room (2.5 m x 3 m, 3-5°C).

False codling moth were obtained from X-SIT (Pty) Ltd., a mass-rearing facility situated in Citrusdal, Western Cape, that has been in operation since 2007. The larvae were reared on an artificial diet described in Moore et al. (2014), at a temperature of 25°C with 50% relative humidity and a photoperiod of 12 h:12 h (L:D). At both Entomon and X-SIT, wild individuals (overwintering pupae) were introduced into the existing colonies to avoid inbreeding depression and to diversify the genetic make-up of the colony.

Location of experiments

The field experiments were conducted in 1-hectare orchards at three different locations, namely a pear orchard situated on a slope at Welgevallen (WEL) in Stellenbosch and a covered (c) and uncovered (u) apple orchard (both on relatively level ground) at Oak Valley Estate (OVE) in Elgin (WEL = S 33° 56’ 52.696”, E 18° 52’ 23.286”; OVE(c) and OVE(u) = S 34° 8” 44.07”, E 19° 3’ 30.373”) (Figure 21). The covered apple orchard had a closed canopy and walls of fine netting that were used to protect the fruit from sunburn. The WEL orchard was planted in 1998 with a 4.5 m inter-row and 1.25 m in-row spacing of the trees. The OVE orchards were planted in 2010 with a 3.5 m inter-row and 1.3 m in-row spacing of the trees. OVE(u) was treated with pheromone disruption, whereas the other two orchards were managed with chemical control.

Handling and transportation of the insects

A ‘cold-chain’ series of handling activities is standard practice at most SIT facilities, as it ensures that the quality of the insects is not diminished during preparation and transportation (Dyck 2010). Moths were kept chilled throughout the processes of collection, storage and sorting at the mass-rearing facilities. During transit and radiation, moth-filled Petri dishes (plastic, 150 x 10 mm) were placed in polystyrene containers (400 x 300 x 300 mm) with icepacks. The Petri dishes were isolated from the icepacks with pieces of cardboard to prevent the direct exposure of the moths to the freezing temperature of the icepacks. The cardboard sections also prevented the excessive movement of the Petri dishes inside the container during transportation.

Radiation procedure

Moths were radiated at Fruit Fly Africa in Stellenbosch. The polystyrene container holding the Petri dishes with the moths was placed on one of the smaller turntables (diameter 20 cm) imbedded in a larger turntable (diameter 1.2 m). The larger turntable revolved around the panoramic Cobalt^{60} point source that delivered a radiation rate of 7.67 Gy/min. The smaller turntable counter-rotated to ensure a 360° treatment of the Petri dishes.

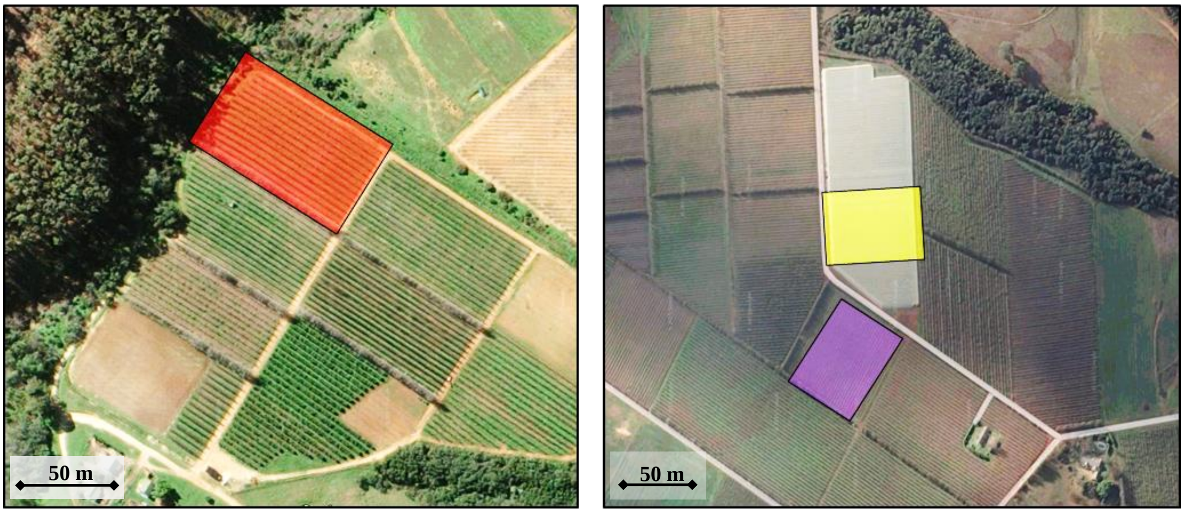

Figure 21: Aerial photographs of the Welgevallen (WEL) orchard in Stellenbosch (indicated by the red square) and covered (c) and uncovered (u) orchards (indicated by yellow and purple squares respectively) at Oak Valley Estate (OVE) in Elgin. Coordinates: WEL = S 33° 56’ 52.696”, E 18° 52’ 23.286”; OVE(c) and OVE(u) = S 34° 8” 44.07”, E 19° 3’ 30.373”.

Experiments and treatments

Three experiments were conducted using the field release/recapture assessment method. Codling moth were used for experiments 1 and 2 and false codling moth for the third experiment. These experiments were conducted to assess the effect of radiation dose, age and handling during transport on the flight ability of the mass-reared moths. The treatment specifications of each experiment were as follows (see summary in Table 12):

Experiment 1: Radiation dose and bran-handling

In the first experiment, three Petri dishes (150 x 10 mm) for each treatment were filled with 32 g (approximately 1000 individuals) of chilled 24-hour-old male moths. The standard and bran treatments received the standard radiation dose (150 Gy) and the high radiation (HighRad) treatment received double the standard radiation dose (300 Gy). The bran treatment moths were then mixed (by hand) with 1ℓ of bran (food grade wheat bran) until an even distribution of moths in the bran was achieved (after approximately 1 minute of mixing). The moths (still in the bran mix) were then placed in a 1ℓ container in which they were transported to the field.

Experiment 2: Ages

For the second experiment, three Petri dishes (150 x 10 mm) for each treatment were filled with 32 g (approximately 1000 individuals) of 24-hour-old moths and stored in a cold room (<6°C). This was done every day for four consecutive days. After the four days, the 24-hour-, 48-hour- and 96-hour-old moths received the standard radiation dose (150 Gy).

Experiment 3: Radiation dose (false codling moth)

In the false codling moth experiment, three Petri dishes (150 x 10 mm) for each treatment were filled with 19 g (approximately 1000 individuals) of chilled 24-hour-old male moths. The non-radiated (NoRad) treatment did not receive any radiation, the standard treatment was treated with 150 Gy and the high radiation (HighRad) treatment received double the standard radiation dose (300 Gy).

