MASS TRAPPING AND OTHER MANAGEMENT OPTIONS FOR MEDITERRANEAN FRUIT FLY AND QUEENSLAND FRUIT FLY IN AUSTRALIA

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Summary

This review examines a number of fruit fly management strategies based on luring flies to a trap, including mass trapping, lure and kill, lure and infect, and lure and sterilize. Their possible role in suppression of two major fruit fly species in Australia, Mediterranean fruit fly and Queensland fruit fly is examined. We review the current trapping systems used in Australia for male and female monitoring, commercially available systems, and discuss the possible application of the sterile insect technique in lure and infect and lure and sterilize strategies.

Keywords: Medfly, Qfly, area wide management, surveillance, male annihilation technique, *Ceratitis capitata*, *Bactrocera tryoni*

INTRODUCTION

True fruit flies (Diptera: Tephritidae) are considered to be one of the most destructive pests of fruit and vegetable crops in Australia, with an annual estimated cost of $4.8 billion (Plant Health Australia 2008). Whilst more than 280 Tephritid fruit fly species are established in Australia, few are responsible for economic damage (Drew 1989). The important pest species are Queensland fruit fly (*Bactrocera tryoni* (Froggatt)) (Qfly) in eastern Australia, and Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann)) (Medfly) in Western Australia (WA) (Dominiak and Daniels 2012). Both species have a wide host range, damaging tropical (e.g. mangoes and papaya), sub-tropical (e.g. citrus and avocadoes) and temperate (e.g. apples and blueberries) crops grown commercially and in home gardens (Hancock *et al.* 2000). Both species can cause up to 100% crop loss in unmanaged orchards. In addition to physical and economic damage, fruit flies impede interstate and international trade as they are regarded to be pests of quarantine significance.

Until recently, the organophosphates dimethoate and fenthion were the main products used for pre- and post-harvest treatments. First developed in the 1950s, these insecticides were removed from use by the Australian Pesticides and Veterinary Medicines Authority (APVMA), the national authority responsible for registering and reviewing all pesticides sold in Australia. In 2011, dimethoate was suspended from use due to public health concerns (APVMA 2011). In October 2015, fenthion was removed due to public health, occupational health and safety, and environmental concerns (APVMA 2014). Maldison (malathion) and trichlorfon, currently used in bait and cover sprays, are also being reviewed by the APVMA and may be removed from use in the future as human health concerns continue to influence pesticide use patterns (Dominiak and Ekman 2013).

Wherever possible, pesticides that are removed from use are being replaced with safer, narrow-spectrum pesticides. However it is unlikely that either fenthion or dimethoate will be replaced with one of equivalent efficacy. This is partly due to the economics of developing new pesticides, with the total development cost per new product estimated at AUD 205 million (€189 m) in 2010 (McDougall 2010), coupled with higher standards of potency and safety (Casida and Quisad 1998). For these reasons, different approaches to fruit fly control are required.
One possible approach is trapping and the more recent development of mass trapping. In a world-wide review of fruit fly eradications, Suckling *et al.* (2016) reported that sterile insect technique (SIT), bait sprays, host removal, male annihilation technique (MAT) and quarantine controls were the five most reported fruit fly management strategies (in that order). Mass trapping was the next in the order and this may be because it is a relatively recent development. Much of the work on mass trapping has been focused on Lepidoptera and Coleoptera rather than Diptera, with the majority of programs aimed at pest management rather than eradication (El-Sayed *et al.* 2006). With regard to fruit flies, the technique has been tested more extensively on *Bactrocera* than *Ceratitis* (from Figure 3 in Suckling *et al.* 2016).

For any lure and kill strategy such as mass trapping to be successful, El-Sayed *et al.* (2009) reported that there should be either a low-density or isolated target population, a competitive lure, high lure density, optimal lure placement and deployment before the main flight period. Inadequate pheromone lure and insufficient trapping density were likely to result in failure of the strategy.

