UNIVERSITY OF SOUTHAMPTON

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

Centre for Biological Sciences

The development of a lure and kill system for control of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae)

by

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ABSTRACT

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THE DEVELOPMENT OF A LURE AND KILL SYSTEM FOR CONTROL OF THE MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE)

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The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is a polyphagous pest of global economic importance. As a result, many systems have been proposed to reduce their impact and population spread, each of which has its limitations. Lure and kill systems are extensively used to combat medfy infestations. However, widely used bait spray applications indiscriminately contaminate the target area with insecticide, having harmful effects on beneficial and other non-target organisms. Alternative systems rely on traps that lure flies in where they are killed, these require regular maintenance and rely on either a single sex attractant (which only have limited effect on the female population) or the use broad spectrum attractants that attract and kill beneficial, pest controlling, insects. This work allows the development of a lure and kill control strategy based on insecticide formulated electrostatic powders that can be autodisseminated through a pest population. Laboratory survival experiments were used to compare the LT50s of two insecticides (chlorpyrifos and spinosad) formulated with different powders (Entostat™ and Entomag™) and to show secondary transfer of insecticide from contaminated males to females through courtship. The combination of Entostat™ powder and 2% spinosad gave the best performance allowing sufficient time for transfer between conspecifics before mortality and rapid mortality to secondary contaminated females. Field studies were undertaken to establish a suitable prototype electrostatic powder container. The proposed system would have benefits over other existing systems, as the targeted nature of the application method limits contamination of produce and the environment with insecticide. Secondly, the autodissemination nature of the system would target female members of the pest population not initially attracted to the insecticide. Three stations were tested, with the traditional delta design proving to be the most effective, with higher numbers of medly contacts on the area that would house the insecticidal agent, suggesting greater numbers of primary transmission from this design.

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Declaration of authorship

I, ……., [Craig Rogers]

declare that the thesis entitled 'The development of a lure and kill system for control of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae)' and the work presented in it are my own. I confirm that:

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Chapter 1: Introduction

1.1 Mediterranean fruit fly

Ceratitis capitata (Wiedemann, Diptera: Tephritidae), the Mediterranean fruit fly (medfly), is found in most tropical and subtropical areas. It originated in Africa and spread from there to many countries through the Mediterranean basin, the Middle East, Western Australia and South and Central America (White & Elson-Harris, 1992; Bonizzoni *et al*., 2001; Bonizzoni *et al*., 2004). Since the 1980s, infestations in the continental United States are limited to two states, California and Florida while it has been established in Hawaii since 1910 (Back & Pemberton, 1918; Burk & Calkins, 1983). The medfly is a pest of a reported 253 varieties of fruits, nuts and vegetables (Christenson & Foote, 1960; Hagen *et al*., 1981; Liquido *et al*., 1991) including pomme and citrus fruits, cocoa and guava (Weems, 1981). Damage is caused by the larvae boring through the fruits destroying them or by secondary infection by fungi and bacteria.

Eggs are laid in groups just under the pericarp using the females protrusible ovipositor. Egg incubation takes two to three days, and are approximately 1 x 0.2 mm in size and white in colour when freshly laid (Ros, 1988). The egg stage lasts for two days (Boller, 1985), after which the larvae bore through the pulp of the fruit and then take 6-10 days to develop through three instars depending on the host (Rivnay, 1950; Carey, 1984; Krainacker *et al*., 1987). The first instar is about 2 mm in length, while the third instar larvae achieve a length of between 6 and 9 mm when full developed (White & Elson-Harris, 1992). Pupation usually takes place in the soil under the host tree and lasts for approximately 9-11 days but can be longer depending on adverse conditions (Boller, 1985; Mavrikakis *et al*., 2000). Reproductive maturity in females is reached four days after pupal eclosion (Arita, 1982). First oviposition takes place after eight days and the flies live for up to two months, feeding on sugars from damaged fruits (Diamantidis *et al*., 2009). In tropical

and sub-tropical regions there may be three to seven overlapping generations in a year (Papadopoulos, 2004).

The combination of widespread distribution over most tropical and sub-tropical regions, its polyphagous nature, high adaptability and multivoltine biological cycle make this species an excellent coloniser and ranks medfly as one the most damaging pests globally (Bonizzoni *et al*., 2001). The negative economic impact caused by medfly is not limited to physical damage to commercially produced crops. Financial loss can also be caused through: control or eradication programs, quarantine restrictions that can deny producers entry into export markets or costly disinfestation and fumigation treatments (Siebert & Cooper, 1995).

1.2 Medfly control

There are a number of different methods currently employed to control medfly including mechanical control, chemical control, biological control and the use of good field management e.g. field sanitation.

1.2.1 Current control methods

The use of protein-rich liquid attractants mixed in insecticide sprays is one of the most commonly applied methods of controlling Mediterranean fruit fly populations. The bait insecticide sprays are applied to plants that serve as refuges for Mediterranean fruit fly. Baits serve to encourage the adults to feed on the spray residue resulting in ingestion of the insecticide. This method of control can provide a high kill rate, however, this is a very untargeted method of control that results in large quantities of insecticide entering the environment (Van den Berg *et al*., 1999)

Another method employed to control medfly is mass trapping using baited traps. However, mass trapping is a high cost and high maintenance method of control (Navarro-Llopis *et al*., 2008). As traps need to be serviced regularly to prevent clogging with retained flies and to prevent drying up of the trapping mechanism.

The most widely used biological control method is release of SIT (sterile insect technique) flies. It acts by reducing the reproductive output of the female. Large numbers of mass-reared sterile males are released into an infested area, where they mate with female conspecifics (Hendrichs et al., 2002). During mating the released males pass on sperm carrying dominant lethal mutations, preventing the female from producing viable progeny. SIT is widely believed to be the most environmentally sensitive method of control but there are many problems associated with it such as cost, the possibility of reduced fitness of SIT males and the complicated logistics involved.

1.3 Trap design

A number of trap and designs have been developed to control and monitor the adult medfly. Traps can be broken down into three main components; the structural element, the functional element (i.e. retention area or insecticide treatment) and the attractant. In general, tephritid traps available today are classified as either wet or dry depending on which trapping mechanism is employed. Wet traps are those that retain flies in a reservoir of fluid, usually a form of protein bait. This style of trap is predominantly used against females. Dry traps are those that use a sticky surface or an insecticide to neutralise the fly and employ a synthetic lure such as a parapheromone as an attractant.

McPhail Traps developed in the 1930's (Newell, 1936; McPhail, 1939) are still used for control and monitoring of medfly today. The trap consists of a transparent bell-shaped body with access holes at the top and base. The upper entrance is used for applying liquid baits and is plugged during operation, whilst the hole in the base allows the flies to gain access to the trap and allows release of the attractant. McPhail traps generally use liquid food bait such as hydrolysed protein as an attractant. This attractant also acts as the retainer which classifies the McPhail trap as a wet trap. These traps are mainly used for control purposes as the food bait yields a high percentage of female catches unlike synthetic pheromone/parapheromone traps

(USDA, 2003). However, the female biased catch rates also make them ideal for the monitoring of natural populations of fruit fly during the mass release of sterilised males during SIT control (Midgarden *et al*., 2004). A disadvantage of McPhail traps is that they are very labour intensive as servicing and re-baiting takes much longer than dry traps.

The Multilure trap is an updated version of the McPhail trap. It is a two part plastic container that clips together, the lower section is yellow and the upper part is transparent (USDA, 2003). This contrast has been found to be attractive to the flies (IAEA, 2003).

Like the McPhail trap, the lower section has an entrance hole that also acts to release the attractant. Unlike McPhail's the Multilure may be used either as a wet or a dry trap. When used as a dry trap, a synthetic female lure comprising of ammonium acetate, putrescine and trimethyl amine is combined with an insecticidal dichlorvos (DDVP) strip. The multilure dry system has advantages over traditional McPhail traps; lack of liquid bait makes servicing cleaner and less labour intensive and the lack of water reduces the quantity of non-target insects caught (IAEA, 2003).

Tephri traps are similar to the McPhail and multilure traps except that the yellow lower section of the trap is larger than that of the other two traps and holes are positioned across the top of the base part to facilitate the release of the attractant.

The three traps described above belong to a group of traps known as 'bucket traps'. There are several problems encountered when using bucket traps: the bait solution is easily spilt and any spills will compete with the attractant within the trap itself; fly samples decompose in the retaining fluid making identification difficult for monitoring; and the size and weight of these traps makes deployment difficult. (Heath *et al*., 1996)

A Jackson trap is delta shaped and made up of laminated or waxed cardboard or corex. This dry trap uses a sticky cardboard insert positioned on the floor of the trap to retain the flies. As the Jackson trap cannot be used with liquid protein, it is generally used for the control and monitoring of male flies using the para-pheromone Trimedlure^{TM} as an attractant (USDA, 2003). Some variations of the trap use an insecticide such as malathion or DDVP impregnated in wicks or panels to aid fly retention (Dantas & Andrade, 2005). Historically the Jackson trap was white but experiments by Greany *et al*. (1982) have shown that this basic style of trap can be improved for use with tephritids by altering the colour to yellow.

Yellow panel traps simply consist of a rectangular, yellow cardboard panel coated in glue. A dry synthetic lure is used as an attractant. Although labour saving and inexpensive, Yellow panel traps are limited to control as the glue destroys samples and makes identification for surveys and monitoring more difficult (USDA, 2003).

ChamP traps are yellow sticky panels covering an attractant impregnated polymeric panel, perforations in the sticky panel allow release of the attractant (IAEA, 2003).

The Cook and Cunningham trap is made up of three panels spaced 2.5 cm apart (IAEA, 2003). The internal panel is polymeric and impregnated with a dry synthetic lure. The external sides of the outer panels are coated in glue. These are fastened to the middle panel with clips.

There are many disadvantages associated with panel traps; the lack of housing around the sticky board can result in fouling of the trap with foliage and other plant debris, reducing the efficiency of the trapping mechanism, the trap will quickly become saturated in instances of high infestation, resulting in premature trap shut down. As a result of these problems, panel traps must be checked and replaced frequently which increases labour costs. Finally, these traps tend to catch large numbers of non-target insects including beneficial species.

Open bottom traps are vertical green cylinders made of either plastic or waxed cardboard, with a transparent top. Three holes are positioned around the circumference half way up the cylinder to allow release of the dry synthetic lure. The base of the trap is open and allows flies access to the sticky board situated there.

Steiner traps (Steiner, 1957) are horizontal clear cylinders, with open ends. The cylinder contains a dry synthetic lure and an insecticide.

1.4 Attractants

1.4.1. Visual attractants

Trap designs for medfly have relied on chemical lures rather than visual cues such as colour and shape (Gilbert *et al*., 1984), however, visual cues are required for optimal catches (Epsky & Heath, 1997). There is evidence that the colours and shapes attractive to medfly are different depending on the sex and sexual maturity of the fly and may also change through the season. Food-seeking flies will respond to cues indicating a ripe fruit (Katsoyannos, 1986) whilst ovipositing females are attracted to shapes and colours representing egg laying sites. (Katsoyannos, 1987)

In trap design, attraction to colour is the most exploitable visual cue utilised by the medfly as it is easiest to incorporate into trap design. Prokopy and Economous (1975) found that McPhail traps that had been painted fluorescent yellow were more attractive than those painted enamel yellow, red or grey. Studies by Epsky and Heath (1997) show that traps painted with a green strip catch more female medfly compared to a clear trap or those with an orange or yellow strip whereas males in this experiment have a preference for yellow over orange and green. Yellow sticky inserts for Jackson traps have also been used with success (Epsky *et al*., 1996)

Shape is also an important cue for medfly but more difficult to incorporate into trap design. Both male and female medfly have been shown to prefer black or yellow

spheres over cylinders, rectangles and cubes of the same colour and surface area (Nakagawa *et al*., 1978).

1.4.2 Chemical attractants

Most traps do not rely on visual cues alone to catch fruit flies. The addition of a chemical attractant to a trap can increase its catch efficiency and effective range (Jones *et al*,. 1983). Economopoulos (1979) and Katsoyannos (1989) have shown that yellow traps that employ an olfactory cue, catch more flies than those possessing only the yellow visual cue. Potential chemical attractants that are used against medfly are pheromones, para-pheromones and liquid food baits.

1.4.3. Pheromone

Pheromones were originally defined as "substances secreted to the outside by an individual and received by a second individual of the same species in which they release a specific reaction , for instance a definite behaviour [releaser pheromone] or development process [primer pheromone]" (Karlson & Lüscher, 1959). Although pheromones are widely used in the control of tephritids, for example, Spiroketal (1,7-dioxaspiro-[5,5]-undecane) for *Bactrocera oleae* (IAEA, 2003), this is not so for medflies. The sex pheromone of medfly is emitted by the male during calling. The first components of the pheromone blend were identified by Jacobson *et al*. (1973) and Baker *et al.* (1985). During electroantennograph (EAG) experiments, excised female antennas have responded to over sixty components emitted by calling males (Baker *et al*., 1985). The three major components of the medfly sex pheromone are ethyl (E)-3-octenoate, geranyl acetate and E,E-α-farnesene. A field study by Heath *et al.* (1991) found that sphere traps baited with this pheromone blend were more attractive to medfly than unbaited traps. The addition of Δ-1 pyrroline to the blend further increased the relative catch rates (Heath & Epsky, 1993).

However, these pheromone blends have not been found to be as effective as the more complex blend under field trial conditions (Delrio & Ortu, 1988; Heath *et al*., 1991).

When tested against calling males, the synthetic pheromone blend was shown to be far less attractive. It is likely that the complex pheromone blend of male fruit flies out-compete the attractants, which are simple synthetic <five part blends (Howse & Knapp, 1996). This partly explains the poor field performance. It is also suggested that failure of synthetic pheromone lures in citrus orchards could also be a result of the volatiles released from citrus fruits. Thirty-one of the main compounds attributed to the male sex pheromone are also present in the peel of oranges. Thus, the odours produced by oranges may well mask the weaker synthetic pheromone.

1.4.4 Para- pheromones

Para-pheromones elicit the same response as a pheromone, however, they are not produced by the responding species (Epsky & Heath, 1998). These chemicals are often similar in structure to the pheromone that they mimic inducing a similar response from the target organism. Para-pheromones are used to attract a number of Tephritid species. For example from the genus *Bactrocera*, melon fly (*B. cucurbitae*) and Queensland fruit fly (*B. tryoni*) are attracted to cuelure, while methyl eugenol is used to capture Oriental fruit fly (*B. dorsalis*), peach fruit fly (*B. zonata*), carambola fruit fly (*B. carambolae*), Philippine fruit fly (*B. philippinensis*), and banana fruit fly (*B. musae*). (Howlett, 1915; Alexander *et al.*, 1962)

Medfly have been found to be attracted to mixtures of the isomers of tetr-butyl 4 (and 5)- chloro-2-methylcyclohexane-1-carboxylate, known as trimedlure (Beroza *et al*. 1961). Medfly display a lek mating system, males aggregate around host plants and defend territory on the underside of leaves (Prokopy & Hendrichs, 1979). From these leaves the males emit a combination of chemical, visual and auditory signals that attract male and female conspecifics to their territory (Shelly & Whittier, 1997).

Trimedlure™ is a para-pheromone that appears to mimic the pheromone produced by the males during lekking. That trimedlure attracts medfly is beyond doubt, Steiner traps baited with trimedlure were found to capture significantly more flies than

unbaited Steiner traps (Nakagawa *et al*., 1971a). However, catch rates using this attractant are strongly sex biased (Nadel & Peleg, 1965). Only 0-1% of flies caught during studies were female. Trimedlure is seen as one of the strongest attractants for male medfly and consequently is widely used in detection and eradication programs. Ceralure™, a mixture of the ethyl esters of 4-(and 5-)-iodo-2-methylcyclohexanecarboxylic acids, acts in a similar way to Trimedlure and was successfully trialled by McGovern and Cunningham (1988).

1.4.5 Food baits

There is a long history of food bait use for the attraction of tephritids. Early traps used protein and fermenting sugar mixes as attractants (Gurney, 1925). Volatiles released from the protein and sugar act as food cues to the flies.