In each experiment, three colours of fluorescent powder (pink, green and blue) were used to mark the different treatments before their release into the cylinders. The moths were marked by placing 1 mg of coloured powder into the Petri dish of the specific treatment, and then slowly and gently rotating the Petri dish until the powder has dispersed evenly within the Petri dish.All three of the experiments were repeated three times. The codling moth field assessments were conducted from October to December 2014, which coincided with the codling moth laboratory and semi-field trials (Chapters 2 and 3). The false codling moth assessments were conducted in March and April 2015.

Table 12**:** Summary of the treatments used in the field release/recapture assessments. The moths were exposed to various treatments of age (days after eclosion), radiation dose and handling to produce moths of different quality. Male codling moths were used in experiments 1 and 2 and male false codling moths were used in experiment 3.

Experiment 1	Age of moths	Radiation dose	Transportation of moths
Standard	< 24 h	150 Gy	Standard
HighRad	< 24 h	300 Gy	Standard
Bran	< 24 h	150 Gy	In bran mix

Experiment 2	Age of moths	Radiation dose	Transportation of moths
1DayOld	< 24 h	150 Gy	Standard
2DayOld	24–48 h	150 Gy	Standard
4DayOld	72–96 h	150 Gy	Standard

Experiment 3	Age of moths	Radiation dose	Transportation of moths
NoRad	< 24 h	0 Gy	Standard
Standard	< 24 h	150 Gy	Standard
HighRad	< 24 h	300 Gy	Standard

Field release/recapture assessment

In all three of the orchards a trapping grid consisting of eight yellow delta traps (20 cm x 20 cm x 16 cm, Chempac, South Africa) baited with the codling moth pheromone lure were used to recapture the moths. In OVE(u), 10 mg codlingmone lures (with E8-E10 Dodecadienol (5.25 g/kg), Chempac, South Africa) were used (as pheromone disruption was implemented in this orchard) and 1 mg codlingmone lures (with E8-E10 Dodecadienol (5.25 g/kg), Chempac, South Africa) were used in OVE(c) and WEL. The traps were spaced approximately 15 m apart to form an inner and an outer circle of four traps (Figure 22), and were hung in the upper third of the tree (± 2 m height off ground). Moths from one Petri dish (150 x 10 mm, approximately 1000 individuals) for each treatment were released from the central point in the orchard and the sticky pads (20 cm x 20 cm, Chempac, South Africa)in the traps were collected and replaced three and seven days after release. In each orchard, two temperature loggers (Model DS1921G iButton, ColdChain Thermodynamics, Fairbridge Technologies, South Africa) were placed at the release point of the moths and recorded the temperature throughout all the trials.

The treatments of the moths caught in the traps were determined by viewing them under a black light, which revealed the fluorescent powder markings. When no fluorescent powder could be detected on a moth, it was removed from the pad by using a scalpel to carefully scrape the moth of the sticky surface. Once removed from the sticky pad, the moth was crushed and placed in a 15 mℓ glass vial containing a strip of filter paper (analytical grade, 1 cm x 4 cm) and 10 mℓ acetone (analytical grade, Sigma-Aldrich). The acetone would be drawn into the filtration paper, carrying with it traces of Calco Red (internal dye used at the Entomon and X-SIT facilities to assist with the identification of mass-reared moths).The vial was placed in a fume hood and 24 hours later the filter paper was examined for traces of the red dye.. The number of moths of each treatment caught and the trap in which they were caught were recorded.

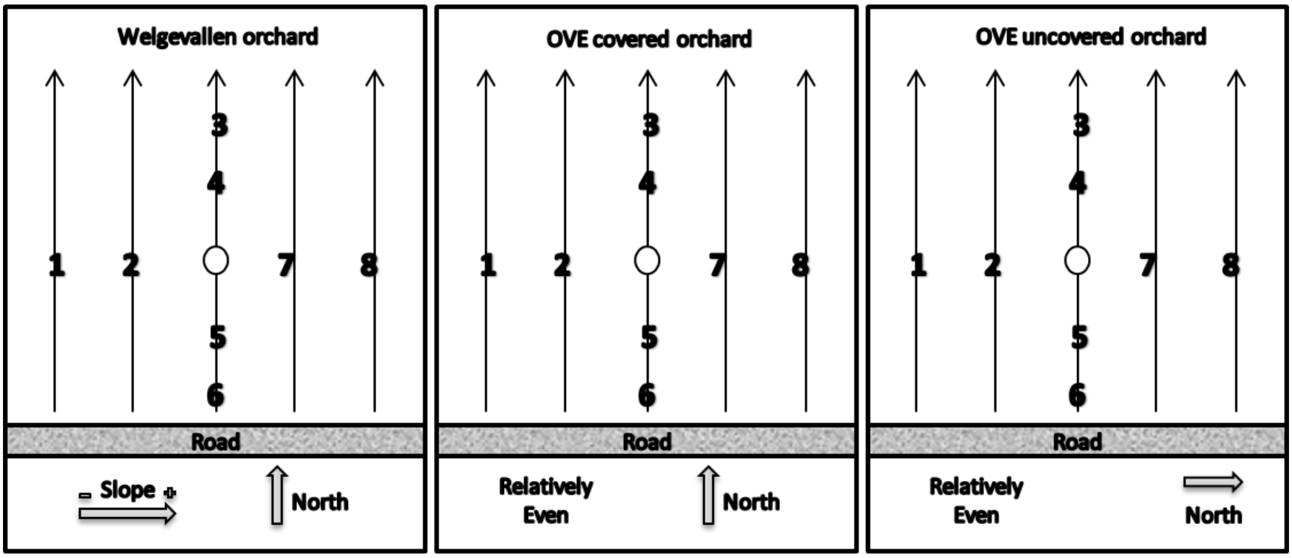

Figure 22: The experimental setup at each of the three orchards. Each of the numbers represents a delta trap baited with pheromone and the traps were spaced 15 m apart. The arrows (↑) indicate a small number of the rows and the row direction in the 1-hectare orchards. The circles (○) represent the central points of release in the trapping grids. The slope, true north and the location of the road are indicated below each diagram. OVE: Oak Valley Estate.

Statistical analysis

The raw data for the field release/recapture experiments were recorded as the number of moths of each treatment caught in each trap at either three or seven days after release. The totals for each trap placed at the same distance were then added (traps 2, 4, 5 and 7 for 15 m and traps 1, 3, 6 and 8 for 30 m) to produce the variable for the number of moths caught for each treatment at each distance. The two collection periods (three and seven days) were also added together, as initial probing of the data revealed that the time periods did not contribute to the analysis, interpretation and understanding of the results.

The derived data were analysed using a mixed model repeated measure ANOVA. The dependent variable was number of moths caught, with treatment, orchard and distance (and all possible interactions) as the independent variables. For each analysis the normality of data distributions was checked and it became evident that the data for experiment 3 required a log-transformation to achieve normality. The difference in the analysis results between transformed and untransformed data was negligible and, therefore, the results of the untransformed data of experiment 3 are reported. Fisher's LSD post hoc tests were used to separate means at $p = 0.05$. The effect of replicate date on the number of moths caught was also tested for each experiment using factorial ANOVA. No replication effect was found. The data analysis software used was STATISTICA 12 (StatSoft, USA).