Although traditionally used to monitor fruit fly as part of surveillance or orchard management programs, trapping is increasingly being adapted worldwide for use in pre-harvest control of fruit flies. Techniques include mass trapping, lure and kill, attract and kill, and lure and infect. All techniques utilise a lure or bait to attract adult fruit flies to a device where they are killed and retained (mass trapping) or receive a lethal dose of insecticide (lure and kill, attract and kill). Lure and infect (attract and contaminate) describes a system whereby flies are attracted to an inoculation device and infected with a pathogen such as the fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) (Navarro-Llopis *et al.* 2015). Lure and sterilize, as the name suggests, requires flies to feed on a sterilizing agent such as the insect growth regulator lufenuron (Navarro-Llopis *et al.* 2004, Moya *et al.* 2010). Many of these options were summarized by Suckling *et al.* (2012). In this review, techniques such as mass trapping, sterile insect technique and lure and kill were considered as density dependent and worked better on smaller populations. In contrast, techniques such as spot bait spraying, host removal and quarantine measures were density independent strategies that removed a proportion of the population (Suckling *et al*. 2012). Fruit fly management or eradication is optimized by using a combination of both techniques: density dependent and density independent. Although mass trapping was ranked as moderate to low suitability for eradication, it has the advantage of high social acceptance in urban areas.

There are many advantages of using lure and kill over conventional methods. Perhaps the most significant is the reduction in pesticide applied within the orchard and to the environment. Insecticides are retained in closed containers or only applied in small quantities to exposed devices. Other advantages include potentially reduced labour costs as orchardists are able to adopt a ‘set and forget’ approach, more targeted pest control, and reduced exposure of beneficials to insecticides. Systems have been developed for some species of *Bactrocera* (*B. cucurbitae* (Coquillett), *B. dorsalis* (Hendel), *B. carambolae* Drew & Hancock, *B. oleae* (Gmelin)), *Rhagoletis* (*R. pomonella* (Walsh), *R. mendax* Curran), *Anastrepha* (*A. suspensa* Loew, *A. ludens* (Loew)), and Medfly (Broumas *et al.* 2002, Leza *et al*. 2008, Navarro-Llopis *et al*. 2008, Heath *et al*. 2009, Peñarrubia-Maria 2010, Pinero *et al.* 2010, Hafsi *et al.* 2015). However, no equivalent system has been developed for Qfly other than perhaps the system developed by Lloyd *et al.* (1998).

In this paper, we review trap and lure components available within Australia that have potential for use in mass trapping or lure and kill systems. We also discuss their possible use in combination with the sterile insect technique.

MALE-TARGETED TRAPPING SYSTEM

Male targeted trapping systems are currently used to demonstrate area freedom and areas of low pest prevalence within production districts, guide management practices in fruit fly endemic areas, and provide early warning of possible exotic incursions (Dominiak *et al*. 2003, Dominiak and Nicol 2010, Dominiak *et al.* 2015). The most widely used trap in south eastern Australia is the modified Lynfield trap, a non-sticky disposable pot-type trap (Cowley *et al.* 1990). It is the only trap recommended for monitoring both Qfly and Medfly in Australia’s Fruit Fly Code of Practice (Wijesuriya and De Lima 1995) replacing the Jackson trap which was a delta type trap with a sticky insert. One advantage of the Jackson trap was that it reduced the likelihood of predation by ants and other insects. In Medfly sterile insect programs, the Jackson trap is used instead as it reduces the likelihood that fluorescent dyes used to mark sterile flies can cross-contaminate unmarked wild flies (Broughton *et al*. 2015). The potential cross-contamination problem for Qfly was first reported by Gilchrist *et al.* (2004) where over 200 ambiguously marked flies were found to be wild flies and not sterile Qfly. Chen *et al.* (2016) refined the microsatellite technique developed by Gilchrist to create a faster and simpler PCR technique to test for this occurrence.

In northern Australia, traps used to monitor *Bactrocera* species include the Steiner trap, an open horizontal plastic cylinder used in high rainfall areas such as far north Queensland. The Paton trap is used in areas of high rainfall or wind, or where traps may need to be set for longer periods (such as a month) between collections. Paton traps are generally used on Cape York Peninsula and the Torres Strait Islands in Queensland, as they are rain resistant, preventing flies falling out of the trap in windy situations. Due to their large volume, they are able to hold about 10,000 flies (the Steiner trap can only hold approximately 6,000).