Protein baits such as Nulure™, Torula yeast™ and Buminal™ are commonly used against a range of tephritid pests including medfly (IAEA, 2003). When protein baits are used in bucket-style traps, the liquid protein also serves as the retainer of flies entering the trap. Additives such as borax are used to slow down the decomposition of captured flies. Unlike pheromones and para-pheromones, food baits are not sex specific, although the percentage of females captured by food baited traps is substantially higher than males (IAEA, 2003). Females require protein for reproduction (Christenson & Foote, 1960) and seek it out more readily than males. However, the water associated with these lures can attract beneficial insects.

Synthetic food lures are also used to attract medfly. Ammonium acetate, putrescine and trimethylamine (Epsky *et al.*, 1999) are combined in ampoules to make Tripack™. Synthetic food lures are more species-specific than liquid protein baits whilst still maintaining dual sex attraction. The lack of water reduces the catch rates of beneficial insects.

Numerous chemicals have been suggested and trialled for use as an attractant for medfly including: kerosene (Severin & Severin, 1913), angelica oil (Steiner *et al*., 1957), ginger oil (Shelly & Pahio, 2002) and citrus peel oils (Levinson *et al*., 1990). However, the liquid protein baits Tripack ™, Trimedlure ™ and Ceralure ™ are the only attractants used commercially.

1.5 Lure and kill technologies

Traditional insecticide spray methods indiscriminately deposit large amounts of insecticide into the environment and the resulting drift and runoff sometimes contaminate non-target areas, soils and aquatic systems, as well as produce. Lure and kill technologies use lower quantities of pesticide and target the pest species more effectively than these methods. (Lanier, 1990).

Killing agents are sometimes housed within a container or trap and target species are lured to the pesticide source with a species-specific attractant. Lure and kill technologies, therefore, have the potential to reduce insecticide residues on produce and also reduce the impact on non-target organisms such as beneficial predators and parasites.

1.6 Electrostatic powders

Certain powders have been shown to display high electrostatic potential that causes adherence to an insect's cuticle (which also carries an electrostatic charge that is achieved through tribocharging) (McGonigle, 2002). The use of electrostatic powders within a lure and kill system allows the delivery of insecticides to the target pest and also gives the added benefit of potential transmission of the insecticide onto conspecifics through social activities, autodissemination.

Entostat ™ is a proprietary powder developed by Exosect Ltd., Winchester, United Kingdom, which is being developed for a wide variety of delivery systems for pest management applications (Burns *et al*., 2005, Nansen *et al*., 2007). Entostat ™ is a

refined carnauba wax powder produced from the Brazilian wax palm, *Copernica cerifera* (Martius Palmae) and Entomag™ (Exosect Ltd., Winchester, UK) is a proprietary metallic powder that displays electrostatic properties.

Adhesion of these electrostatic powders to medfly (Armsworth *et al*., 2006, Barton *et al*., 2006) as well as transfer of Entostat to conspecifics (Barton *et al*., 2006) has been proven, indicating potential for autodissemination of insecticides through the population. However, no work has been carried out on the compatibility and suitability of these powders for delivery and dissemination of insecticides through lure and kill.

1.7 Killing agents

Spinosad, released by Dow AgroSciences under the name Tracer ™ in 1997, was the first insecticide identified in the Naturalyte class and is currently widely used to target medfly, commercially referred to GF-120 (Burns *et al*., 2001; Stark *et al*., 2004). Spinosad is a naturally occurring mixture of two macrolides, spinosyns A and D. These are the two most active metabolites derived from the soil actinomycete *Saccharopolyspora spinos* (Kirst *et al.*, 1992). They are commercially produced by the aerobic fermentation of *S. spinosa* on nutrient media (Boek *et al.*, 1994)

Activity which was originally used to target Lepidoptera, is proven against the orders Coleoptera, Diptera, Homoptera, Hymenoptera, Isoptera, Orthoptera, Siphonoptera, Thysanoptera and Acari (Thompson *et al.*, 1995). The insecticide can work through topical application or through ingestion (Cisneros *et al.*, 2002). No significant toxicological effect was found for many insecticide and drug target sites, and as a result, spinosad is described as having a novel mode of action. Spinosyns are neurotoxins which affect the nicotinic acetylcholine receptor and the GABA receptors (Watson, 2001) by an unknown mechanism. Due to the novel mode of action there is no cross-resistance with current biological and synthetic insecticides (Salgado, 1997; 1998).

Contaminated insects display involuntary muscle contractions caused by excitation of neurons of the central nervous system. The hyperexcitation results in paralysis attributable to neuromuscular fatigue due to overuse, but not due to the activity of spinosad on the neuromuscular system (Salgado, 1998). Furthermore, Watson (2001) describes a shut down of feeding in cockroaches in response to intoxication through ingestion of spinosyns.

Spinosad shows, despite the broad spectrum toxicity against pest insects, reduced toxicity against beneficial insects such as ladybirds and lacewings (DeAmicis *et al.*, 1997; Williams *et al*., 2003). It was also demonstrated that toxicity to birds and mammals is minimal (Bret *et al.*, 1997; Breslin *et al.*, 2000). The organic nature of the compound coupled with its low toxicity towards non-target organisms and short microbial and photodegredation have resulted in spinosad being listed as an environmentally and toxicologically reduced risk material by the U.S. Environmental Protection Agency (Saunders & Bret, 1997).

Toxicity has been tested on a number of tephritids. Adan *et al.* (1996) showed mortality of medfly when exposed to low concentrations of spinosad, whilst King and Hennessey (1996) tested it on Caribbean fruit fly (*Anastrepha suspense*). Moreno *et al.*, 2000 described comparable mortality of medfly when testing the product SolBait ™ which contained spinosad) against the malathion containing Nu-Lure ™. The bait spray product GF-120 which contains spinosad has also been used effectively against medfly.

The DOW Chemical Company launched chlorpyrifos in 1965 and it has become one of the most widely used insect control agents (Mori *et al*., 2006). Chlorpyrifos belongs to the organophosphate class of insecticides. It causes mortality by inducing excessive transmission of nerve impulses. This is a result of the accumulation of acetylcholine at nerve endings due to acetylcholinesterase enzyme inhibition.

Currently chlorpyrifos is the killing agent contained in the commercial products Lorsban™ and Dursban ™. It is used to protect pomme, stone and citrus fruits as well as a wide range of other crops (Dow AgroSciences, 2000).

Methoxyfenozide belongs to the class of insecticides known as the diacylhydrazines. These were first classified by the Rohm and Haas Company in the 1980s (Wing *et al.*, 1988) and are a novel class of insect growth regulators (IGRs) (Hsu, 1991). RH-5849 was the first of this class to be used and shows insecticidal activity against larvae of lepidopteran, coleopteran and dipteran species (Aller & Ramsay, 1988). This class of insecticide mimics the natural insect moulting hormone 20-hydroxyecdysone (ecdysone), binding to ecdysone sensitive nuclear receptors (EcRs). Ecdysone regulates genes integral to development and reproduction so that when diacylhydrazines are applied to larvae it causes mortality by interrupting or initiating the moulting process. Contaminated larvae display a range of cuticle malformations including premature apolysis and inhibition of ecdysis (Trisyono & Chippendale, 1997).

RH-5849 was later replaced by RH-5992 (tebufenozide) and RH-0345 (halofenozide). These show greater efficacy and selectivity than RH-5849 and are sold commercially under the trade names Mimic ™ and Mach 2 ™.

Methoxyfenozide (RH-2485) was described by Le *et al.*, 1996. Traditionally, methoxyfenozide has been used as a larvicide through ingestion by lepidopteran pests as uptake through ingestion is more efficacious than topical application (Carlson *et al.*, 2001). Exposed larvae show symptoms consistent with those of earlier diacylhydrazines.

Methoxyfenozide, which is not lethal to adults, can be used for chemo-sterilisation of lepidoptera. Application to females causes disruption of oogenesis resulting in the reduction of fecundity (Sun *et al.*, 2000). Application to males cause eggs produced

by females fertilised by these individuals to be unviable (Sun *et al.*, 2000). There are a number of theories put forward to explain this; Dhadialla (1998) suggests diacylhydrazines disrupt the spermatogenic processes while Carpenter and Chandler (1994) believe treated males are incapable of transferring sperm during mating. Hoelscher and Barrett (2003) reported that Codling moth males' locomotor activity was affected when exposed to surfaces treated with methoxyfenozide. This resulted in a reduction in males' ability to respond to a pheromone-producing female causing a reduction in her egg viability.

Carlson *et al.* (2001) has shown that methoxyfenozide binds to the lepidopteran EcRs six times more effectively than tebufenozide and 400 times more than 20-hydroxyecdysone. However, when binding to drosophila, EcRs methoxyfenozide displays only half the affinity of 20-hydroxyecdysone. For use against dipteran pests such as medfly a higher or more targeted dose would be required than those used against lepidopteran pests.

1.8 Aim of the research

There are a number of lure and kill systems currently available for use against tephritid fruit flies and medfly specifically. However, such systems primarily target only one sex. Food baits are used to mainly attract females while para-pheromones such as Trimedlure ^{TM} are used to attract males, but no system can access both sexes effectively without having adverse affects on beneficial organisms.

Medfly are known to display polyandry and polygyny (Nakagawa *et al*., 1971b; Hendrichs & Hendrichs, 1990). Males of this species also display in a lek mating system (Prokopy & Hendrichs, 1979) where they will weakly defend their territories (Whittier *et al*., 1992; Whittier & Kaneshiro, 1995). These behavioural traits give rise to frequent contact between conspecifics. These social interactions result in a number of opportunities for insecticide-laced electrostatic powder to be transferred from one individual to another. Medfly were used as the preliminary target for this lure and kill system due the potency of commercially available attractants for this

species and a social behaviour that lends itself to being targeted through autodissemination by allowing secondary transfer of insecticide to both sexes through transmission during courtship, mating and lekking events. It is hoped that this will resolve the problem of single sex attraction encountered by other control systems.

The primary aim of the current research is to develop a lure and kill system for the Mediterranean fruit fly that targets both sexes whilst using an attractant that targets only one. The efficacy of the killing agents spinosad, chlorpyrifos and methoxyfenozide will be trialled on their own and in combination with the electrostatic powders Entostat ™ and Entomag ™ at different concentrations. No work has been carried out on the compatibility and suitability of these powders for delivery and dissemination of insecticides through lure and kill. The optimum combination and concentration will be selected and the possibility of secondary transfer and subsequent mortality to conspecific male and female medfly during social interactions will be examined. Powder dispensing bait station prototypes will also be developed and will then be tested against standard delta bait stations in field-based experiments.

1.9 Aims

- **Establish the most suitable killing agent for inclusion in the system by;**
    - Quantifying carrier powder uptake by male and female medfly
    - Assessing the use of methoxyfenozide as a sterilant to medfly
    - Assessing the effect of spinosad on medfly
    - Assessing the effect of chlorpyrifos on medfly

- **Establish the most suitable carrier powder for the system by;**
    - Assessing the effect of Entostat electrostatic powder on medfly
    - Assessing the effect of Entomag electrostatic powder on medfly

- **Establish the most suitable carrier powder/killing agent dose combination**

- **Confirm lethal contamination from male to females through courtship**

- **Assess the efficacy of open and closed container designs through field trials**

- **Establish what aspects are important in container design through behavioural observations**

Chapter 2: The potential of electrostatic powders and killing agents for inclusion in an autodissemination control system for the medfly, *Ceratitis capitata* (Wiedemann)

2.1 Introduction

Increased consumer awareness of food safety and the environmental issues surrounding agricultural practices has increased consumer demand for organic and low pesticide input produce (Michaelidou & Hassan, 2008). This shift in public opinion and the associated increase in political pressure have resulted in restrictions on the application of many traditional pesticides, with some having been withdrawn from use altogether. This shift in cultural perception of how we produce food has led to a drive towards more targeted solutions to pest problems.

There are a number of control strategies currently employed to limit damage incurred by medfly (USDA, 2003). Bait sprays are extensively applied and the use of mass trapping and sterile insect technique (SIT) are also widespread (Enkerlin, 1984; Peck & McQuate, 2000; Navarro-Llopis *et al*., 2008). These methods have their limitations and recent developments have looked towards the prospect of infield sterilisation by chemosterilising baits, primarily lufeneron (Navarro-Llopis *et al*., 2007).

Traditional medfly control techniques have generally utilised lure and kill methodology where a protein rich liquid attractant combined in an insecticide spray is applied to harbourages and host crops. Malathion and other organophosphates are commonly used within bait spray systems (Burns *et al*., 2001). These chemicals and application methods are effective but non-specific, killing many beneficial insects (Chueca *et al*., 2007). They have implications for human health and have shown resistance problems within some insect populations (Magaña *et al*., 2007; Vontas *et*

al., 2011). Spinosad based bait sprays such as GF-120 (Dow AgroSciences, Indianapolis, USA) have shown to be less toxic to some beneficial insects (Michaud, 2003). The sugar based attraction component of the control system, however, is associated with spot damage and an increase in sooty mould growth in orchards (Chueca *et al*., 2007).

Mass trapping is also implemented for control of medfly (Navarro-Llopis *et al*., 2008). Baited traps, however, incur a high economic cost due to the component parts and maintenance (USDA, 2003). These traps need to be serviced regularly to keep them operational due to inactivation of the trapping/killing mechanism or due to loss of attraction.

Attractants utilised in these systems are either general sugar based formulations that attract a broad spectrum of insects, many beneficial to the natural control of medfly, while the species-specific trimedlure para-pheromone almost entirely attracts only male flies.

The most promising chemical sterilent identified for medfly is lufeneron (Casaña-Giner *et al*., 1999; Navarro-Llopis *et al*., 2004), a phenylbenzoylurea insect growth regulator (IGR) that has previously been used to control lepidopteran and coleopteran larval pests. Methoxyfenozide which belongs to the diacylhydrazine class of IGR, has also been shown to be active against lepidopteran, dipteran larvae topically and through ingestion, (Pineda *et al*., 2007). Further to this, activity was described against adult codling moth (*Cydia pomonella*) in which reductions in fertility and fecundity were recorded (Sun *et al*., 2000). Carlson *et al*. (2001) have shown that in Diptera (drosophila), methoxyfenozide activity is the result of binding of the compound to the ecdysone sensitive nuclear receptors (EcRs), mimicking the natural insect moulting hormone 20 hydroxyecdysone (ecdysone), that regulates genes integral to development and reproduction (Hagedorn, 1985). This suggests potential activity against adult medfly.

A control method that can reduce the amount and distribution of the killing agent while also affecting both sexes but not containing a broad insect attractant such as a sugar formulation that can potentially cause further crop damage, would be advantageous. A powder-based lure and kill/sterilise container could fill this niche. Species-specific lures could be used to attract male medfly to containers which included a killing agent-impregnated electrostatic powders. These powders would adhere to the insects' body and be passed on to conspecifics through courtship and other social interactions or allow sterilised males to leave the container to compete with male conspecifics.

This study tested the suitability of two different adhesive powders, Entostat and Entomag, as pesticide carriers for use in a lure and kill pest control system. Both of these powders have been shown to adhere to medfly adults visiting bait stations in the field but it has not been ascertained whether or not these powders can be combined with killing agents to kill medfly (Armsworth *et al*., 2008). In this study Entomag was formulated with chlorpyrifos or spinosad and Entostat only with the latter. The efficacies of these three powders were compared using dose response bioassays conducted on medfly. The killing agents were also applied with solvent to assess the affect of the carrier powders on the performance of the pesticides.

The efficacy of methoxyfenozide on egg production and viability in medfly was also assessed. Methoxyfenozide was applied directly onto the cuticle of both male and female medfly and the effect on the fertility and fecundity of the flies was considered. A novel dosing technique was developed to simulate uptake of the powder formulations by medfly under field conditions. Prior to dosing, the powders were mixed with a fluorescent dye to allow uptake to be quantified using a fluorometric assay. Lethal time to 50% mortality were calculated for male and female medfly contaminated by each of five different doses of the insecticides (chlorpyrifos and spinosad) formulated with each carrier powder to give an idea of which doses would be most appropriate for use in a system reliant upon autodissemination to conspecifics and associated secondary knockdown. The

optimum dose and formulation were selected and further bioassays were conducted to allow quantification of secondary transfer of the formulated powders to male and female medfly during social interaction. Delayed action of the pesticide is vital to ensure transfer of killing agents to conspecifics, however, sub-lethal dosing during primary transfer must be avoided to prevent resistance to the insecticide from occurring.