Results

Experiment 1: Radiation dose and bran-handling

The mean number of moths caught was significantly influenced by ‘orchard’, ‘treatment’ and ‘distance’. Moth recapture in the orchards OVE(c) and WEL were statistically alike, but significantly fewer moths were caught at the OVE(u) orchard (Figure 23). The standard treatment yielded a significantly higher number of moth catches compared to the HighRad and bran treatments, which did not differ from each other (Figure 24). The number of moths caught at a distance of 15 m from the point of release was significantly higher than at the distance of 30 m (Figure 25). The interactions of ‘orchard x treatment’ and ‘orchard x distance’ were not found to be significant, nor was the three-way interaction of ‘orchard x treatment x distance’. This can mainly be attributed to the small number of catches at OVE(u), as these interactions could be observed for orchards OVE(c) and WEL (Figure 26). See Table 13 for F-statistics and significance values of effects.

Table 13: The factorial analysis of variance results for codling moth males (initial n ≈1000) under three different radiation and handling treatments recaptured at two distances from the release point (15 m and 30 m) in three different orchards (1 ha). Two of the orchards were apple orchards located at Oak Valley Estate (OVE) in Elgin: one uncovered (u) and the other covered (c). The other orchard was an uncovered pear orchard located at Welgevallen (WEL) in Stellenbosch. The codling moth received either the standard radiation dose (Standard), a high radiation dose (HighRad) or the standard radiation dose with bran-handling during transit (Bran). Num. d.f.: degrees of freedom of the numerator; Den. d.f.: degrees of freedom of the denominator.

Effect	**Num. d.f.**	**Den. d.f.**	**F**	**p**
Orchard	2	6	19.506	0.002
Treatment	2	12	6.892	0.010
Distance	1	6	6.355	0.045
Orchard x Treatment	4	12	1.387	0.296
Orchard x Distance	2	6	2.015	0.213
Treatment x Distance	2	12	0.821	0.463
Orchard x Treatment x Distance	4	12	0.985	0.451

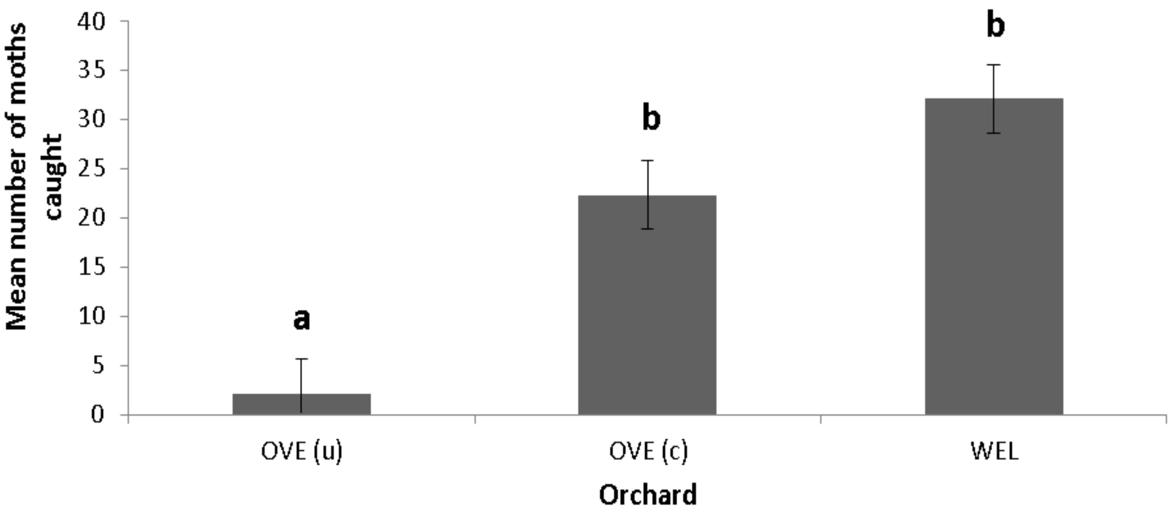

Figure 23: Mean number (±SE) of codling moth males (initial n ≈ 1000) under three different radiation and handling treatments caught at two distances from the release point in the covered (c) and uncovered (u) orchards at Oak Valley Estate (OVE), as well as in the uncovered orchard at Welgevallen (WEL) . The mean numbers of each orchard with the same lowercase letters above the histogram are not significantly different ($F_{2,6}$ = 19.506, p = 0.002). SE: standard error.

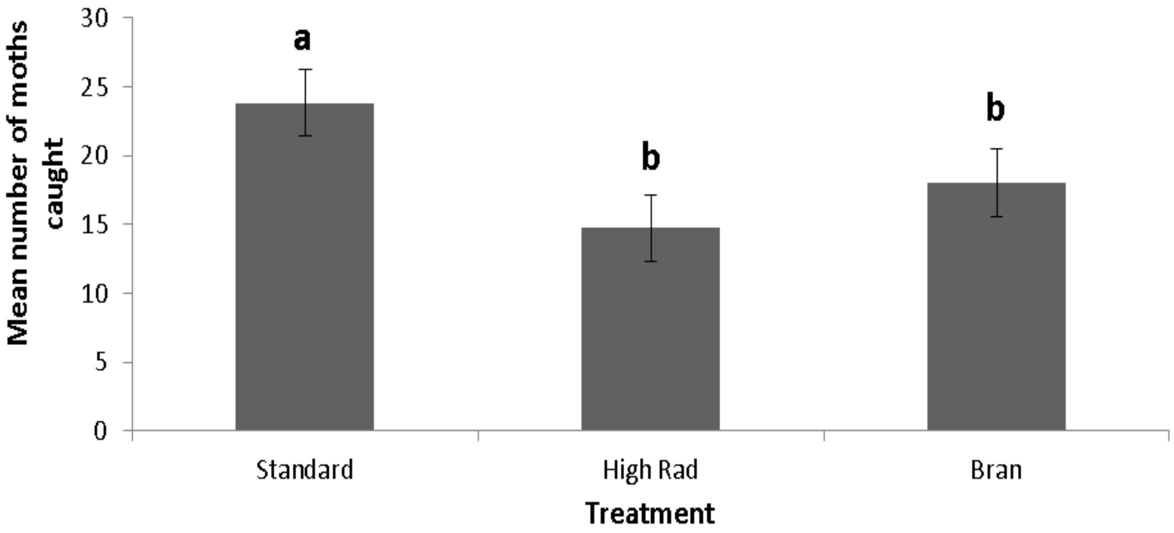

Figure 24: Mean number (±SE) of codling moth males (initial n ≈ 1000) under three radiation and handling treatments caught at two distances from the release point in three different orchards. The codling moth received either the standard radiation dose (Standard), a high radiation dose (HighRad) or the standard radiation dose with bran-handling during transit (Bran). The mean numbers of each treatment with the same lowercase letters above the histogram are not significantly different ($F_{2,12}$ = 6.892, p = 0.010). SE: standard error.