Recent developments in trap design include the production of different shapes, colours and sizes with plastic foldable traps such as the cone trap (see Table 1 for manufacturers of traps), the globe trap, and a pot type trap becoming available in Australia in the last few years. Limited field testing has been conducted with these traps, which can also be used in female-targeted trapping. In preliminary field trials, cone traps were are at least as effective at trapping and retaining Qfly as the modified Lynfield trap (Dominiak unpub. data 2015).

LURES

Male Qfly and Medfly respond to para-pheromones which are of anthropogenic origin and thought to chemically resemble or mimic male aggregation or female sex pheromones. Species in the genus *Bactrocera* respond primarily to either cuelure (4-(p-acetoxyphenyl)-2-butanone) or methyl eugenol (ME) - although there are exceptions where some species have a small response to the other lure (Dominiak *et al*. 2011a). There is a small group of *Bactrocera* that do not respond to either of these two main lures. Qfly responds to cuelure with males reported to feed on it: virgin females are also reported to respond weakly to cuelure (Drew 1989). Cuelure is chemically related to raspberry ketone and found in the plant families Rosaceae, Asteraceae, Lamiaceae and Orchidaceae. In male-trapping systems, cuelure is usually applied to a cotton wick incorporating a killing agent such as malathion (Dominiak *et al.* 2003).

Male Medfly are attracted to trimedlure (TML), a mix of isomers of ceralure (Jang *et al*. 2001). TML liquid is highly volatile and in Australia, Capilure®, a mixture of TML plus extenders, replaced TML for use in monitoring and surveillance in the 1980s (Hill 1987). However, the longevity of TML has been improved by incorporating it into solid dispensers, thereby reducing evaporation rates. Experiments in WA comparing Capilure® with TML plugs, cones and wafers, show that TML outperformed Capilure® in trials in stone and pome fruit (Broughton *et al.* 2015). Ceralure B1, an idio-analog of TML, is reported to be more attractive to Medfly than TML (Warthen *et al.* 1998, Jang *et al.* 2005), however it is not used in Australia due to its higher cost.

MALE ANNIHILATION TECHNIQUE (MAT)

This technique utilizes a dispenser loaded with a male lure and insecticide for species that respond to ME and cuelure. Males are attracted to the lure and actively feed on it, thereby acquiring a lethal dose of insecticide. The main function of MAT is to remove a high proportion of males from the population and minimize the chance of many males forming a mating site, as single males are less likely to complete a mating (Weldon 2007). However it is well recognized that MAT is not a stand-alone treatment, with other parallel treatments needed to increase its effectiveness. MAT is often combined with protein bait spraying.

In WA, MAT was combined with protein bait spraying and SIT to eradicate Qfly from Perth over an area of 100 km^2 (Sproul 2001). The MAT dispensers were 5cm x 5cm caneite blocks loaded with 2 mL cuelure and 2 ml Hy-mal® (1150g/L malathion) and nailed or wired to trees at a rate of 30/ha. Similar formulations have been used in New South Wales and Victoria (Dominiak and Nicol 2012). In Queensland, MAT was a key component to the eradication of *B. dorsalis*, a ME attracted species (Lloyd *et al*. 1998).

More recent applications of the technique have been in area-wide management programs such as in the Central Burnett (Lloyd *et al.* 2010). In this program, dental wicks impregnated with 1 mL cuelure and 1 mL malathion EC were retained in a plastic ‘cup’ (6 cm diameter and 2 cm high) (Bugs for Bugs, Mundubbera, Australia), distributed at the rate of 10/ha, and replaced three times per year.

Other commercial products include Amulet C-L® which are molded paper fiber stations impregnated with cuelure and fipronil (Vargas *et al*. 2005). SPLAT (Specialized Pheromone and Lure Application Technology) incorporating a waxy coating with insecticide (spinosad) and lure, can be applied with a hand-held sprayer, from a truck, or by air. Spinosad is an insecticide derived from a soil actinomycete and regarded to be reduced environmental and human risk compared to organophosphates. It has been used to suppress cuelure responsive melon fly *B. cucurbitae* and oriental fruit fly *B. dorsalis* , which responds to ME, in area-wide programs in Hawaii (Vargas *et al.* 2010a), but is not widely used in Australia at present. Recent research has demonstrated promise of SPLAT MAT for *B. tryoni* (Reynolds *et al.* early view).