2.1.1 Aims

- **Assess the use of methoxyfenozide as a sterilant to medfly**
- **Quantify carrier powder uptake by male and female medfly**
- **Establish the most suitable killing agent for inclusion in the system**
- **Establish the most suitable carrier powder for the system**
- **Establish the most suitable carrier powder/killing agent dose combination**
- **Confirm lethal contamination from male to females through courtship**

2.2 Material and Methods

2.2.1 Study organisms

Medfly used in this study were cultured within the quarantine facility at Exosect Ltd., Winchester, UK, and originated from a strain maintained at the Moscamed mass-rearing factory in Guatemala, established in 1984 (Rendón, 1996). Rearing of medfly and all experiments were conducted at 25±3°C, 65±5% relative humidity (RH) and 16:8 hour light:dark (L:D). Adults and larvae were reared according to Armsworth, *et al*., 2006.

In all experiments, adult flies of unknown mating status were used in trials four to eight days after eclosion and individuals were only used once. Except the 'powder transfer and sterilisation experiments' where individuals were separated into male and female cages within 24 hours of eclosion to ensure that only virgins were used, and these were used in trials two to six days after eclosion. Each experiment was repeated on ten occasions giving a sample size of ten, except the powder transfer experiment which was replicated three times.

2.2.2 Formulation of carrier powders

Two powders were investigated; the first is an electrostatic powder called Entostat (Exosect Ltd, Winchester, UK), a refined carnauba wax produced by the fronds of the Brazilian wax palm, *Copernica cerifera* (Palmae). The second is a proprietary metallic powder called Entomag™ (Exosect Ltd, Winchester, UK). These powders were each formulated with technical grade spinosad (Dow Agrosciences, Oxford, UK) at five different insecticide concentrations (2, 1, 0.5, 0.1 and 0.05% w/w) using proprietary formulation techniques developed at Exosect Ltd. To quantify how much powder medfly took up during dosing, a fluorometric assay was used in which a fluorescent dye, Glo-Brite® AW Powder (Himar, Bradford, UK) was combined with each powder at a concentration of 10% w/w (Armsworth *et al*., 2006; Barton *et al*., 2006).

2.2.3 Powder uptake quantification

Dyed Entostat or Entomag powder was applied as a fine layer to a foil dish by finely dusting powders onto the dish surface and gently tapping off the excess powder. The foil dishes were housed inside upturned plastic containers with a hole on the side for access. Individual flies were captured in glass vials and shaken gently onto the fine layer of powder. Only flies that landed, contacted the powder once and then flew off were included in the experiment. After dosing, the flies were recaptured individually in Eppendorf tubes containing 1 ml 95% ethanol and then run through a Luminescence Spectrometer LS5OB (Perkin Elmer LAS, UK Ltd) as in Armsworth *et al*., 2006 to find out the average quantity of powder uptake.

2.2.4 Methoxyfenozide Sterilisation

Flies were collected and anaesthetised by placing them into a freezer (-14ºC) for 2 to 3 min. Fifteen male and fifteen female adult flies were selected for each of the treatment groups: control (acetone only), acetone methoxyfenozide 1000 ppm. 1 µl of the appropriate treatment was applied to the ventral surface of the abdomen of each fly using a micro-applicator (Burkhard Manufacturing Co., Ltd.). Adults were kept in Perspex cages (33 cm × 20 cm × 22 cm) (www.PetsDirect.com) and fed on a 75% sugar and 25% yeast paste. Water was provided in a pot with a sponge wick. A 12 cm diameter Petri dish containing tap water was placed under the oviposition screen to collect the eggs. Eggs were removed at 24 hour intervals and counted then fly mortality was assessed to adjust for decreasing fly population. Eggs were retained for five days at which point egg viability was assessed by counting hatched larvae. This experiment was replicated three times giving a sample size of 45, error bars were used to assess differences between the treatment groups.

2.2.5 Toxic powder application assays

Foil dishes were coated in a fine layer of Entomag or Entostat at each spinosad/chlorpyrifos dose (2, 1, 0.5, 0.1 and 0.05% w/w) using the same application method as in the previous experiment (see above). Two trays were also treated with unformulated blank Entostat or Entomag to act as positive controls. Ten male and ten

female medfly were dosed on each tray (as in Section 2.2.3). After dosing, the treated flies were recaptured and housed individually in 25 ml, ventilated pots (SHC Web POT25ml) and were provided with water and a diet of sucrose and yeast (3:1) mixture *ad libitum.* The condition of the treated flies, as well as the group of untreated controls, were assessed at regular intervals. Individual flies were recorded as alive, dead or moribund (irreversible knockdown).

2.2.6 Toxic solvent application assays

Acetone (>99.5%) was used as a carrier for spinosad and chlorpyrifos in this experiment. Solutions at doses of 0.1, 0.05, 0.025, 0.005 and 0.0025 % w/w of both spinosad and chlorpyrifos were prepared. These doses were selected in order to expose the flies to equivalent doses of pesticide to those expected to be delivered by the carrier powders as indicated by the results of the fluorometric assays in Experiment 1 (Fig. 3.1.). A Burkard microapplicator (Burkard Manufacturing Co. Ltd. UK) was used to administer a 1 µl droplet of formulation or acetone control onto the ventral surface of 10 male and 10 female medfly (anaesthetised by placing in a freezer for a few minutes) for each treatment. After dosing, the medfly were housed individually as in Experiment 2 (see above). The condition of the treated flies as well as the group of untreated controls was assessed at regular intervals. Individual flies were recorded as alive, dead or moribund.

2.2.7 Secondary transfer of spinosad in Entostat to conspecifics

Virgin male medfly were dosed with Entostat powder formulated with 2% spinosad w/w on foil trays (as in previous experiments - see above). The male flies were then introduced into a Perspex cage (43 cm x 28 cm x 33 cm) containing virgin female flies at a ratio of at least 1:3 males to females. This cage was observed and when a copulation event began, the mating pair was removed and transferred to a separate holding pot. For comparison, an unmated female was removed for observation each time a mating pair was collected. Upon termination of copulation the males and females were housed separately. All medfly were housed individually in small, ventilated containers with food and water, observed at regular intervals and assessed

as alive, dead or moribund as before. This experiment was repeated three times giving a sample size of 51.

2.2.8 Statistical analysis

One-way analysis of variance was used to compare the mean uptake of Entomag and Entostat for males and females. Prior to analysis, data were transformed by $\log_{10}$ to Normalise residuals. Error bars were used to assess differences between the treatment groups in the sterilisation experiment. In the toxicity studies the lethal time to 50% mortality (LT50) was estimated for each application method this was done by applying a Probit model to the data using Minitab 14 software (Finney, 1971), a commonly used assessment of killing agent efficacy (Gupta *et al*., 2009; Bürgi & Mills, 2010; Hardke et al., 2011). Time of initial moribundancy (the onset of abnormal behaviour leading to death, for example 'twitching' and the dragging of appendages) was used to calculate the dose of the toxicant expected to kill 50% of population (LT50) as flies were irreversibly knocked down after this point. Non-overlapping 95% fiducial limits were used to determine the significance of any differences between groups. The LT50 was only calculated for doses that had caused >50% mortality except in the secondary transfer of spinosad in Entostat to conspecifics experiment.

2.3 Results

2.3.1 Powder uptake quantification

The average pick-up of dyed Entostat powder by medfly was found to be 25.5 µg for females and 24 µg for males (fig. 2.1.). However, no significant difference was found between the sexes. Pick-up of dyed Entomag powder was 46.5 µg for females and 44.8 µg for males but again this difference was not significant. However, Entomag was found to adhere to medfly far more readily than Entostat ($F_{1,8} = 11.03$, $P<0.01$).

The average powder pick-up values were used to estimate the quantity of spinosad that would be transferred to the flies during the toxic powder application experiments. The quantity of spinosad picked up by an individual fly during treatment with 0.05, 0.1, 0.5, 1 and 2% Entostat powder was estimated at dosages of 0.012, 0.024, 0.12, 0.24, 0.48 µg respectively for males and 0.013, 0.026, 0.13, 0.26, 0.52 µg respectively for females. The estimated quantity of spinosad picked up during treatment with Entomag for doses of 0.05, 0.1, 0.5, 1 and 2% were 0.023, 0.045, 0.225, 0.45 and 0.48 µg respectively for males and 0.024, 0.047, 0.235, 0.47 and 0.52 µg respectively for females.

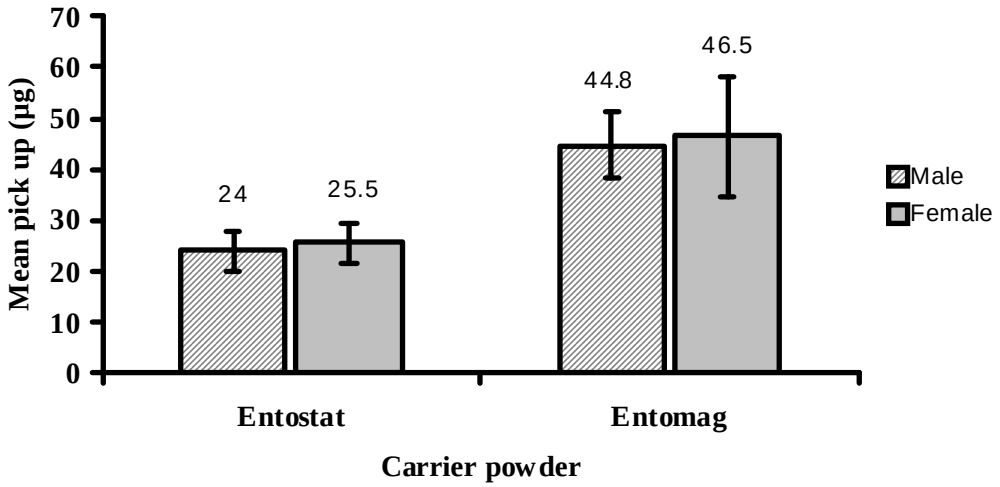

Figure 2.1 Mean quantity of dyed carrier powder picked up by male and female Medfly.

2.3.2 Methoxyfenozide sterilisation

Overlapping error bars imply that the values observed during the fertility (fig. 2.2) and fecundity (fig. 2.3) assessments for the two treatments, control and methoxyfenozide did not differ significantly. Results in the fertility experiments were highly variable meaning that any differences in treatments would be difficult to elucidate.

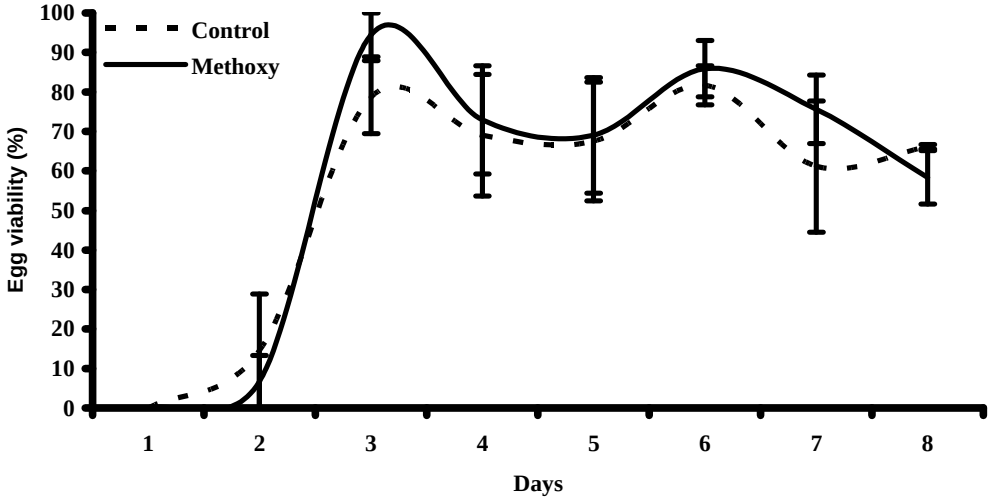

Figure 2.2 Fertility of medfly post application of methoxyfenozide.

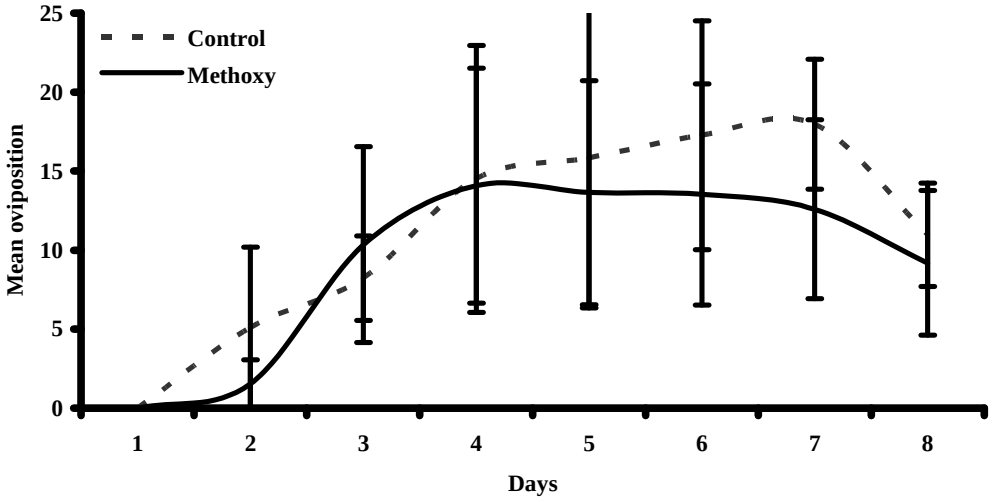

Figure 2.3 Fecundity of Medfly post application of methoxyfenozide.

2.3.3. Toxic powder application assays

Chlorpyrifos formulated with Entomag produced 100% mortality at doses of 0.5% and above in females and at 0.1% and above in males. LT50's were calculated through Probit analysis for all chlorpyrifos doses that provided 100% mortality (Table 2.1). There was no significant difference between LT50's for any dose administered to either female or male flies and this was due to the overlapping of Fiducial limits. The slowest acting dose that resulted in 100% mortality to both male and female flies was 0.5%.

The two lowest dosages of spinosad formulated Entostat and spinosad formulated Entomag (0.1% and 0.05%), were sub-lethal to both male and female flies (table 2.2 and 2.3). However, doses of 0.5, 1 and 2% spinosad gave rise to 100% mortality in both males and females allowing LT50 values to be calculated using Probit analysis. Male flies displayed a greater susceptibility to the spinosad powder, with LT50 values being lower than those of the females at corresponding doses. LT50 values for males dosed with 0.5% and 2% Entostat powder were significantly lower than those

of the females although the fiducial limits of the LT50 of males and females dosed at 1% were not distinct (fig. 2.4).

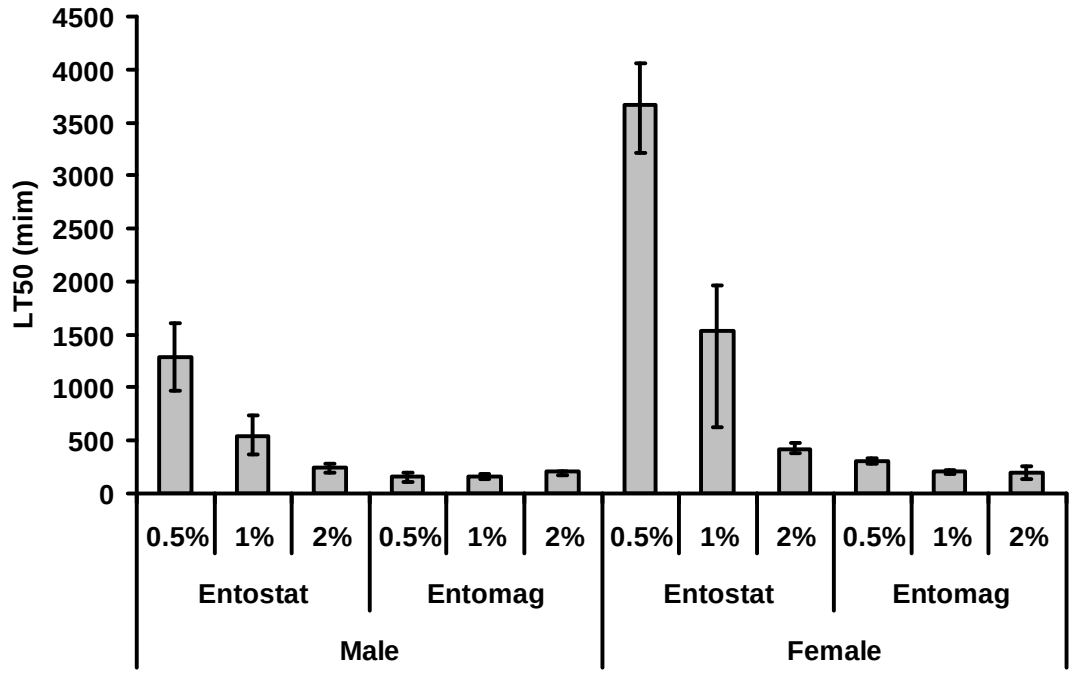

Figure 2.4 LT50 of male and female medfly contaminated with the carrier powders Entostat and Entomag formulated with varying doses of spinosad.