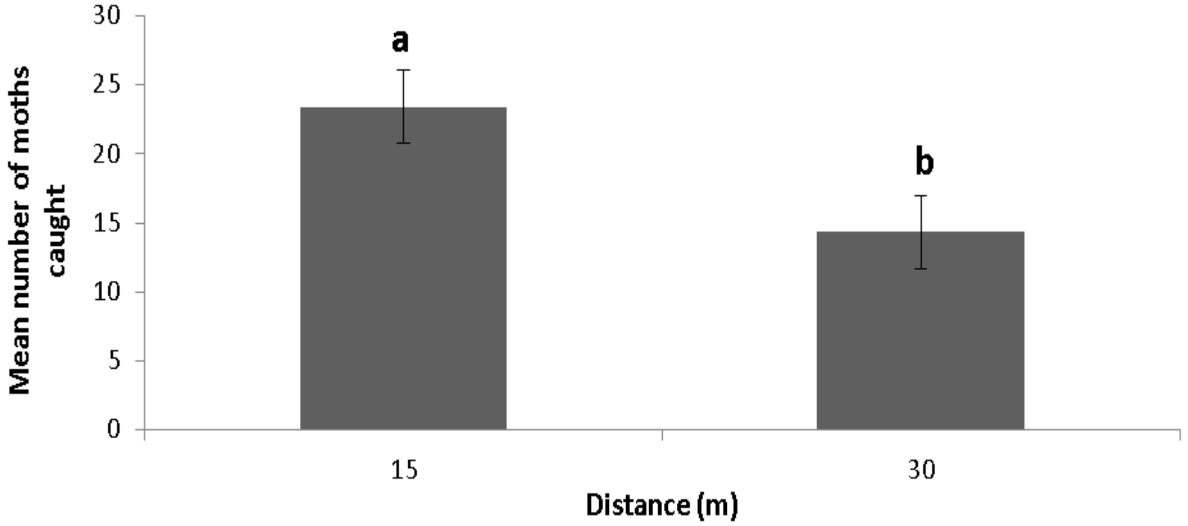

Figure 25: Mean number (±SE) of codling moth males (initial n ≈ 1000) under three radiation and handling treatments caught at two distances from the release point (15 m and 30 m) in three different orchards. The mean numbers of each distance differs significantly, as indicated by the differing lowercase letters above the histogram ($F_{1,6} = 6.355$, $p = 0.044$). SE: standard error.

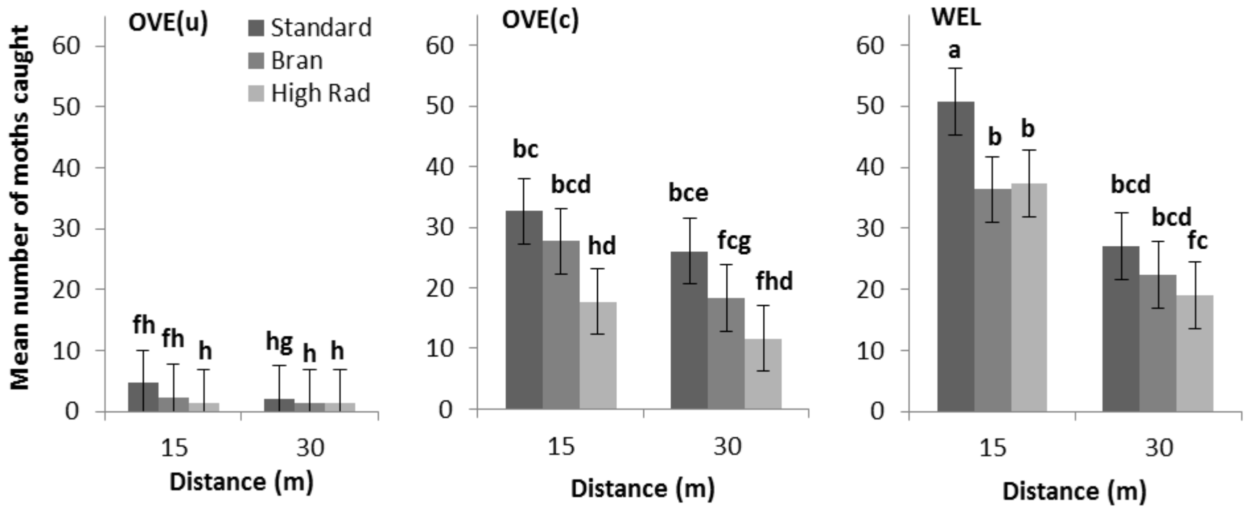

Figure 26: Mean number (±SE) of codling moth males (initial n = ±1000) under three different radiation and handling treatments recaptured at two distances from the release point (15 m and 30 m) in three different orchards (1 ha). Two of the orchards were apple orchards located at Oak Valley Estate (OVE) in Elgin: one uncovered (u) and the other covered (c). The other orchard was an uncovered pear orchard located at Welgevallen (WEL) in Stellenbosch. The codling moth received either the standard radiation dose (Standard), a high radiation dose (HighRad) or the standard radiation dose with bran-handling during transit (Bran). The mean numbers with the same lowercase letters above the histogram are not significantly different across all three graphs ($F_{4,12} = 0.985$, $p = 0.451$). SE: standard error.

Experiment 2: Ages

‘Distance’ was the only factor to significantly influence the number of moths caught in the field assessments for experiment 2. Significantly more moths were caught at a distance of 15 m from the point of release than at 30 m (Table 14). The factors ‘treatment’ and ‘orchard’ did not have significant effects on moth captures. No significant effects on moth captures were found for the interactions of ‘orchard x treatment’, ‘orchard x distance’, ‘treatment x distance’, nor for the three-way interaction of ‘orchard x treatment x distance’. Post hoc tests did, however, reveal that a significant difference could be observed between the number of moths caught at a distance of 15 m compared to 30 m within WEL orchard (Figure 27). See Table 14 for F-statistics and significance values of effects.

Table 14**:** The factorial analysis of variance results for codling moth males (n ≈ 1000) of three different age treatments recaptured at two distances from the release point (15 m and 30 m) in three different orchards (1 ha). Two of the orchards were apple orchards located at Oak Valley Estate (OVE) in Elgin: one uncovered (u) and the other covered (c). The third orchard was an uncovered pear orchard located at Welgevallen (WEL) in Stellenbosch. The codling moth were either aged 24 hours (1DayOld), 48 hours (2DayOld) or 96 hours (4DayOld). Num. d.f.: degrees of freedom of the numerator; Den. d.f.: degrees of freedom of the denominator.