Whereas Qfly actively feeds on cuelure, Medfly does not actively feed on TML. Laboratory observations suggest that male Medfly will perch near, but not on TML wicks (pers. obs. Broughton). MAT has not been successful for Medfly when used without other control methods (Avery *et al*. 1994) and is not used commercially. However, Vargas *et al.* (2012) recently tested a TML dispenser with a large surface area with promising results.

FEMALE TARGETED TRAPS - QUEENSLAND FRUIT FLY

McPhail traps were the standard trap used for trapping female Qfly in Australia. Consisting of a glass or plastic flask-shaped container with an invaginated entrance at the base, they were used with liquid lures as a 'wet trap'. Liquid attractants were based on food or host odours such as orange-ammonia, fruit juices and proteinaceous solutions, which both attracted and killed flies (by drowning) (reviewed by Navarro-Llopis *et al*. 2015). As attractant fluids need to be replaced at least weekly (Dominiak 2006), McPhail traps were too time consuming for use in mass trapping or lure and kill. Dominiak and Nicol (2010) reported that McPhail traps using protein or orange juice lures caught six times more males than females. These trap and lure combinations also attract non-target species, adding to identification costs (Dominiak 2006). These traps have been discontinued in most areas due to their relatively poor performance.

Dry lures for female Qfly are not commercially available. Some early work by Vickers (1999) demonstrated one formulation had merit in the laboratory, though was not confirmed with field-testing. Biotrap® has recently developed a gel that has shown promise in field trials in Somersby, New South Wales and Swan Hill, Victoria (Jessup, unpubl. data 2015).

FEMALE TARGETED TRAPS - MEDITERRANEAN FRUIT FLY

Effective dry synthetic attractants were developed for Medfly in 1995 (Epsky *et al.* 1995, Heath *et al*. 1995), enabling mass trapping and lure and kill to be developed. The attractants consist of two (ammonium acetate (FFA)) and putrescine (FFP, 1,4-diamiobutane or butanediamine) or three components (FFA, FFP and trimethylamine (N,N-diethylethanamine). Supplied as dispensers with an adhesive back, they are easily attached to the inside of the trap. Commercially available lures in Australia include three (BioLure®) and single component dispensers (BioLure® Unipack; Ceratitis® Unipack). Unipack and 3 part-BioLure dispensers are equivalent in terms of capture of Medfly (Navarro-Llopis *et al.* 2008, Holler *et al.* 2009). As the attractants are species specific, they attract fewer non-target organisms (Katsoyannos *et al.* 1999), though Island Fruit Fly (*Dirioxa pornia*), crickets, cockroaches and ants have also been recorded from traps baited with BioLure® (Broughton unpubl. data. 2015).

Several female-targeted McPhail-type traps are available commercially: all have yellow or orange bases, as Medfly and other fruit fly species are reported to respond to yellow or fluorescent yellow (520-540 nm peak reflectance, Navarro-Llopis *et al.* 2015). Queensland fruit fly is attracted primarily to yellow and green (Meats 1983, Weldon 2003). All traps also have a translucent lid, as traps with clear lids have been demonstrated to retain flies in the upper part of the trap (Navarro-Llopis and Vaca 2015). Current traps include a globe trap, Maxi® trap, Tephri® trap and Cone® trap.

Field trials conducted in stone and pome fruit orchards showed that all aforementioned trap types out-performed the Tephri® trap (Broughton *et al.* 2015) with the Maxi® trap catching the most Medfly.

NEW AND FUTURE SPECIES-BASED MANAGEMENT OPTIONS

Magnet MED®, cone traps and Ceratrap® are currently available in Australia. Ceratrap® is the only product currently available to the home grower designed to attract female Qfly. The predominantly yellow plastic bottle contains a liquid attractant, in which the captured flies drown. Magnet MED® and cone traps may be used by commercial growers under a trial permit supplied by the APVMA, though registration is being sought for both devices.