Table 2.1 Mortality (LT50) through Entomag applied chlorpyrifos.

Carrier/KA	KA (%)	Approx. Weight of AI (µg)	Sex	LT50 (min)	Fiducial limits (95%)		Slope (± SE)	Mortality (%)
					Lower	Upper		
Entomag/ chlorpyrifos	0.05	0.0225	Male	*	*	*	*	50
		0.0235	Female	*	*	*	*	20
Entomag/ chlorpyrifos	0.1	0.045	Male	34ab	-48	67	0.00078 ±0.00024	100
		0.047	Female	407c	177	769	0.00078 ±0.00024	80
Entomag/ chlorpyrifos	0.5	0.225	Male	49ab	31	56	0.08532 ±0.00136	100
		0.235	Female	54ab	-42	103	0.00506 ±0.00105	100
Entomag/ chlorpyrifos	1	0.45	Male	27a	-150	37	0.06109 ±0.29279	100
		0.47	Female	49b	37	57	0.05114 ±0.01291	100
Entomag/ chlorpyrifos	2	0.9	Male	31ab	22	39	0.05539 ±0.01277	100
		0.94	Female	46ab	22	51	0.04595 ±0.01283	100

LT50 values with unlike letters were significantly different (P≤0·05)

Table 2.2. Mortality (LT50) through Entomag applied spinosad.

Carrier/KA	KA (%)	Approx. Weight of AI (µg)	Sex	LT50 (min)	Fiducial limits (95%)		Slope (± SE)	Mortality (%)
					Lower	Upper		
Entomag/ spinosad	0.05	0.0225	Male	*	*	*	*	10
		0.235	Female	*	*	*	*	0
Entomag/ spinosad	0.1	0.045	Male	*	*	*	*	40
		0.047	Female	*	*	*	*	20
Entomag/ spinosad	0.5	0.225	Male	159a	109	190	0.00740 ±0.00136	100
		0.235	Female	307b	285	335	0.01085 ±0.00216	100
Entomag/ spinosad	1	0.45	Male	164a	135	184	0.01364 ±0.00299	100
		0.47	Female	205a	178	227	0.01217 ±0.00265	100
Entomag/ spinosad	2	0.9	Male	205a	169	207	0.06436 ±0.01797	100
		0.94	Female	191a	139	257	0.01777 ±0.00403	100

LT50 values with unlike letters were significantly different (P≤0·05)

Table 2.3. Mortality (LT50) through Entostat applied spinosad.

Carrier/KA	KA (%)	Approx. Weight of AI (µg)	Sex	LT50 (min)	Fiducial limits (95%) Lower	Upper	Slope (± SE)	Mortality (%)
Entostat/ spinosad	0.05	0.012	Male	*	*	*	*	0
		0.013	Female	*	*	*	*	0
Entostat/ spinosad	0.1	0.024	Male	*	*	*	*	30
		0.026	Female	*	*	*	*	30
Entostat/ spinosad	0.5	0.12	Male	1286a	970	1601	0.00091 ±0.00015	100
		0.13	Female	3661b	3210	4060	0.00068 ±0.00011	100
Entostat/ spinosad	1	0.24	Male	543c	373	733	0.00172 ±0.00046	100
		0.26	Female	1537ac	626	1961	0.00086 ±0.00024	100
Entostat/ spinosad	2	0.48	Male	248d	195	280	0.00931 ±0.00178	100
		0.52	Female	420c	385	476	0.007722 ±0.00206	100

LT50 values with unlike letters were significantly different (P≤0·05)

2.3.4 Secondary transfer of spinosad in Entostat to conspecifics

Females that mated with contaminated males were shown to have a significantly faster mortality rate when compared to unmated females, LT50 mated 1712 min and unmated LT50 3636 min (table 2.4). Mortality in the unmated females was 41% compared to 75% mortality for mated females. Mortality rates for female flies were significantly slower than that of the artificially contaminated male flies LT50 191 min.

Table 2.4 Conspecifics transfer of 2% spinosad Entostat.

Status	Dose	LT50 (Min)	Fiducial Limits (95%)		Slope (± SE)	Mortality (%)
			Lower	Upper		
Contaminated Male	Entostat/ spinosad 2%	191a	186	197	0.01029 ±0.00045	100
Mated Female	Entostat/ spinosad 2%	1712b	1553	1905	0.00068 ±0.00005	75
Unmated Female	Entostat/ spinosad 2%	3636c	3234	4242	0.00045 ±0.00005	41

LT50 values with unlike letters were significantly different (P≤0·05)

2.3.5 Toxic solvent application assays

All doses of spinosad and chlorpyrifos applied using an acetone carrier resulted in 100% mortality.

LT50 values for spinosad at dosages of 0.0025, 0.005 and 0.1% active ingredient showed significant differences between males and females at corresponding doses (table 2.6). 0.025µg of spinosad applied through a 1µl droplet of acetone gave 100% mortality to both males and females in contrast to the 30% mortality achieved as a result of the application of similar quantities of spinosad via Entostat powder.

Chlorpyrifos applied through acetone gave LT50 values that were significantly different between sexes at every corresponding dose except 0.025% where Fiducial limits slightly overlapped, with males having a lower LT50 than females (Table 2.5). LT50 values were lower for dosages of chlorpyrifos applied with acetone than with Entomag, but the variability of the Entomag data gave wide-ranging Fiducial limits so that no significance was found.

Table 2.5 Mortality (LT50) through acetone applied chlorpyrifos.

Carrier/KA	KA (%)	Approx. weight of AI (µg)	Sex	LT50 (min)	Fiducial limits (95%) Lower	Upper	Slope (± SE)	Mortality (%)
Acetone/ chlorpyrifos	0.0025	0.025	Male	44a	40	48	0.08397 ±0.01807	100
			Female	128a	86	168	0.00861 ±0.00235	100
Acetone/ chlorpyrifos	0.005	0.05	Male	40a	35	45	0.07183 ±0.01465	100
			Female	74a	69	79	0.08873 ±0.02404	100
Acetone/ chlorpyrifos	0.025	0.25	Male	28b	25	31	0.13615 ±0.03195	100
			Female	35a	30	40	0.07710 ±0.01584	100
Acetone/ chlorpyrifos	0.05	0.5	Male	19c	15	23	0.09152 ±0.01913	100
			Female	34b	30	37	0.12823 ±0.03323	100
Acetone/ chlorpyrifos	0.1	1	Male	13c	10	15	0.17167 ±0.03661	100
			Female	28b	22	31	0.11728 ±0.03262	100

LT50 values with unlike letters were significantly different (P≤0·05)

Table 2.6 Mortality (LT50) through acetone applied spinosad.

Carrier/KA	KA (%)	Approx. Weight of KA (µg)	Sex	LT50 (min)	Fiducial limits (95%) Lower	Upper	Slope (± SE)	Mortality (%)
Acetone/ spinosad	0.0025	0.025	Male	123a	108	136	0.01405 ± 0.0023	100
			Female	193b	179	211	0.01569 ±0.00218	100
Acetone/ spinosad	0.005	0.05	Male	74c	34	90	0.02024 ±0.00526	100
			Female	125a	110	136	0.01935 ±0.00333	100
Acetone/ spinosad	0.025	0.25	Male	55c	50	58	0.08183 ±0.01493	100
			Female	54c	42	60	0.04625 ±0.01009	100
Acetone/ spinosad	0.05	0.5	Male	52c	44	59	0.03452 ±0.00613	100
			Female	70c	51	84	0.01295 ±0.00173	100
Acetone/ spinosad	0.1	1	Male	31abc	22	337	0.06295 ±0.01473	100
			Female	48c	42	53	0.04840 ±0.00898	100

LT50 values with unlike letters were significantly different (P≤0·05)

2.4 Discussion

2.4.1 Sterilisation

Overlapping error bars in the fecundity experiment indicate that there was no significant difference in the oviposition of methoxyfenozide treated flies. High variability in the fertility experiment means that any differences in treatments would be difficult to expose. Variability in this experiment may have been down to the methodology or working on a laboratory strain of medfly or potentially, inconsistency in the activity of the killing agent. The dose of killing agent used was the highest possible that could be formulated into the two electrostatic powders considered for use in this lure and sterilise system. Higher concentration or oral application of methoxyfenozide may offer clear sterilising effects on medfly but were not trialled in this study.

2.4.2 Toxic powder application assays

2.4.21 Chlorpyrifos

The LT50 for male medfly dosed with chlorpyrifos applied via the proposed carrier powder Entomag was 48.6 minutes. A previous study on the reaction of medfly to contamination by Entomag showed that 50 minutes post powder application, as few as 2.5% of males had participated in mating events (Armsworth *et al*., 2006). This experiment was carried out in flight cages under laboratory conditions and it is expected that the figure for mating within the 50 minute time period under natural conditions would be much reduced due to the lower densities of conspecifics. This rapid knockdown rate provided by chlorpyrifos would limit the flies to a very narrow window of opportunity to leave the container and participate in social interaction before succumbing to the effects of the insecticide. For this reason, chlorpyrifos was not deemed suitable for inclusion in an autodissemination lure and kill system.

2.4.22 Spinosad

Spinosad dosages of 0.1% and 0.05% killing agent formulated with either powder were sub-lethal on both females and males and were, therefore, discounted for inclusion in the lure and kill system. The slowest acting dosage applied through Entomag was 0.5% (LT50 of 158.6 minutes). Armsworth *et al*. (2006) found that there was a significantly smaller proportion of flies observed mating in Entomag contaminated flies compared to control flies under laboratory conditions 120-200 minutes post contamination. Entomag formulation with spinosad was, therefore, deemed unacceptably fast for use with an autodissemination lure and kill system as the short time frame post contamination would limit potential transfer to conspecifics. As a result, Entomag was discounted for use in the system and was not used in further trials.

Spinosad doses of 0.5% and 1% on Entostat powder gave 100% mortality, the LT50 for both doses were over 9 hours (table 2.3) which would allow powder transfer to conspecifics. However, the LT50s at these doses were over 24 hours for females. The >24 hour window given by 0.5% and 1% spinosad would permit the female to mate and begin to oviposit.

The 2% dose was selected for inclusion in the system due to the lower LT50 values associated with this dosage which limits the female's egg-laying window to approximately seven hours, but allows the males approximately four hours to lek and mate transferring powder to conspecifics. Armsworth *et al*. (2006) found that Entostat contaminated males had recovered sufficiently by 260 minutes post application that there was no significant difference in the proportion of observed matings by control or Entostat coated males. Thus, by the time of death of males at 2% spinosad, the flies had returned to normal levels of mating frequency, thereby, allowing greater carrier powder transfer. The 2% spinosad formulation, therefore, was selected for further testing.

2.4.3 Toxic solvent application assays

All quantities of spinosad applied via acetone resulted in a much faster knockdown time than comparative quantities of spinosad formulated with Entostat and Entomag. This may be a result of the loss of carnauba wax powder through time (Armsworth *et al*., 2006). The carrier powder becomes dislodged through movement and grooming, and results in a lower effective dose of spinosad which leads to reduced mortality. It is also possible that the acetone carrier could have aided absorption of spinosad through the cuticle of the fly which would increase the activity speed of the spinosad, whereas in the powders the insecticide is bound up in the formulation.

2.4.4 Secondary transfer of spinosad in Entostat to conspecifics

The mortality of females mating with artificially contaminated males was not 100% but the LT50 was significantly faster than that of the unmated females in the cage. This is evidence supporting the transfer of powder from males to females during mating. The mortality observed in the unmated females suggests that powder transfer is also present whether or not mating occurs. Barton *et al.* (2006) also found that dyed Entostat powder was transferred from contaminated male medfly to female conspecifics, with mating events increasing transfer by approximately 400%. Powder transfer to females that did not mate with contaminated conspecifics may in part be due to interactions such as mating attempts. However, it is also likely that transfer to non-mated individuals was a result of loss of powder from males to the cage environment, thus transfer from males to females with which mating did not take place is expected to be much lower under field conditions.

2.4.5 Insecticide

Spinosad and chlorpyrifos were tested as potential insecticides for use within the lure and kill system. In dose response assays chlorpyrifos was found to be very effective resulting in 100% mortality in medfly at low concentrations and displaying very fast knockdown times. Spinosad also produced 100% mortality at most doses, but the knockdown times were slower than those of chlorpyrifos. Autodissemination systems require slow knockdown times to allow for social interactions to occur between the

target insects and thus transfer the insecticide powder from one individual to the next. The slower knockdown times observed with spinosad would allow more transmission opportunities to occur therefore spinosad was considered to be the more suitable killing agent for inclusion into the system.

An added advantage of the use of spinosad rather than chlorpyrifos is that spinosad is of natural origin, shows limited toxicity to non-target organisms and breaks down rapidly in the environment on exposure to UV radiation. In contrast, chlorpyrifos is a broad-spectrum organophosphate and its use could, therefore, be associated with many health and environmental risks. One of the main aims of the lure and kill system is to reduce the adverse effects on non-target organisms and the environment usually associated with insect pest control methods. The ecologically sound nature of spinosad combined with the low dose, targeted action of the lure and kill system will provide a safe and ecologically sound method of control for medfly.

2.4.6 Carrier Powder

Two carrier powders were tested for inclusion into the lure and kill system. These were Entostat, a fine carnauba wax powder and Entomag, a metallic powder, chosen due to their electrostatic properties. Both were found to adhere to medfly in suitable quantities. However, all doses of insecticide applied using Entomag were either sub-lethal to some or all flies, or caused mortality too rapidly to allow social interaction to occur. Thus it was unsuitable for inclusion within an autodissemination control system. In contrast, Entostat was found to give rise to 100% mortality in combination with spinosad at 2%, and at this concentration, the time-frame between contamination and knockdown was such that social interactions could occur but that the potential for successful oviposition by contaminated females would be limited.

2.4.7 General

Previous autodissemination studies have mainly focused on social or highly gregarious insect pests, such as Hymenoptera, Isoptera and Dictyoptera. Many species belonging to these orders live in close association with each other often

living within a central nest or communal harbourages where frequent conspecific interaction leads to higher killing agent transfer and dissemination. Coprophage and necrophage play an important role in systems used to target Dictyoptera species. Killing agents formulated into food baits are ingested by individuals and upon returning to communal harbourages defecate and die allowing further conspecifics to contaminate themselves.

Current systems used for the control of medfly control are problematic for a number of reasons. Firstly, systems containing broad spectrum organophosphate insecticides such as malathion are acetylcholinesterase inhibitors (Silva *et al*., 2008). Exposure, ingestion or misapplication can be hazardous (Brown & Brix, 1998; Blain, 2001) and as a result are being phased out of use in many countries (Jones *et al*., 2010). These chemicals are also highly deleterious to the environment including high toxicity to fish and beneficial insects (Fountain *et al*., 2007). Spinosad based bait sprays, although displaying no toxicity to vertebrates and reduced toxicity to beneficial insects, are incorporated into systems that either only target males of the species or use general protein and sugar based attractants that are attractive to non-target organisms (Bret *et al.*, 1997; Breslin *et al.*, 2000; Langewald *et al*., 2010).