Effect	**Num. d.f.**	**Den. d.f.**	**F**	**p**
Orchard	2	6	1.423	0.311
Treatment	2	12	1.102	0.363
Distance	1	6	9.699	0.020
Orchard x Treatment	4	12	0.190	0.938
Orchard x Distance	2	6	3.216	0.112
Treatment x Distance	2	12	0.524	0.605
Orchard x Treatment x Distance	4	12	0.959	0.464

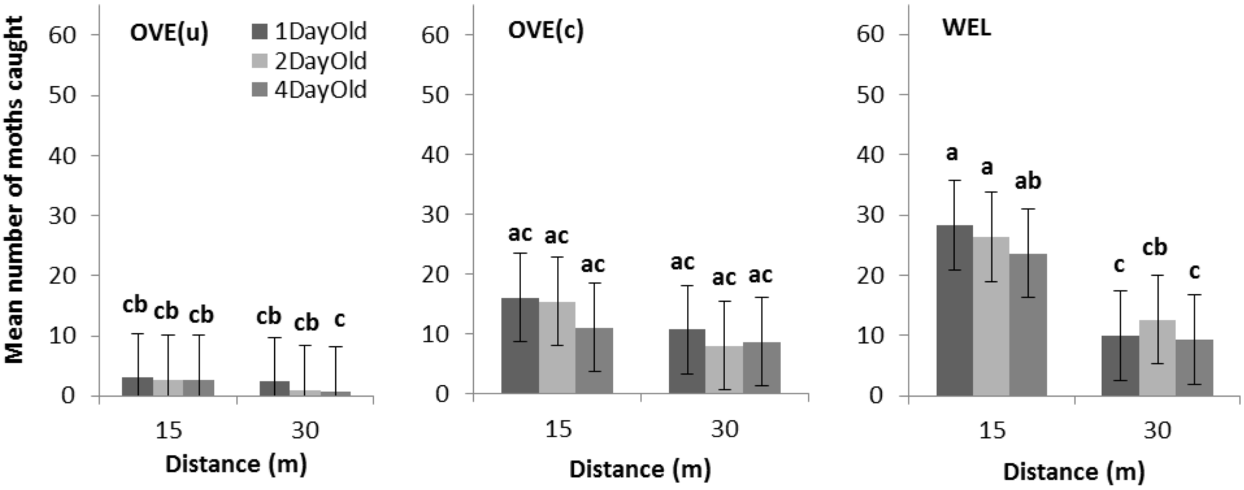

Figure 27: Mean number (±SE) of codling moth males (initialn = ±1000) under three different age treatments recaptured at two distances from the release point (15 m and 30 m) in three different orchards (1 ha). Two of the orchards were apple orchards located at Oak Valley Estate (OVE) in Elgin: one uncovered (OVE(u)) and the other covered (OVE(c)). The other orchard was an uncovered pear orchard located at Welgevallen (WEL) in Stellenbosch. The codling moth received either the standard radiation dose (Standard), a high radiation dose (HighRad) or the standard radiation dose with bran-handling during transit (Bran). The mean numbers with the same lowercase letters above the histogram are not significantly different ($F_{4,12}$ = 0.959, p = 0.464). SE: standard error.

Experiment 3: Radiation dose (false codling moth)

The number of moths recaptured was significantly affected by 'treatment'. Moth capture for the treatment NoRad was significantly higher compared to the treatments HighRad and Standard (Figure 28). Unlike for experiment 1 and 2, the factor 'distance' did not significantly influence moth captures in experiment 3. Neither the factor 'orchard' nor the interactions of 'orchard x treatment', 'orchard x distance' and 'treatment x distance' had any significant effect on moth captures. The three-way interaction between 'orchard', 'treatment' and 'distance' also had no significant influence on moth capture. At OVE(u), however, post hoc tests indicated a significant difference between the number of NoRad treatment moths caught at 15 m, compared to all other treatments at both distances (Figure 29). See Table 15 for F-statistics and significance values of effects.

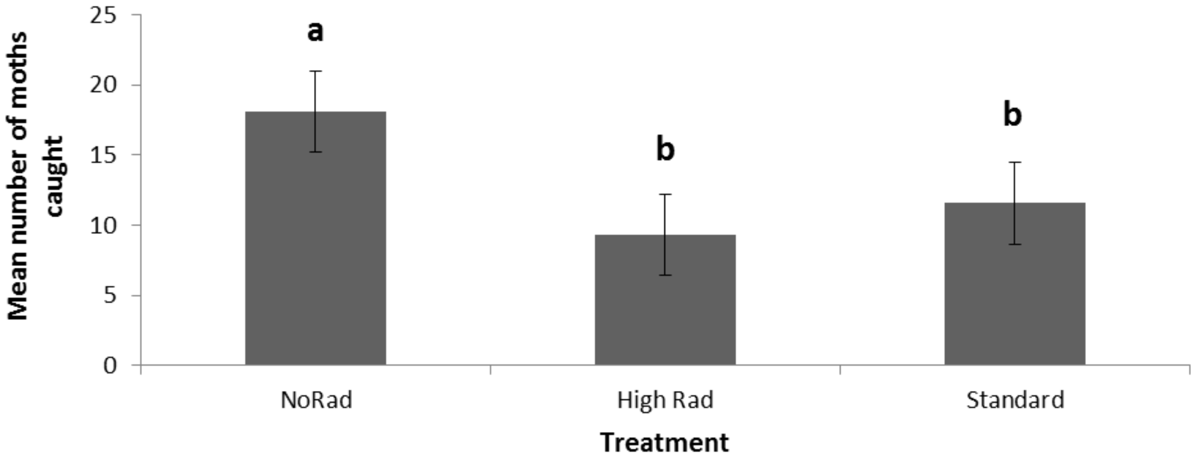

Figure 28: Mean number (±SE) of false codling moth males (initial n ≈ 1000) under three radiation treatments caught at two distances from the release point in three different orchards. The false codling moth received either no radiation (NoRad), the standard radiation dose (Standard) or a high radiation dose (HighRad). The mean numbers of each treatment with the same lowercase letters above the histogram are not significantly different ($F_{2,12} = 4.605$, $p = 0.032$). SE: standard error.