It has been suggested that fast-acting insecticides result in fewer individual exposures and thus fewer total mortalities when autodissemination techniques are implemented in termite control (Thorne & Breisch, 2001, Saran & Rust, 2007). Chlorpyrifos Entomag formulations in this study gave a rapid knockdown time which limited the number of social interactions that contaminated individuals could participate in before succumbing to the effects of the insecticide. Soeprono and Rust (2004) found that slow-acting insecticides allowed ants greater amount of time foraging in the application area, recruiting a greater number of nest mates to the contamination area. In the proposed system a slower acting insecticide/delivery system like spinosad and Entostat at 2% would allow the medfly greater time to recover from the initial dosing and participate in courtship, mating and leking behaviours before dying. This would mean that more individuals are contaminated and has the benefit of transferring

killing agent to female medfly while using a predominately male only, species specific attractant such as the para-pheromone Trimedlure, limiting exposure to non-target organisms.

This study showed that it was possible to transfer a lethal amount of insecticide from one conspecific to another. This allows the prospect of using single sex attractants that are highly specific, to contaminate males and then transfer a lethal dose to females in the population. Such a system would prevent the luring of beneficial insects to the insecticide area of the container and target both sexes.

2.4.8 Conclusions

High variability in experiments carried out on methoxyfenozide meant that any differences in treatments would be difficult to expose. Although the experiment did not indicate any effect of the killing agent on medfly it cannot be ruled out given the variability of results and the limited maximum quantity of killing agent delivered to the insect via the carrier powder. It was concluded that the insecticide chlorpyrifos was incompatible for use in this lure and kill system due to the fast rate of knockdown which limits potential transfer to conspecifics. The carrier powder Entomag is also unsuitable for use within a lure and kill autodissemination system as the knockdown time was also too fast. Future fieldwork on this system should focus on the carrier powder Entostat. Entostat has been proven to be compatible with the insecticide spinosad. When formulated with 2% spinosad, Entostat will result in 100% mortality to primary contaminated male medfly, whilst still allowing sufficient time for the male to transmit a lethal dose of insecticide to a female during mating before knockdown takes place.

The results of the experiment with conclude that;

- **Flies pick up between 25 and 46.5 µg of electrostatic powder when contaminated**
- **Methoxyfenozide displayed no noticeable activity against medfly in this study**

- **Chlorpyrifos knocks down medfly males too rapidly and is therefore unsuitable for inclusion in a autodissemination system**
- **All doses of spinosad applied using Entomag were either sub-lethal to some or all flies, or caused mortality too rapidly to allow social interaction to occur**
- **Entostat formulated with 2% spinosad gave the mortality rate that best fitted the autodissemination system**
- **Mortality through powder (2% spinosad Entostat) transfer during mating proven**
- **Entostat 2% spinosad formulation chosen for inclusion in the system**

Chapter 3: Carrier powder container design for inclusion in an autodissemination control system for the medfly, *Ceratitis capitata* (Wiedemann)

3.1 Introduction

The medfly is a pest of a reported 253 varieties of fruit, nuts and vegetables (Hagen *et al*., 1981; Liquido *et al*., 1991) including pomme and citrus fruits, cocoa and guava (Weems, 1981). Damage is caused by the larvae boring through the fruits destroying them or by secondary infection by fungi and bacteria.

Lure and kill systems are extensively used to combat tephritid fly infestations (Koyama *et al*., 1984; Tsolakis *et al*., 2011). The standard bait spray applications usually used for the control of the Mediterranean fruit fly indiscriminately contaminate the target area with insecticide, and have harmful effects on beneficial and other non-target organisms (Michaud, 2003). Many lure and kill systems involve spraying an attractive protein/sugar based solution formulated with an insecticide, this is applied directly to the host crop or harbourages (Vargas *et al*., 2002). Such systems attract and kill non-target organisms potentially causing a reduction of beneficial insects while crop damage can occur due to fungal growth induced by sugar based solutions (Chueca *et al*., 2007). Alternative systems rely on traps that lure flies which are then killed and retained in the trap through the use of glue boards, insecticides etc., such as McPhail and delta traps (Dantas & Andrade, 2005). Traditional tephritid control traps like the McPhail and other styles of bucket trap have successfully been used and adapted for control of medfly since the 1930's (Newell, 1936). Delta traps have also successfully been used for the control of medfly for many years and in addition, they have now been used effectively for autodissemination/autoconfusion techniques for the control of Lepidopteran pests such as *Lobesia botrana* and *Cydia pomonella* (Howse *et al*., 2007).

Medfly have an innate preference for yellow coloured objects (Prokopy & Economous, 1975), and curved surfaces are favoured resting places for this species (Nakagawa *et al.*, 1978), most probably as a result of the likeness to the fruit that the flies use as food and for oviposition sites. These cues can be incorporated into container design in order to increase the likelihood of attracting medfly. Commercially available species-specific lures are also available for medfly and these should provide a reliable method of attracting flies into the containers. Two such attractants are Trimedlure and Tripack. Tripack is a synthetic food-bait lure that is found to be very attractive to medfly, especially to females (Beroza *et al*., 1961). Timedlure is a parapheromone that mimics a lekking pheromone, which causes males to aggregate around the source (Beroza *et al*., 1961) and is shown to be a very effective attractant for use in traditional control systems for medfly (IAEA, 2003).

The current control systems have a number of problems, they rely on either a single sex attractants like the parapheromone trimedlure which only has limited effect on the female population or the use broad spectrum attractants that attract and kill beneficial pest- controlling insects. An improved lure and kill system would affect both male and female medfly and not beneficial insects while retaining as much of the insecticidal agent within the system to limit adverse effects on the crop and the environment.

It is shown that electrostatic powders such as Entostat can contaminate medfly and be transferred to conspecifics (Barton *et al*., 2006). Further to this, when formulated with the insecticide spinosad at 2% w/w the powder is toxic to male medfly and powder transferred to females during mating events with contaminated males sufficient to cause secondary mortality. An electrostatic powder formulated with insecticide could be incorporated into a new lure and kill system. This would be an improvement on the current methodology for a number of reasons. The insecticide would be housed in a container which limits contamination of the environment. Species-specific lures could be used to target males that would subsequently contaminate females through social interactions. This is an advantage over current

systems which can only target females while utilising broad spectrum attractants that kill beneficial organisms. The container design for such a powder-based system would require a modification of the current trap designs currently in use for medfly.

In order to develop an autodissemination lure and kill system for medfly, it is necessary to produce a container capable of transferring the killing agent to the fly. The bucket trap style is inappropriate for modification as a container for autodissemination. The traps can easily be modified to prevent fly mortality within the trap but the addition of fine powder to this style of trap would inhibit the escape of contaminated flies from the bucket. Delta trap design used for lepidopteran autodissemination control was chosen to be trialled for use in this lure and kill system alongside two modified delta style container prototypes: open and closed (fig. 3.1; 3.2; 3.3). Field trials were used to assess the efficacy of each container design and behavioural observations were used to determine the mechanism by which the containers achieved success. The suitability of both Trimedlure and Tripack were also assessed for use in this system.

The lure and kill system proposed in this thesis functions by dosing individual flies with an electrostatic powder laced with insecticide which adheres to the cuticle of the insect leading to eventual mortality. The powder is housed in a number of containers, which are designed so that the powder is retained, but that contaminated flies are able to leave the containers and autodisseminate the killing agent through the population to conspecifics through social interactions such as courting, mating and lekking. The targeted dosing system employed in this control method coupled with the containment of the killing agent within the containers ensures that environmental contamination is kept to a minimum.

3.2 Aims

- **To assess the efficacy of open and closed container designs through field trials**

- **To establish what aspects are important in container design through behavioural observations**

3.2 Materials and methods

3.2.1 Field sites

Field sites were located at two mixed citrus orchards in Algarve, Portugal (fig 3.1).

Figure 3.1 Field site location, image taken from Google Maps - ©2011 Google.

Prototype container field trials and behavioural observations were carried out in a commercial orchard near Conceição de Tavira (fig. 3.2) and at the agricultural research station 'Centro de Experimentação Agrária' (CEAT), Tavira (fig. 3.3). The trials were carried out between May and July, 2006. In both cases citrus trees were planted in rows with 6 m between each row and 2 m between each tree within a row.

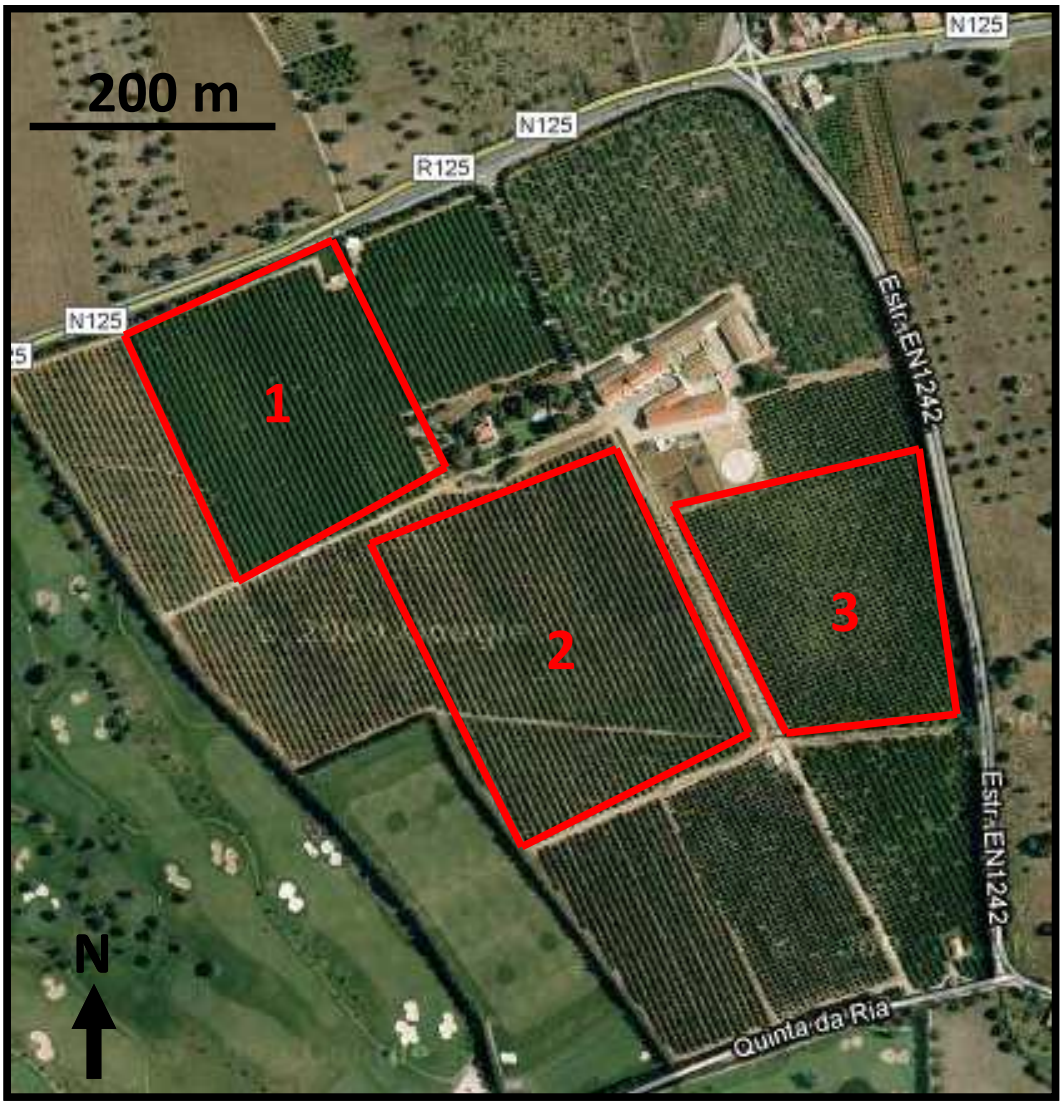

Figure 3.2 Field plot location, commercial orchard near Conceição de Tavira, taken from Google Maps - ©2011 Google.

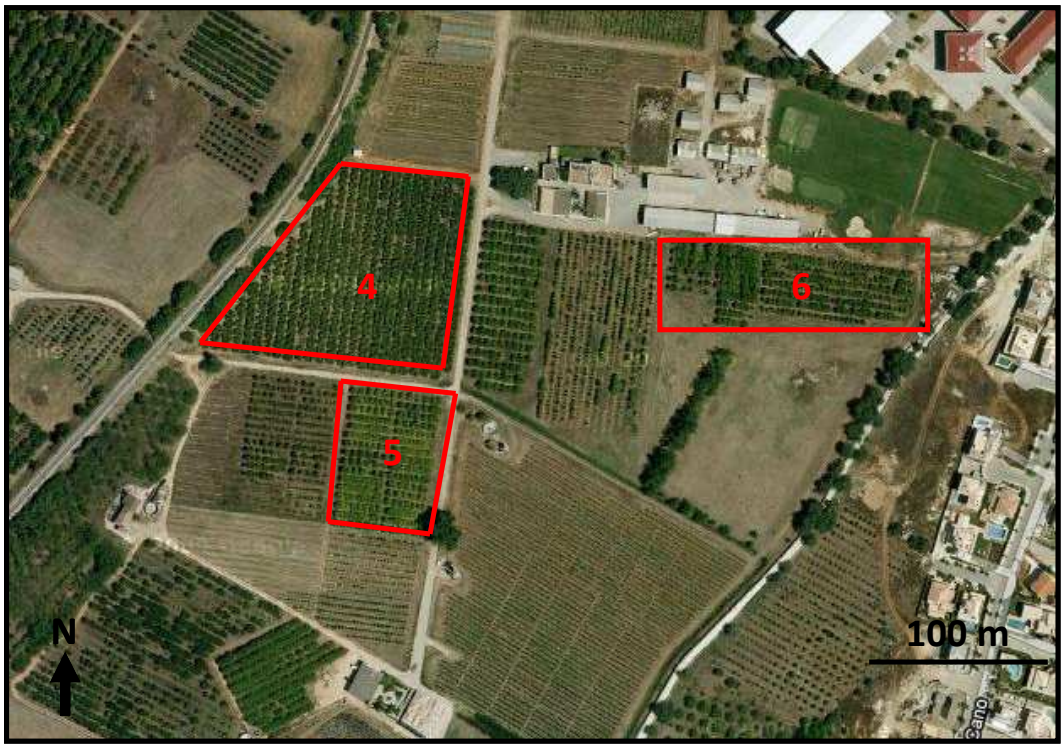

Figure 3.3 Field plot location, at the agricultural research station 'Centro de Experimentação Agrária' (CEAT), Tavira, taken from Google Maps - ©2011 Google.

3.2.2 Containers

The delta container (fig. 3.4) consisted of the standard delta traps available for monitoring insect pests, but without the adhesive insert used for trapping the target organism. Instead, a tray was inserted into the base of the trap to be used for the retention of killing agent-laced powder (referred to hereafter as the powder retention tray). An attractant plug was placed into the centre of the powder retention tray to lure the flies into the container.

The open style container (fig. 3.5) were modified from standard delta traps and consisted of two parallel surfaces suspended one on top of the other. The powder retention tray and attractant plug were placed on the lower surface of the container. It was hoped that this design would allow increased airflow through the container causing the attractant to spread over a wider area, thus attracting more flies.

The closed style containers (fig. 3.6) were also modified from standard delta traps. The two side walls of the trap were folded across each other resulting in a container with a smaller volume than the previous designs and with arc-shaped open ends. The powder retention tray and attractant plug were again placed on the floor of the container. In this case it was hoped that the shape of the container would inhibit escape thus increasing the likelihood of contact between the flies and the powder, therefore increasing contamination rates.

For the purpose of these trials, the powder retention tray was replaced with an adhesive monitoring board. This allowed easy observation of the number of flies that may become contaminated with powder in a day.

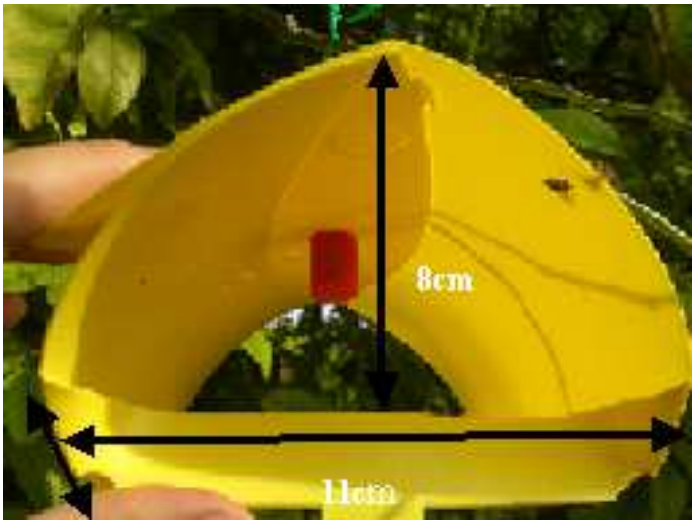

Figure 3.4 Delta container design.

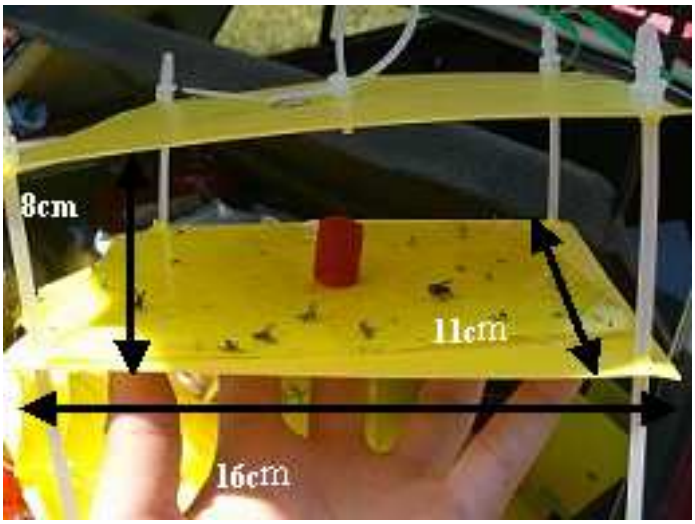

Figure 3.5 Open container design.

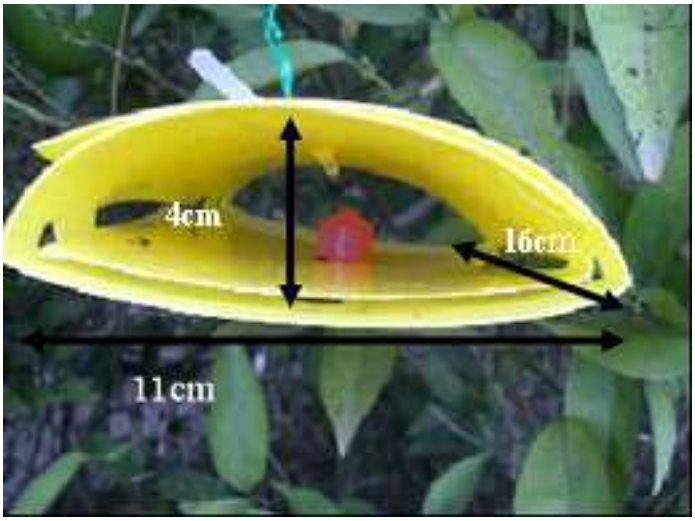

Figure 3.6 Closed container design.

3.2.3 Field trial: Prototype containers

Prototype container designs were tested in a citrus grove against wild, naturally occurring medfly. The open and closed designs were tested independently against the delta design using both male (Trimedlure) and female (Tripack) attractants.

3.2.31 Field trial: Prototype containers delta vs. open

Six treatments consisting of all possible combinations of the two designs - open and delta- and three attractants- Trimedlure, Tripack and a blank control, were hung in rows at three different plots (plots 1, 2 and 3 see fig. 3.2). The treatments were placed in randomised Latin square of six replicate sets (fig 3.7) at each of the three plots, giving 18 replicates in total. Each position in the square was 12 m distance from the next position. Containers were checked for catches, cleaned and position rotated daily, this was continued for one complete rotation of six days, allowing each style of container to occupy each position in the Latin square. For each day, the number of fly catches in all the containers were recorded. This approach was used to limit the impact of daily fluctuations of fly numbers.

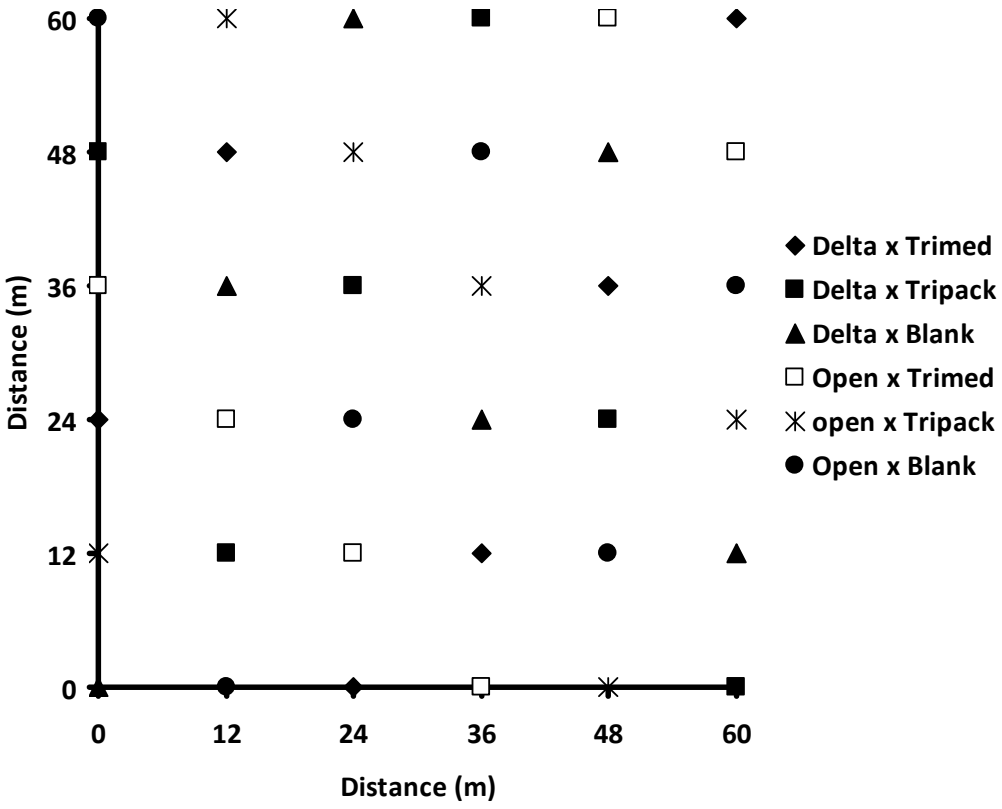

Figure 3.7 Example of a randomised Latin square field plot for the delta and open prototype container design experiment.

Observations were Normalised using a square root transformation. Fly catch rates were then analysed using a General Linear Model to test for significant effects of prototype design, attractant and plot location as well as interactions between prototype design and attractant on fly catches. A pairwise comparison (Tukey's) was run to look at differences between the groups.

3.2.42 Field trial: Prototype containers delta vs. closed

Four treatments consisting of all possible combinations of the two containers- closed and delta- and two attractants- Trimedlure, Tripack were hung in rows at two different plots (plots 4 and 5 see fig. 3.3). The treatments were placed in a randomised Latin square of four replicate sets (fig. 3.8) at two plots, giving 8 replicates in total. Each position in the square was 12 m distance from the next position. Containers were checked for catches, cleaned and rotated daily, and this was continued for one complete rotation of four days). For each day, the number of flies caught in all the containers were recorded. This approach was used to limit the impact of daily fluctuations of fly numbers.

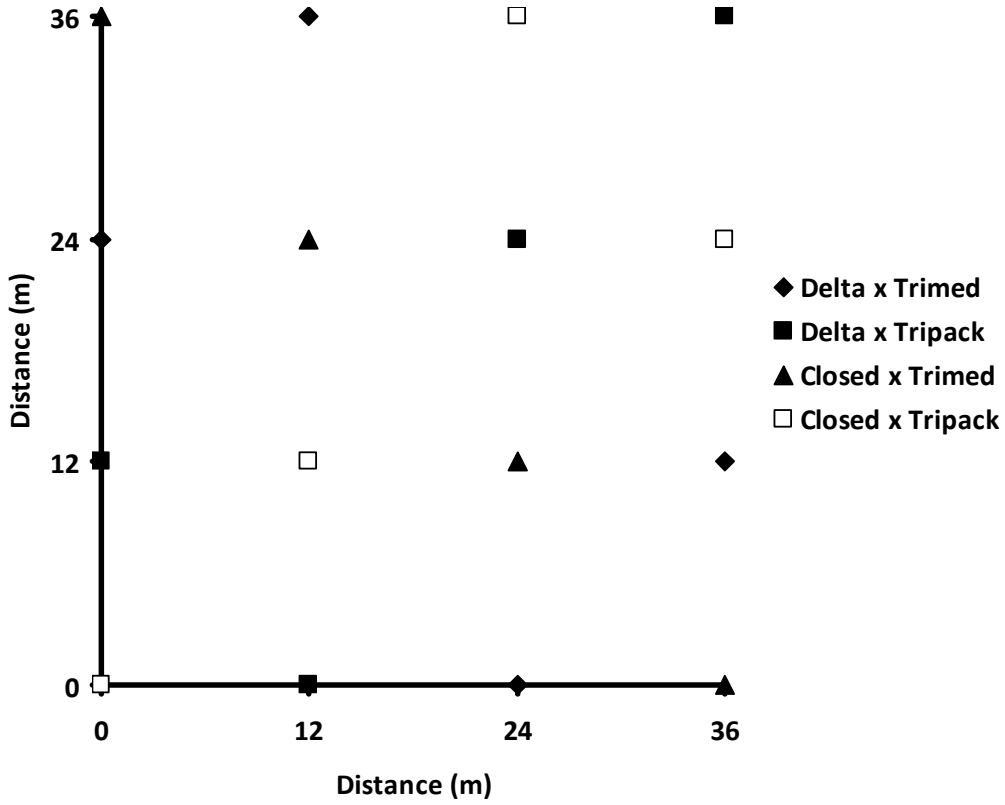

Figure 3.8 Example of a randomised Latin square field plot for the delta and closed prototype container design experiment.

Observations were Normalised using a square root transformation. Fly catch rates were then analysed using a General Linear Model to test for significant effects of prototype design, attractant and plot location as well as interactions between prototype design and attractant on fly catches. A pairwise comparison (Tukey's) was run to interpret these data.

3.2.4 Behavioural observations: Prototypes open, delta and closed lekking study

This experiment was carried out with Trimedlure alone, as Tripack was found to be ineffective for recruitment of flies to the powder retention board. Two of each of the three container designs (delta, open and closed) were baited with a single Trimedlure plug and hung at a randomly selected position within the citrus orchard (plot 6 see

fig. 3.2). Each container was observed for ten minutes each day for five consecutive days. Containers were relocated after each observation.

During the 10 min observation period, the total number of flies within the container, the total number of lekking events that occurred within each container and the number of lekking events that resulted in one or more flies falling onto the base of the container (where the powder retention tray would be positioned) were recorded. The latter is hereafter referred to as a 'hit'. A Kruskal-Wallis (K-W) test was used for analysis as the data was not Normally distributed, therefore, a one-way analysis of variance could not be used, as this is an analysis of the median these are shown on the graph, ±95% confidence intervals (CI) of the median.

3.2.4 Statistical analysis

All statistical analysis was conducted using Minitab 14th edition (Minitab Inc.).

3.3. Results

3.3.1 Field trial: Prototype container open vs delta

Containers baited with the blank attractant control caught no flies during any of the experiments. Delta containers were found to catch significantly more flies than the open-style containers (fig. 3.11). When delta containers were trialled against the open-style containers, mean catch rate of medfly for the delta was 7.3 (±1.9) flies over the six day period compared to only 3 (±0.8) for the open-style (ANOVA: $F_{1,71} = 7.53$, $P = 0.008$).

The Tripack attractant displayed low catch rates throughout the trial with a mean catch of 0.5 (±0.2) medfly overall (fig. 3.10). The Trimedlure-baited containers caught a mean of 9.8 (±1.8) flies over the trial period (ANOVA: $F_{1,71} = 161.07$, $P \leq 0.001$). Both container designs performed better when combined with Trimedlure than with Tripack, however, the combination of the delta design and the Trimedlure attractant proved to be the most efficacious set-up (ANOVA: $F_{1,71} = 15.07$, $P \leq 0.001$) (fig. 3.9), with a mean catch rate of 14.3 flies compared to that of 5.4 flies with the open-style container-Trimedlure combination.

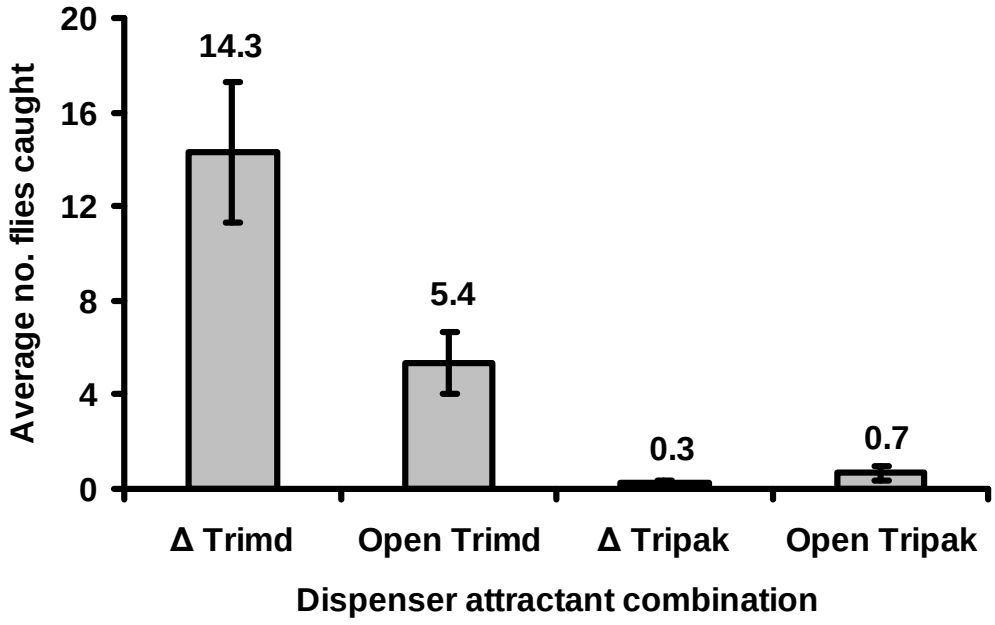

Figure 3.9 Field trial catches design, delta vs. open, Trimedlure vs. Tripack. Mean (±SE) number of flies caught by delta and open container designs baited with Tripack or Trimedlure.

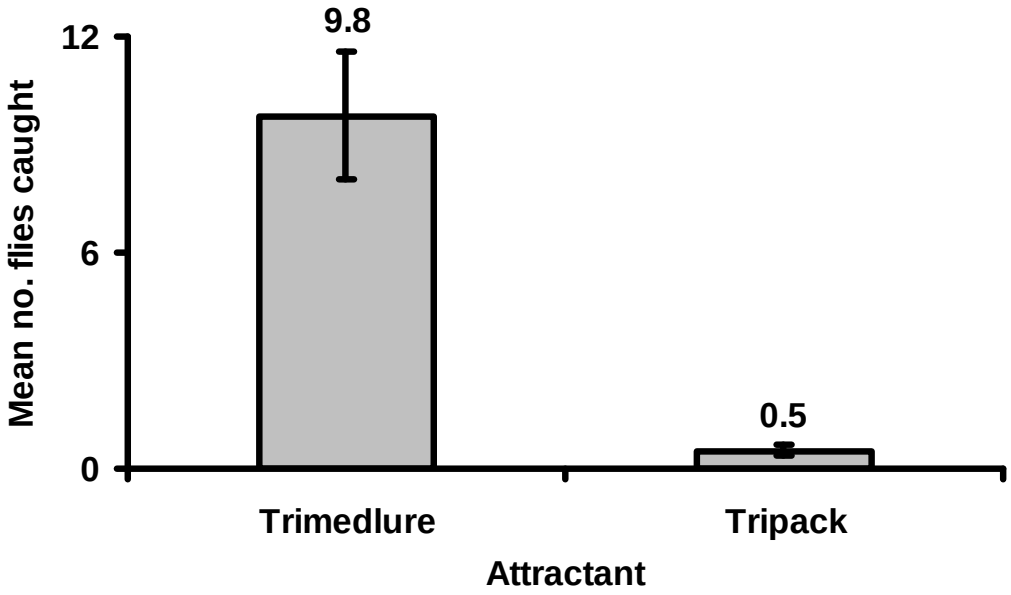

Figure 3.10 Field trial catches Trimedlure and Tripack. Mean (±SE) number of flies caught by Trimedlure and Tripack attractants (delta and open).