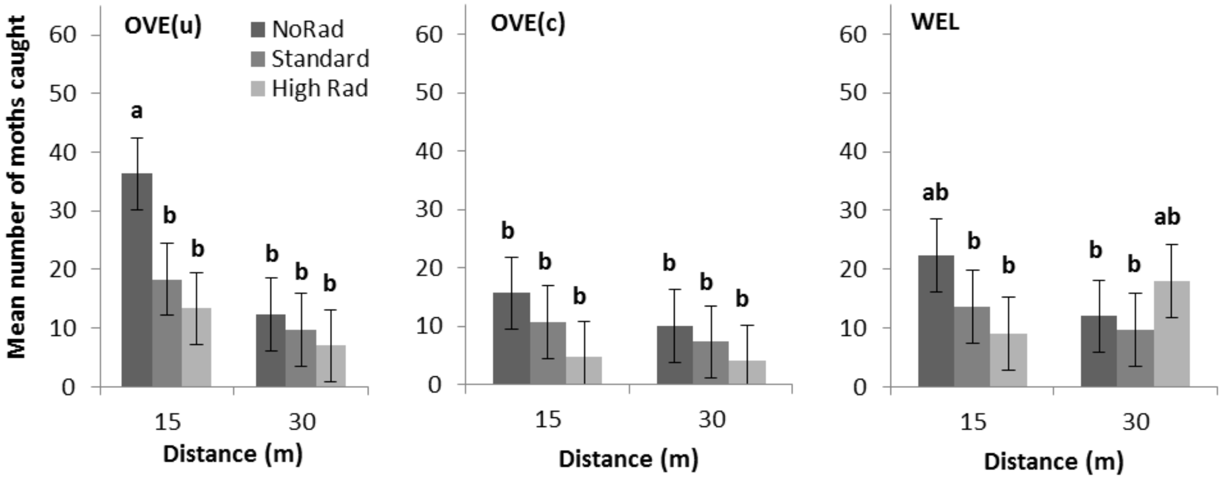

Figure 29: Mean number (±SE) of false codling moth males (n ≈ 1000) under three different radiation treatments recaptured at two distances from the release point (15 m and 30 m) in three different orchards (1 ha). Two of the orchards were apple orchards located at Oak Valley Estate (OVE) in Elgin: one uncovered (u) and the other covered (c). The third orchard was an uncovered pear orchard located at Welgevallen (WEL) in Stellenbosch. The false codling moth received either no radiation (NoRad), the standard radiation dose (Standard) or a high radiation dose (HighRad). The mean numbers with the same lowercase letters above the histogram are not significantly different across all three graphs ($F_{4,12} = 0.580$, $p = 0.682$). SE: standard error.

Table 15**:** The factorial analysis of variance results for false codling moth males (n ≈1000) under three different radiation treatments recaptured at two distances from the release point (15 m and 30 m) in three different orchards (1 ha). Two of the orchards were apple orchards located at Oak Valley Estate (OVE) in Elgin: one uncovered (u) and the other covered (c). The other orchard was an uncovered pear orchard located at Welgevallen (WEL) in Stellenbosch. The false codling moth received either no radiation (NoRad), the standard radiation dose (Standard) and a high radiation dose (HighRad). Num. d.f.: degrees of freedom of the numerator; Den. d.f.: degrees of freedom of the denominator.

Effect	**Num. d.f.**	**Den. d.f.**	**F**	**p**
Orchard	2	6	0.856	0.470
Treatment	2	12	4.605	0.032
Distance	1	6	5.214	0.062
Orchard x Treatment	4	12	0.633	0.648
Orchard x Distance	2	6	1.799	0.244
Treatment x Distance	2	12	3.615	0.059
Orchard x Treatment x Distance	4	12	0.580	0.682

Table 16: The average, highest and lowest values recorded for the amount of rainfall, maximum and minimum temperatures from September 2014 to April 2015. Data captured at the Oak Valley Estate (-34°09'32.5800", 019°04'22.2240") and Welgevallen (-33°55' 23.8800", 018°52'23.9160") weather stations. (ARC-Institute for Soil, Climate and Water (ARC-ISCW)

	Oak Valley Estate			Welgevallen		
	Maximum temp (°C)	Minimum temp (°C)	Rainfall (mm)	Maximum temp (°C)	Minimum temp (°C)	Rainfall (mm)
Sept 2014						
Average	18.82	8.24	0.02	23.83	7.79	1.12
Highest	29.27	14.38	0.6	33.27	13.46	16.51
Lowest	11.62	3.65	0	16.06	1.77	0
Oct 2014						
Average	22.04	10.37	0	28.38	10.75	0.29
Highest	31.37	14.33	0	36.37	18.25	4.06
Lowest	11.71	4.53	0	19.45	3.48	0
Nov 2014						
Average	24.23	10.91	0	28.88	12.44	1.4
Highest	32.15	14.25	0	36.26	19.55	19.56
Lowest	16.13	5.98	0	20.39	7.85	0

Dec 2014						
Average	25.98	12.84	0.01	29.89	13.72	0.23
Highest	33.05	16.86	0.3	36.63	19.68	6.6
Lowest	19.11	10.07	0	24	8.86	0
Jan 2015						
Average	27.93	14.18	0.13	32.4	14.28	0.53
Highest	35.09	17.91	2.2	39.53	21.74	14.22
Lowest	20.46	9.5	0	24.69	8.92	0
Feb 2015						
Average	24.77	13	0.74	31.26	12.93	0.19
Highest	32.92	17.46	14.9	38.41	20.91	4.57
Lowest	17.7	7.71	0	24.39	7.26	0
Mar 2015						
Average	25.96	13.66	0.42	31.83	13.35	0.06
Highest	40.03	16.46	5.3	45.43	20.83	1.78
Lowest	19.09	10.42	0	26.46	9.06	0
Apr 2015						
Average	21.95	11.07	0.87	27.94	9.89	0.13
Highest	31.76	18.21	6.1	37.94	15.08	1.52
Lowest	13.74	6.96	0	20.26	6.06	0

Discussion

Objective 1: Determine if field release/recapture assessments are able to detect differences in moth quality.

In this study, the same experimental design was repeated in three one-hectare orchards that were selected for their diverse characteristics. The WEL orchard was located on a slope at the foot of Stellenbosch Mountain near Stellenbosch. This area has a warmer, drier climate compared to OVE in Elgin, where OVE(c) and OVE(u) orchards were located on relatively level terrain. Apart from the regional differences in climate, Middleton & McWaters (1996, 2000) and Lloyd (2003) also demonstrated the changes in microclimate caused by mesh net covering of an orchard. The former authors listed changes such as a reduction of wind speed, an increase in humidity and a reduction of light intensity, whereas the latter author reported that some nets caused an increase in temperatures. The differences between these three orchards would demonstrate how environmental conditions and orchard characteristics influence the outcome of field trials.

The analysis of the results of the release/recapture experiments, however, revealed that the number of moths caught in each orchard only differed significantly in experiment 1 (Figure 23). The total number of moths recaptured in WEL were twice as high as for OVE(c), suggesting that the warmer climate in Stellenbosch (average temperature difference between the two locations was 4.96°C, Table 16) was more conducive to moth dispersal than in Elgin. The temperature loggers in the orchards indicated that there were no noticeable temperature differences between OVE(u) and OVE(c). Therefore, the low trap catches recorded in OVE(u) during experiments 1 (radiation dose and bran-handling) and 2 (ages) can be credited to the codling moth mating disruption employed in that orchard. Even with the use of 10x lures in OVE(u), the mating disruption so limited the number of moths recaptured that no treatment differences could be observed, indicating that the release/recapture method is not suitable for use in orchards utilising this control technique.

Although the effect of the different orchards was shown not to be statistically significant in the analysis of experiment 2 (ages), it would be misguided to infer that, different to experiment 1 (radiation dose and bran-handling), the conditions and orchard characteristics had no impact on the recapture averages of moths in experiment 2. The lack of significance can rather be attributed to the overall low recapture percentages and high variance in the data (Figure 27). The mean trap catches for the standard treatment at each orchard declined markedly from 6.66, 58.66 and 77.66 for OVE(u), OVE(c) and WEL respectively in experiment 1, to 5.33, 26.66 and 38.33 in experiment 2. This decrease in captures of the standard treatment moths indicates that an external variable affected the quality of the moths in experiment 2. The examination of the data revealed that noticeably lower recapture percentages were observed in OVE(c) and WEL in two different replications, which are likely due to adverse weather conditions (possibly strong wind in the region). Although the difference between the mean numbers of moths caught at OVE(c) and WEL was not statistically significant for the codling moth experiments, the recaptures were consistently higher at WEL than at OVE(c) (Figure 23), corroborating the observation for experiment 1 in that the higher temperatures in Stellenbosch encouraged better dispersal in the orchards.

The trapping grid and central release point used in this trial made it possible to assess whether the trap catches at two distances (15 and 30 m) could reveal a difference in the flight performances of good and poor quality moths. These distances were selected by considering that the spacing between releases of sterile insect in orchards is commonly no further than 30 m. Theoretically, sterile moths should only have to be able to fly 15 m to achieve a uniform distribution in the orchard (Bloem et al. 1998). Although it has been established that codling moth has the ability to disperse great distances (Schumacher and Weber 1997; Schumacher et al. 1997), it would not be sensible to assess the ability of sterile moths to do so if it would not contribute to their efficacy in the field.

Although a significantly higher number of moths was caught in the traps placed at a distance of 15 m compared to the traps at a distance of 30 m in experiments 1 and 2 (Figure 25 and Figure 27), the actual result of interest was the number of moths of each treatment caught at the two distances. The treatment x distance interaction was not significant in any of the experiments, indicating that there was no difference in the ability of high and low quality moths to disperse up to a distance of 30 m. This correlated well with the results of the flight tunnel experiments, which also indicated that flight distance alone could not be used as a measure of moth quality. In their field release/recapture trials using radiation dose treatments, Carpenter et al. (2013) also did not detect any treatment differences at either a distance of 9 m or 22.5 m.

Traps were serviced three and seven days after release in the field to establish whether there was a difference in the time period that moths of different quality remained active in the orchard. The findings provided no evidence that degraded quality affected the recapture of moths within one week and as sterile insect releases are made at least twice weekly in most SIT programmes, it was fruitless to consider the effect over a longer time period. It was, therefore, decided to analyse the trap catches over a single period of seven days instead of two periods of the first three and the latter four days.

Objective 2: Evaluate the effects of factors such as age, radiation dose and handling on moth performance in field.

Treatment had a significant effect on the mean number of moths recaptured for experiment 1 (radiation dose and bran-handling), but not for experiment 2 (ages). The results attained in the field release/recapture trials for the radiation dose treatments confirmed what was observed in the flight cylinder assessments, i.e. standard radiated moths exhibited significantly better flight performance than moths that received a high radiation dose (Figure 24). Carpenter et al. (2013) could also observe that the results gathered from the flight cylinders correlated well to their field trial results, and found that an increase in radiation dose caused a decrease in codling moth flight ability. Interestingly, the moths transported in bran showed a significantly lower recapture rate in the field, compared to moths of the standard handling treatment (Figure 24 and Figure 26). This is contrary to what was observed in the flight cylinder assessments, which indicated no difference in the flight performance of the standard and bran-handling treatments. It may be that the transportation to the field extended the period in which moths were exposed to the bran handling compared to the moths destined for the flight cylinder and wind tunnel assessments. This result underscores the importance of including both laboratory and field level assessments in the QC protocol at insect mass-rearing facilities.

The moths that were aged for either one, two of four days in cold storage did not display significantly different flight ability in the field assessments, which correlates well with the results that were obtained for the age treatments in the flight cylinder matrix (Figure 27). This is in contrast to other studies which have reported that extended periods in cold storage had detrimental effects on the quality of the mass-reared moths (Leopold 2000, 2007; Calkins and Parker 2005; Parker 2005; Dyck 2010). It is possible that chilled moths are more susceptible to the effects of other quality-degrading factors (e.g. handling) and that cold storage and age alone do not have significant effects on moth flight ability (Bloem and Bloem 1995, 2000; Bloem et al. 1998; K. Bloem et al. 2002; S. Bloem et al. 2002; Blomefield et al. 2011). This conclusion is supported by the fact that some studies failed to find a degrading effect for cold storage as a single factor (Bloem et al. 2006a; Chidawanyika and Terblanche 2011b). However, the chilled moths in experiment 2 were subjected to radiation and some handling during transportation to the field and it is not known why no effect was observed in the field release/recapture trials.

Objective 3: Determine whether the release/recapture assessment developed for codling moth can be used for false codling moth.

Experiment 3 of the field release/recapture assessments was conducted with mass-reared false codling moth. The field sites and the experimental setup were identical to that for the codling moth experiments, which meant that the field level assessments of the flight performance of false codling moth were conducted in non-host plant orchards. This would allow the release of non-radiated false codling moth, which would provide insight into the effect of radiation on the performance of false codling moth in the field.

There was no significant difference in the mean number of false codling moth caught in each orchard, in spite of the differences in average temperatures observed for the two locations (Oak Valley Estate had an average temperature of 23.95 °C, compared to 29.88 °C for Welgevallen, Table 16). Whereas the recapture rate was consistently highest at the WEL orchard for the codling moth experiments, slightly more false codling moth were caught in OVE(u) (Figure 29). However, high variation and low recapture percentages were observed in experiment 3 (similar to experiment 2 of the codling moth field assessments), which could be a contributing factor to the lack of orchard differences. The high variation could also clarify why no significant differences were obtained for the number of false codling moth caught at the two distances in the orchards (Figure 29) (although a trend can still be observed where more moths were caught closer to the point of release than in the furthest traps). This variability and low recapture averages are thought to be the result of the fluctuating quality of the moths released for each replication. During some replicates, low recapture rates were observed in all three of the orchards and were not limited to one or two orchards, as was the case for the codling moth in experiment 2. This is indicative of poor quality for the entire cohort of moths released for that replication, regardless of treatment or orchard. This observation parallels the observations in the flight cylinder assessment, where the flight performance of the false codling moth varied significantly between replications.

The radiation treatments had a considerable influence on the recapture percentages of the false codling moth. The numbers of untreated moths recaptured in the orchards were significantly higher than either the standard or high radiation dose treatments. The similar number of moths caught for the standard and high radiation dose treatments was noteworthy, as this differs from what was observed for codling moth. It would seem that even the standard radiation dose has a considerable influence on the flight ability of false codling moth, much more so than for codling moth, but this observation could also be due to the low number of moths recaptured because of the fluctuating quality of the false codling moth (Figure 28).

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CHAPTER 5 : GENERAL CONCLUSIONS

The aim of this study was to identify and develop laboratory, semi-field and field methods that can be used on a regular basis to assess the quality of mass-reared codling moth. Understanding how the expression of codling moth quality changes in linked assessments that increase in scale, duration and complexity is crucial to the development of effective QC protocols for insect mass-rearing facilities. To this end, three objectives were identified: first, to develop flight performance assessments that are effective at determining the quality of mass-reared codling moth, second, to evaluate the effect of factors such as age, radiation dose and handling on moth quality and, finally, to determine whether the methods developed for codling moth can be used for another mass-reared lepidopteran species, the false codling moth.