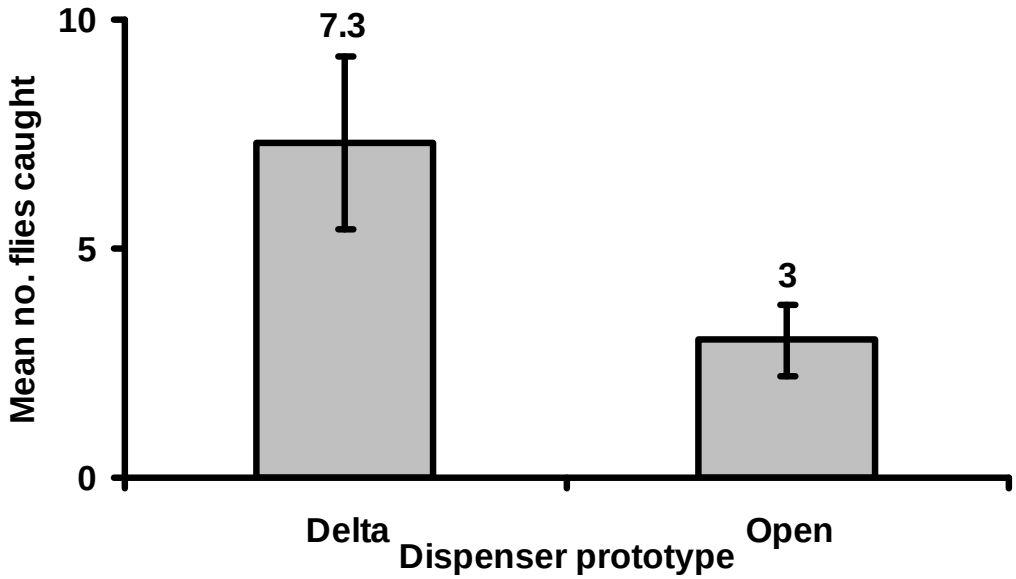

Figure 3.11 Delta vs. open catches. Mean (±SE) number of flies caught by the delta and open container designs using either Trimedlure or Tripack as an attractant.

3.3.2 Field trial: Prototype container closed vs delta

Delta containers were also found to be more efficacious than closed-style containers (ANOVA: $F_{1, 31} = 7.39$, $P = 0.012$) (fig. 3.14). The mean medfly catch for the delta container was 2.5 (±0.5) compared to a catch of 1.4 (±0.4) from the closed-style design. Again, Tripack performed poorly in comparison to Trimedlure (0.2 (±0.1) and 3.7 (±0.5) mean flies caught respectively), ANOVA: $F_{1, 31} = 141.04$, $P \leq 0.001$ (fig. 3.13). There was also a significant interaction effect between container design and attractant with the combination of delta design and Trimedlure being the most effective (ANOVA: $F_{1, 31} = 9.37$, $P = 0.005$) (fig. 3.12).

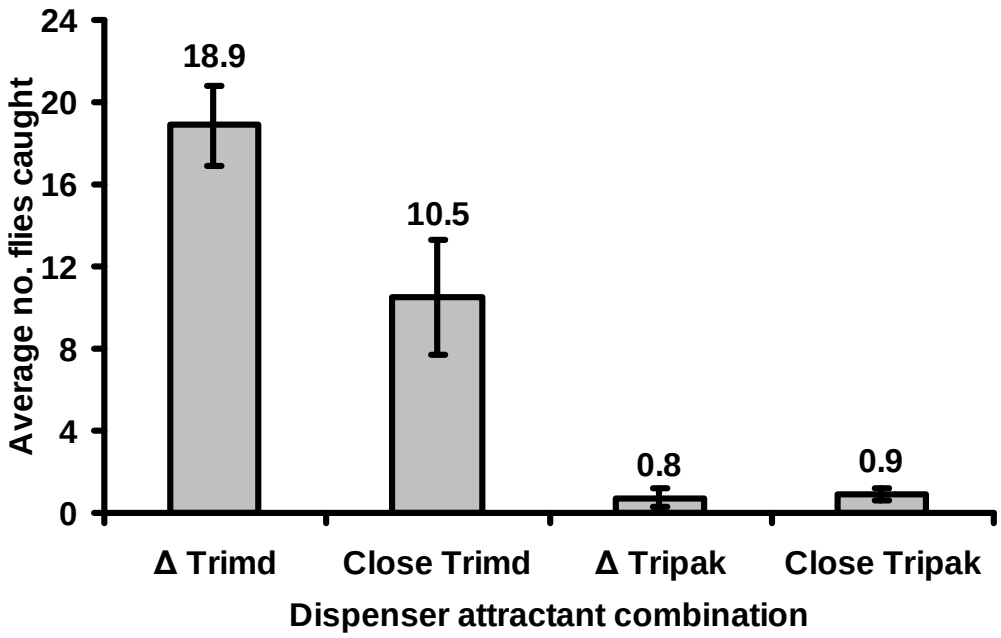

Figure 3.12 Field trial matches combinations. Mean (±SE) number of flies caught by delta and closed container designs baited with Tripack or Trimedlure.

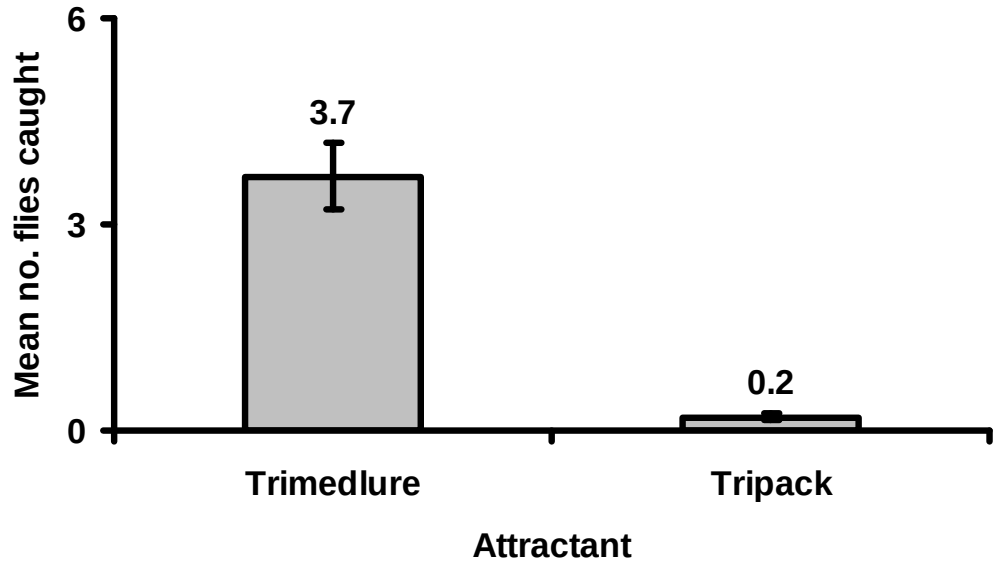

Figure 3.13 Field trial catches Trimedlure and Tripack. Mean (±SE) number of flies caught by trimedlure and tripack attractants (in both delta and closed prototypes).

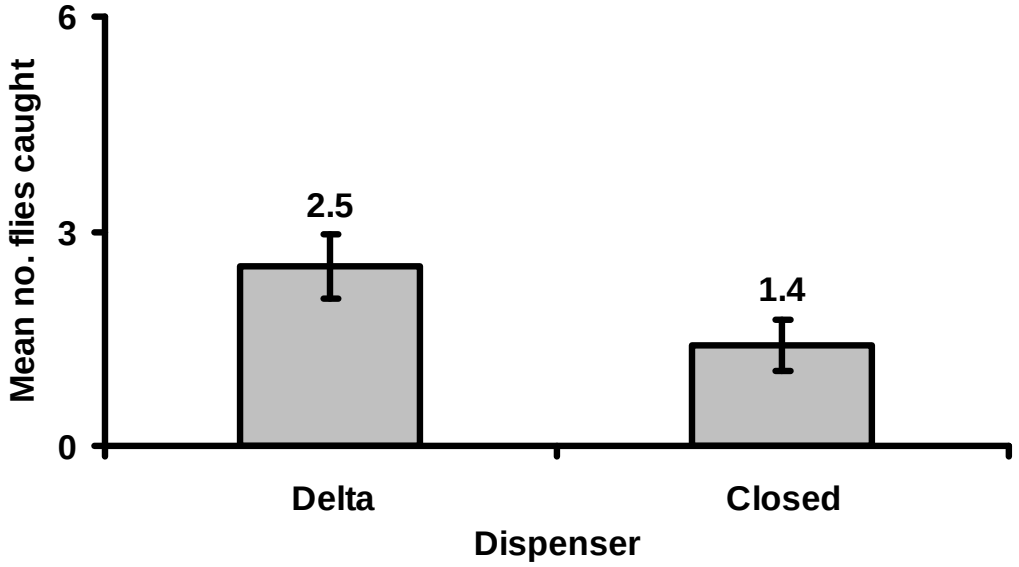

Figure 3.14 Field trail catches design, delta vs. closed. Mean (±SE) number of flies caught by the two container designs delta and closed using either trimedlure or tripack as an attractant.

3.3.3 Behavioural observations: Prototypes open, delta and closed lekking study

There was no significant difference between the number of fly visits observed at each of the different styles of container during the 10-minute observation period (K-W test: $H_{2, 30}$ =3.24, P = 0.198).

However, container design was shown to be an important factor in predicting lek frequency (fig. 3.15). Lek frequency observed in the open-style container (24.5 leks per container) was significantly greater than that of the delta container (17 leks per container) and the closed-style container (3 leks per container), (K-W test: $H_{2, 30}$ =20.48, P ≤0.001). The closed-style container was the least effective for initiating lekking behaviour. There was therefore no relationship between number of flies attracted to the container and frequency of lekking behaviour induced.

Open-style containers, whilst initiating the most lekking behaviour, were found to give rise to the least number of hits (median of 0 hits per container). The delta and closed-style designs had a significantly higher hit rate (a median of 1 hit per container each) (K-W test: $H_{2, 30}$ = 9.39, P=0.009).

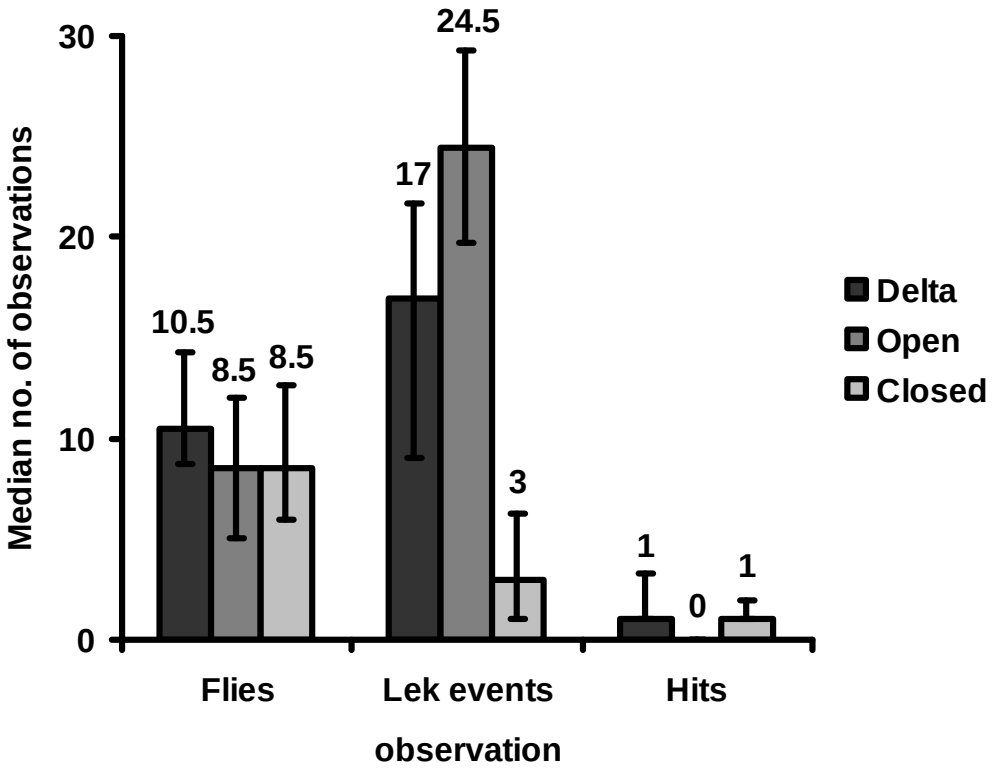

Figure 3.15 Lekking behaviour. Median number of; medfly attracted, lekking events and 'hits' recorded for three container styles over a ten-minute observation period (n=10) (±95% CI of the median).

3.4 Discussion

3.4.1 Field trials: Attractant

Trimedlure and Tripack were trialled as potential attractants for use in the lure and kill system. Trimedlure is a parapheromone that mimics the lekking pheromone used to recruit male medfly to lekking sites, and is shown to be highly attractive to males of this species (Beroza *et al*., 1961). Tripack is a synthetic food lure which is known to attract both females and males although catches tend to be very female biased when this lure is used (Braga-Sobrinho *et al*., 2004)

Trimedlure was extremely effective at inducing lekking behaviour in males and is thus considered to be a highly suitable attractant for this lure and kill system. However, containers baited with Tripack caught very few individuals of medfly so this lure was discarded for use in the system.

3.4.2 Field trials: Container design

Three container designs were tested in field trials in order to assess suitability for use with the lure and kill system. A standard delta design based on those used for monitoring of insect pests was trialled against 'open containers' (two parallel surfaces suspended one on top of the other) and 'closed containers' (delta containers modified so that the open ends of the container are arc-shaped and narrower than the standard delta container.

Containers were set up so that adhesive monitoring traps were used in place of the powder retention tray. The standard design was found to catch far greater numbers of medfly than either of the two modified delta designs. This was initially thought to be due to differences in the attractiveness of the designs to medfly. It was thought that the larger, more curved profile of the standard delta design (medfly have been found to show a preference for curved surfaces (Nakagawa *et al*., 1978) would have attracted medfly from a larger area when compared to the smaller, flatter shape of the closed prototype and the rectangular outline and small profile of the open design.

However, the behavioural study showed that the number of flies attracted to each design were very similar, this discounts attractiveness as an explanation for the disparity in catch rates between the three designs in the earlier trials. During this study two behaviours were recorded; lekking events around each container design, and number of lekking events that resulted in displacement and landing on the container floor (referred to as a 'hit'). Hits represented fly activity that would have resulted in a fly coming into contact with killing agent formulated adhesive powder.

Lek frequency was highest in the open design and lowest in the closed design. The frequency was highest in the designs with the greatest amount of ventilation, the open and delta designs. This suggests that the greater the air flow through the container and thus across the lure, the greater the recruitment of lekking males. Frequency of lekking behaviour was low in the closed containers, possibly due to build up of the lure (Trimedlure) in the container. Work on lepidopterans has shown that exposing adult males to high levels of sex pheromone can make the moth incapable of locating female conspecifics (Nansen, 2007), this can be due to over excitation of the sensory receptors causing sensory fatigue (Cardé & Minks, 1995). It is possible that Trimedlure would have the same effect at high concentrations caused by the conditions of the closed container.

The usual behaviour in lekking male medfly is to rest only on downward facing surfaces usually the underside of leaves in nature (Nakagawa *et al*., 1971b). Lekking males, therefore, rested on the underside of the container tops, rather than in the area that contained the powder retention tray. Thus, catches were only made when males were dislodged from their resting places due to high levels of activity within the lek. Dislodgement resulted in a significantly greater catch rate in those designs which had side walls to direct the flies towards the container floor. Fewer flies became dislodged in the closed design due to lower male activity levels as a result of the effect of the concentrated Trimedlure. However, any flies that were dislodged were deflected onto the powder tray region due to the enclosed nature of the container.