Evaluation of study objectives

Objective 1

The laboratory method used in this study, the flight cylinder matrix, was able to differentiate between codling moth males of good and poor quality, even though daily fluctuations affected the percentage of moth escape from the cylinders. From the flight cylinder results it is evident that cylinder height is more influential of moth escape than cylinder diameter. The best results were achieved at a diameter of 10 cm. Therefore, the nine-cylinder matrix can be reduced to three cylinders of heights 2.5, 15 and 26.5 cm, all with a diameter of 10 cm. This will reduce the number of moths needed per replication and more replications of the test can be conducted, increasing the accuracy of the findings.

The novel method of using a horticultural tunnel as a semi-field assessment was not robust enough to detect treatment differences and is, therefore, not suitable for use as a QC test. The method was based on the wind tunnel model, but the size of the tunnel precluded the generation of a strong, uniform wind flow. In the absence of wind resistance, moths of all quality grades were able to cross the 12 m distance to the trap with relative ease and no treatment effects were observed.

The release/recapture method used in field assessments was able to distinguish between high and low quality moths under milder conditions. These differences could only be measured as the total number of moths recaptured per treatment per orchard, as neither distance nor time could be used to distinguish between treatments. However, treatment differences could only be measured when sufficiently high numbers of moths were recaptured in the assessment, which hinged on environmental conditions, orchard characteristics and the pre-treatment quality of the moths. These factors did not affect the expression of treatment differences, only the ability to detect them. When recapture percentages were low, the differences between treatments were less pronounced and could lead to inaccurate conclusions about the quality of the moths.

Objective 2

An increase in radiation dose is known to cause a decline in the quality of mass-reared codling moth and this could be observed in both the flight cylinder and the field release/recapture method. Although the radiation dose required to induce inherited sterility in codling moth is much lower than that required for full sterility, its effect on moth flight ability could still clearly be observed when compared to untreated moths. This will certainly influence the competitiveness of the moths in the field, but the extent can only be determined once standard radiated moths can be compared to moths from a wild population in the flight cylinder matrix.

A significant difference was observed in the field between the moths transported in bran compared to moths that were treated with standard handling procedures. Comparable results were not obtained in the flight cylinder and tunnel assessments, which suggests that the detrimental effect of bran-handling on the quality of the moths becomes more pronounced the longer the moths are exposed to the bran during transit. The results for the age treatments, which suggested that ageing moths for up to four days in cold storage does not affect flight ability, were surprising, as it differed to what has been reported in previous studies. It is possible that, in isolation, the effect of age and cold storage did not have an excessively negative effect on moth quality, but that the effects of other factors (such as handling) are compounded when moths are chilled for long periods. These findings underscores the importance of storage and handling protocols at mass-rearing facilities. All the efforts exerted at the facilities to minimise quality degradation will come to naught if the moths are damaged during transportation to the field. Finding solutions to improve the storage and handling of moths should, therefore, be a priority for future studies.

Objective 3

The laboratory and field methods employed in this study to evaluate the quality of codling moth also proved successful for another mass-reared Tortricid pest, the false codling moth. Since the semi-field assessment method proved ineffective for codling moth, it was not evaluated for false codling moth. The success of the flight assessment methods on false codling moth will hopefully lead to further investigations into the applicability of the methods at mass-rearing facilities for other lepidopteran species. Streamlining the processes associated with SIT for the existing programmes will encourage the adoption of this pest management strategy for other lepidopteran pests, especially if it can be shown that the developed improvements are applicable to many other species.

Implications of findings

The evaluation of codling moth flight performance in linked assessments in laboratory, semi-field, and field tests has revealed the crucial role each that these tests play in QC. It would be unwise to rely solely on one of these methods to monitor insect quality, as each one has different strengths and weaknesses.

The strength of the flight cylinder matrix lies in its ability to determine where on the spectrum of flight performance the abilities of the mass-reared moths lie. It is able to distinguish between high, medium and poor quality moths, providing more accurate feedback to the mass-rearing facility. The distinctions between these quality grades are not arbitrary – the 'low-medium-high' trend could also be observed in the field trials comparing the flight performance of moths treated with various doses of radiation. Another strength of the laboratory method is its ability to detect the daily fluctuations of moth quality. Using only periodic quality tests might cause a delay in the detection of a greater systemic problem that causes the steady degradation of moth quality, as a poor result could possibly be ascribed to the normal daily quality fluctuations. Laboratory quality tests are generally excellent at isolating and accurately measuring a single quality parameter, which can then be monitored regularly to indicate changes in moth quality. It can, therefore, also be used to evaluate the effect of specific factors (e.g. temperature, mating status and diapause) on moth flight ability and quality. However, it cannot truly provide an idea of the overall moth competitiveness in the field.

The strength of the release/recapture assessment lies in its ability to provide a more holistic idea of the in-field effectiveness of mass-reared moths. This test evaluates many quality parameters simultaneously (e.g. dispersal, longevity and ability to locate females), as well as the ability of the mass-reared insects to cope with changing environmental conditions. Because it incorporates so many different aspects, this type of assessment cannot be relied upon to accurately measure a single quality parameter. The efficacy of release/recapture trials to differentiate between various treatments depends greatly upon the recapture percentage achieved in the trial, as well as the number of replications done for each experiment. As this is a time-consuming and expensive method, not very many replicates can be done on a routine basis. The numerous variables that could affect the number of moths recaptured could lead to inaccurate assumptions about moth quality, which in turn could have dire consequences should these assumptions be used to inform important decisions for the SIT-programme.

This study has shown that the results obtained in the laboratory can be correlated to the performance of the moths in the field, but the semi-field tests should provide a very useful intermediate test, i.e. assessment of a single quality parameter in conditions close to what is obtained in the field. However, the development of a robust and reliable semi-field assessment for flight performance is still ongoing.

Future applications and suggestions for further investigations

It is hoped that the outcomes of this study will contribute to the establishment of a standardised QC protocol based on flight ability for codling moth. This protocol would ideally include assessments for all three levels (laboratory, semi-field and field) and would hopefully prove effective for other mass-reared lepidopteran species. To this end, future studies would do well to assess the performance of wild moths in the selected QC tests, to set the external standard for moth quality. The development of a simple but effective semi-field assessment method and the standardisation of a release/recapture experimental setup should also be prioritised.

The flight cylinder method is simple and inexpensive to apply and provides accurate, easy to interpret results in a relatively short period of time. It is therefore not only ideal for use as a routine QC test at mass-rearing facilities, but could also prove useful in other studies as a method to evaluate the effect of various factors on the flight ability of moths (e.g. new diet formula, different rearing regimes, or extreme temperatures).

Final remarks

This study set out to identify and develop laboratory, semi-field and field methods that can be used to assess the quality of mass-reared codling moth. It succeeded in part and also serves as a confirmation of the fact that good QC is crucial to all SIT programmes.