The open design instigated significantly more male activity within the container but dislodged flies escaped easily by exiting the container out of the open sides so that few flies landed on the powder retention area of the prototype. However, the level of ventilation associated with the delta container was great enough to promote lekking and the relatively enclosed nature of the design also ensured that a high number of displaced flies were forced down into the trapping area. This combination of properties was the reason for the higher catch rates observed in the standard delta containers in the previous study. Wind tunnel trials using standardised designs with graduating ventilation levels may allow a more accurate assessment of how air flow effects male behaviour in containers and containers allowing further optimisation of the delta container.

3.4.3 *Behavioural observations: Prototypes open, delta and closed lekking study*

There was no significant difference between the number of fly visits observed at each of the different styles of container during the 10-minute observation period ($H_{2, 30}$ =3.24, P=0.198), implying that attractiveness of the designs could not explain the differential catch rates observed in the previous trials.

However, container design was shown to be an important factor in predicting lek frequency. Lek frequency observed in the open-style container (24.5 leks per container) was significantly greater than that of the delta container (17 leks per container) and the closed-style container (3 leks per container), ($H_{2, 30}$ =20.48, P ≤0.001). The closed-style container was the least effective for initiating lekking behaviour. There was, therefore, no correlation between number of flies attracted to the container and frequency of lekking behaviour induced. This discrepancy was attributed to relative levels of airflow though each container design. The Lekking pheromone released by males arrests other males and they join in the lek (Villeda *et al*. 1988). Trimedlure acts in a similar way to this pheromone, and arrests attracted flies as if they are participating in a lek. At high concentrations it is possible that the Trimedlure overwhelms the flies and renders them unresponsive or possibly masks naturally produced pheromones that stimulate further lekking behaviour such as

'butting' and 'jousting'. The open-style and delta containers allow the relatively high airflow across the attractant such that the attractant is detected by medfly at concentrations similar to those at which the natural pheromone would be encountered. Conversely, the closed-style container allows relatively little airflow across the attractant, thus the parapheromone is highly concentrated within the container causing males to become unresponsive.

During lekking, male medfly aggregate on the undersides of leaves or fruit and defend a territory against competing males. As a result, males tend to congregate on the underside of the upper surface(s) of the containers and a hit is only achieved if a fly is dislodged and falls onto the powder retention tray. It might be predicted that the greater the number of leks, the greater the number of hits as higher levels of activity should result in a greater number of flies becoming dislodged. However, the open-style containers, whilst initiating the most lekking behaviour, were found to give rise to the least number of hits (median of 0 hits per container). The delta and closed-style designs had a significantly higher hit rate (a median of 1 hit per container respectively) ($H_{2, 30}$ =9.39, P=0.009).

This was observed to be a result of the ability of each container to deflect dislodged individuals into the powder retention tray. Although flies were often dislodged during lekking behaviour in the open-style containers, the open structure allowed displaced individuals to fly out of the container avoiding the powder retention tray. Frequency of lekking behaviour was low in the closed-style containers, but when lekking did occur and result in the dislodgement of an individual, the fly was often deflected into the powder retention tray off the walls of the container. However, the delta containers allowed enough airflow to produce concentrations of Trimedlure to encourage lekking in medfly whilst being enclosed enough to deflect a high number of displaced individuals onto the floor of the container.

3.4.4 Conclusions

Field trials identified the delta container design and Trimedlure as the most effective combination of container and attractant. Observations of male lekking behaviour in the containers showed no significant difference in attractiveness of the three designs. However, differing behaviour of flies within the containers affected catch rates and therefore contamination levels in the final design. Male lekking behaviour was retarded in the closed-style design possibly due to low levels of ventilation giving rise to high concentrations of Timedlure and having an arrestant effect on the flies, similar to the effect of high concentrations of sex pheromone on lepidopterans, that cause over excitation of sensory receptors, leading to sensory fatigue (Cardé & Minks, 1995; Nansen, 2007). In the open-style design, the greater levels of ventilation appeared to promote lekking and social interaction, but when this behaviour caused a competing fly to fall from the underside of the top surface of the container, it could escape from the open sides, preventing catching/contamination. The delta design promoted lekking activity in male medfly and resulted in a greater number of hits per fly dislodged than the other two styles of container. Thus, the delta container was the most effective design for use in the autodissemination system.

The conclusion of the field trials show:

- **The delta container and the Trimedlure were the most effective combination of container and attractant.**
- **The greater the degree of openness of the container design the greater the level of fly activity (lekking) within the container but the lower the hit rate.**

Chapter 4: General discussion

4.1 Aims

This project aimed to develop a control system for Mediterranean fruit fly based on the autodissemination of toxic powders through populations. The specific components assessed for use with this system were carrier powders, insecticides and container structure. In order for such a system to be successful, it was also necessary to ensure carrier powder-pesticide compatibility, delayed mortality of individuals contaminated with the powder-pesticide complex and effective transfer of insecticide powder from primary contaminated individuals to conspecifics.

4.2 Insecticide

Aims and conclusions

- ***Aim: To a*ssess the use of methoxyfenozide as a sterilant to medfly**
    - ***Conclusion*: Methoxyfenozide displayed no noticeable activity against medfly in this study**
- ***Aim*: To establish the most suitable killing agent for inclusion in the system**
    - ***Conclusion 1*: Chlorpyrifos knocks down medfly males too rapidly and is therefore unsuitable for inclusion in an autodissemination system**
    - ***Conclusion 2*: All doses of spinosad applied using Entomag were either sub-lethal to some or all flies, or caused mortality too rapidly to allow social interaction to occur**
    - ***Conclusion 3*: Entostat formulated with 2% spinosad gave the mortality rate that best fitted the autodissemination system**
- ***Aim:* To confirm lethal contamination from male to females through courtship**

- **Conclusion: Mortality through powder (2% spinosad Entostat) transfer during mating proven**

Spinosad and chlorpyrifos were tested as potential insecticides for use within the lure and kill system. In dose response assays, chlorpyrifos was effective; it resulted in 100% mortality in *C. capitata* at low concentrations and displayed very fast knockdown down times. Spinosad also produced 100% mortality at most doses, but the knockdown times were slower than those of chlorpyrifos. Autodissemination systems require slow knockdown times to allow for social interactions to occur between the target insects and thus transfer the insecticide powder from one individual to the next. The slower knockdown times observed with spinosad would allow more transmission opportunities to occur so it was considered to be the more suitable killing agent for inclusion into the system.

***General conclusion*: Spinosad 2% formulation chosen for inclusion in the system**

4.3. Carrier Powder

Aims and conclusions

- ***Aim*: To quantify carrier powder uptake by male and female medfly** ***Conclusion:*** **Flies pick up between 25 and 46.5µg of electrostatic powder when contaminated**

- ***Aim*: To establish the most suitable carrier powder for the system**
  - ***Conclusion*: Spinosad formulation chosen for inclusion in the system due to slower knockdown rate with spinosad**

- ***Aim*: To establish the most suitable carrier powder/killing agent dose combination**

- ***Conclusion*: Entostat formulated with 2% spinosad gave the mortality rate that best fitted the autodissemination system**

- ***Aim*: To confirm lethal contamination from male to females through courtship**
  - ***Conclusion*: Mortality through powder (2% spinosad Entostat) transfer during mating is proven**

Two carrier powders were tested for inclusion into the lure and kill system. These were Entostat, a fine carnauba wax powder and Entomag, a metallic powder, chosen for their electrostatic properties. Both adhered to *C. capitata* in suitable quantities. However, all doses of insecticide applied using Entomag were either sub-lethal to some or all flies, or caused mortality too rapidly to allow social interaction to occur. Thus it was unsuitable for inclusion within an autodissemination control system. In contrast, Entostat was found to give rise to 100% mortality in combination with spinosad at 2%, and at this concentration, the time-frame between contamination and knockdown was such that social interactions could occur but that the potential for successful oviposition by contaminated females would be limited.

***General conclusion*: Entostat 2% spinosad formulation chosen for inclusion in the system. Autodissemination of insecticide shown.**

4.4 Container design

Aims and conclusions

- ***Aim*: To assess the efficacy of open and closed container designs through field trials**

- ***Conclusion*: Field trials identified the delta container design and Trimedlure as the most effective combination of container and attractant**

- ***Aim*: To establish what aspects are important in container design through behavioural observations**
  - ***Conclusion*: It was observed that the greater the degree of openness of the container design the greater the level of fly activity within the container but the lower the 'hit' rate. As a result standard delta designs were selected for inclusion in the system**

***General conclusion*: Standard delta container design and trimedlure selected for inclusion in the system.**

4.5 The autodissemination lure and kill system

It is envisaged that the final set-up for the system will comprise of outer container similar in design to that of a delta trap with an insert on the floor of the container containing Entostat powder formulated with spinosad at 2%. This will be combined with a Trimedlure attractant plug that will attract male medfly into the container (further work trialling food bait based lures may allow the development of a container capable of directly targeting females also). Male medfly visiting the container will position themselves on the underside of the container roof as they would at a natural lekking site. High levels of activity during lekking will result in many flies becoming dislodged and the walls of the container will deflect these individuals downwards onto the Entostat powder at the base of the container. Contaminated flies will then leave the container and have a window of opportunity of approximately 247 minutes to interact with conspecifics and transmit the insecticide to male courtship rivals during lekking and to females during courtship attempts and mating (fig 4.1).

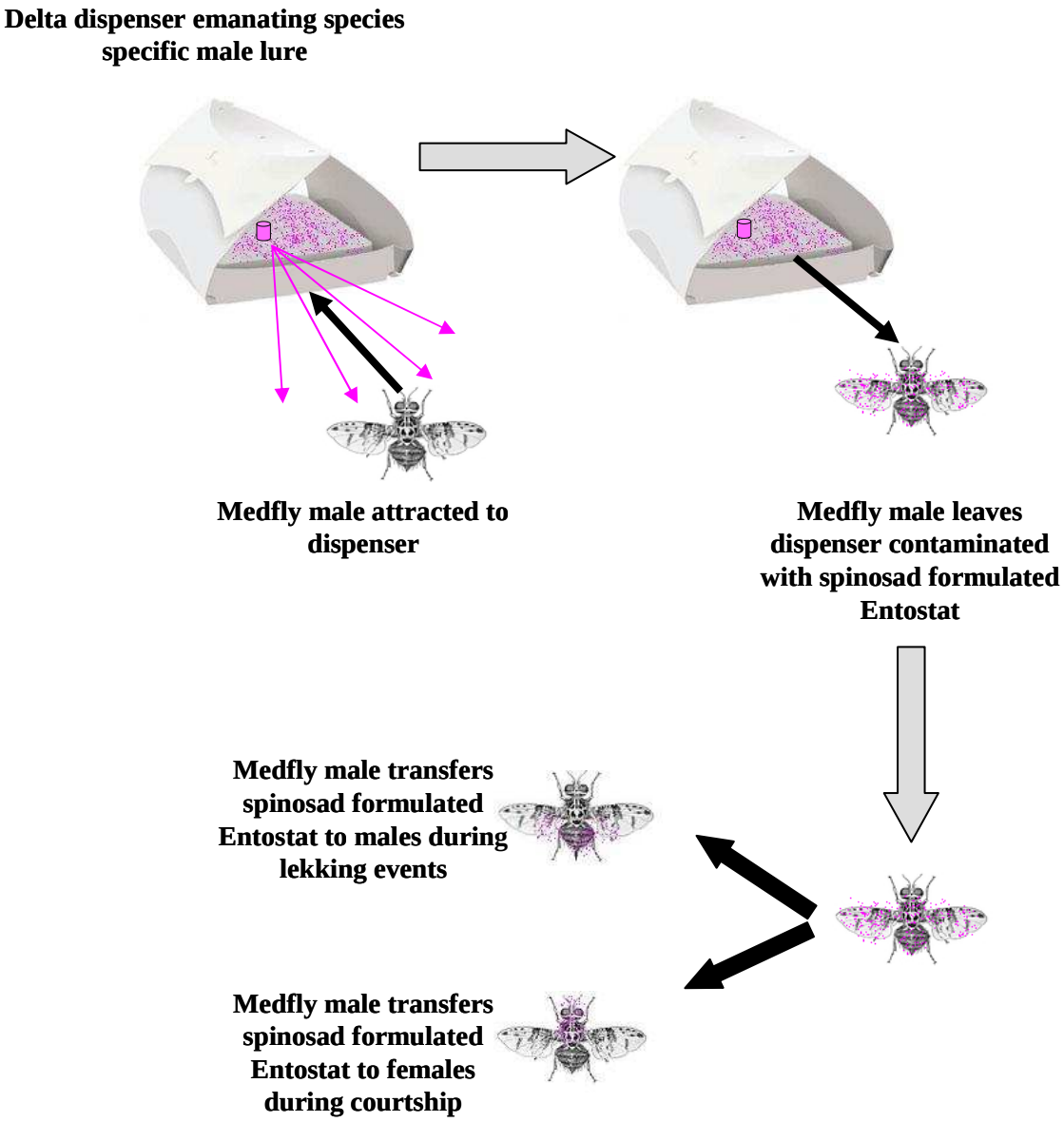

Figure 4.1 Proposed autodissemination lure and kill system Mediterranean fruit fly. Diagram adapted from images taken from exosect.com

The development of the autodissemination lure and kill system offers several advantages over current tephritid control methods. Mass trapping using McPhail and Jackson traps predominantly only target a single sex and if the trapping mechanism is a glue board or a combined trapping/bait liquid, a high number of beneficial insects and other non-target species are also trapped. The lure and kill system promotes the spread of insecticide across the whole adult medfly population with a greatly reduced

risk of contamination of non-target organisms. Another advantage of this system is that servicing is greatly reduced compared to traditional McPhail and Jackson traps. The containers do not get fouled up by dead insects as in the traditional traps and the killing agent formulated Entostat powder is replaced with a simple tray change. This is much easier than the replenishment of trapping and bait liquids common with the use of McPhail and Jackson traps. It is also anticipated that improvements in the formulation techniques used to produce the powder will result in greater longevity so that final formulations may persist and remain active throughout the growing season so that replenishment of the system will not be required at all.

The construction of the container is much the same as traditional Jackson traps that have been widely used for agricultural pest control over almost 100 years. Thus, field personnel and farm technicians will find the container familiar and easy to operate.

Spinosad bait sprays are currently of the most favoured methods of control for medfly in the Mediterranean and suppression of this species during outbreaks. Spinosad bait sprays require reapplication every seven days depending on infestation levels and weather conditions (Dow AgroSciences, 2006). This is time consuming and costly in terms of both consumables and labour. Spinosad is not UV stable and is broken down rapidly when not adequately protected (Saunders & Bret, 1997). During bait spraying much of the spinosad is exposed on leaf surfaces in direct sunlight which results in the in breakdown of the insecticide (Saunders & Bret, 1997). However, when formulated with Entostat powder, the spinosad could become more stable due to naturally occurring UV stabilising properties of the powder (Xia, *et al*., 2007). Further to this, the spinosad is housed within a container casing which provides further protection from UV and other environmental factors that would otherwise speed up degradation. Less application will be required because the killing agent will be sheltered and dispensed directly onto the target organism which will greatly reduce the costs of consumables and labour and will also reduce the amount of killing agent released into the environment.

At this stage of development, the product is probably not robust enough to act as a stand alone product for control of medfly but the system could be employed very successfully as part of an integrated pest management (IPM) scheme. The most effective use of the system would be as a population suppressant, to be employed after the initial use of bait sprays. The lure and kill system would prevent the resurgence of medfly and maintain the reduced population at a commercially acceptable level.

Further work is required to develop the proposed system. The laboratory experiments assessing the transfer of powder from contaminated males to conspecifics would need to be attempted in the field. Weather and other environmental factors may have an effect on powder uptake and transfer, this could be assessed through marking experiments in the field. Male flies could be lured to and contaminated with marked powder from containers containing Trimedlure, the containers would be changed for Tripack or another bait lure and a glueboard after 24 hours. Female flies caught in the new container could then be analysed for marker powder, in a similar way as in Armsworth *et al*. (2008). Proving that transfer of carrier powder occurs in the field would confirm the novel claim of the system of targeting both sexes of the pest population. Studies would have to be conducted to look at the longevity of spinosad when formulated with Entostat powder, Entostat has properties that suggest that longevity of spinosad may be increased but this is yet to be assessed (Xia, *et al*., 2007). These experiment and large scale field trials with spinosad formulated Entostat would show how effective the proposed system is in suppressing medfly populations.

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