Magnet MED® is a lure and kill device, consisting of a paper envelope coated with the synthetic pyrethroid deltamethrin, with holes in the middle of the envelope to emit attractant odours (Casagrande 2009). The attractant used within Magnet MED® is BioLure® Unipak. Flies are killed when they come into physical contact with the envelope. The cone trap consists of a yellow cone base with a clear plastic lid. The lid is pre-treated with alpha-cypermethrin, to kill flies coming into contact with it. Different lures are used depending on the target species.

Trap distribution rates of 80/ha are recommended to manage fruit fly in susceptible crops such as stonefruit. However, field trials in pome and stone fruit in the Perth Hills showed that the devices cannot be used as a 'stand-alone technique'. Economically acceptable levels of crop loss (< 5%) were only attained once devices were combined with conventional fruit fly controls such as baits and cover sprays (Broughton *et al.* 2015).

Devices available overseas, but not currently available in Australia, include a lure and infect device from Spain that incorporates *Metarhizium anisopliae* (Navarro-Llopis *et al.* 2015). The spores are transmitted between flies via contact such as mating. The conidia are supplied on a Petri dish containing a semi-solid gel, to achieve a dose of $1x\ 10^9$ conidia per dish (= inoculation dish). In a trial, contaminant devices (ACD) were constructed by placing the Petri dish in a delta trap, with a TML plug or 3 component female lure Biolure® Medfly, to attract male and female Medfly respectively. The two year study in citrus using an application rate of 24 ACD/ha showed a reduction of the Medfly population in citrus. The technique was more effective than female baiting with malathion and inoculation dishes, only needing to be replaced once mid-season (Navarro-Llopis *et al.* 2015).

In Kenya and South Africa, *Metarhizium* (isolate 69) is used to control several species of fruit fly including Medfly (Dimbi *et al.* 2003, Quesada-Moraga *et al.* 2008, Real IPM 2015). The isolate can be applied to the ground to control pupae. When used in an inoculation device, it takes 2 to 5 days to kill depending on the environment and dose rate (Real IPM 2015). The conidia do not need to be ingested to be effective.

SIT is a species specific form of biological control, where sterilized flies (usually male) are released to overflood the 'wild' male population, reducing the probability of successful wild to wild mating occurring. Females mating with the sterile males produce infertile eggs and the population declines over time. SIT was originally proposed by Knipling (1955). The first half century of world-wide use was reviewed by Krafsur (1988). Dominiak *et al.* (2011b) reviewed SIT for Qfly in eastern Australia.

In Spain, a chemosterilant bait station Adress® trialed in citrus, stone and persimmon showed that SIT + Adress® applied at a rate of 24 devices/ha was more effective than SIT alone in suppressing Medfly and reducing fruit damage (Navarro-Llopis et al, 2004). The Adress® system is a bait station consisting of a yellow vertical cylinder containing the chitin synthesis inhibitor lufenuron, formulated in a phagostimulant gel. Male (TML) and female attractants (N-methyl pyrrolidine and ammonium acetate) are used to lure adult flies to the device (Navarro-Llopis *et al.* 2010). In laboratory and field trials, female Medfly that had fed on lufenuron laid non-viable eggs. Moreover, male Medfly were able to transfer lufenuron via mating. Preliminary tests in WA indicated that the phagostimulant gel dries out under their conditions. However new formulations have been developed that may be more stable (Woods pers. comm. 2015). Field trials are currently being conducted on *B. tryoni* in Queensland (Mess pers. comm. 2015).

INTEGRATING THE STERILE INSECT TECHNIQUE (SIT)

In WA, a male-only strain of Medfly (temperature-sensitive lethal for female eggs) SIT is being trialed with bait spraying to eradicate Medfly from the Carnarvon region. Qfly SIT programs in eastern Australia use a bi-sex strain (Dominiak *et al.* 2011b, Fanson *et al.* 2014). A key objective of the large SIT-plus program is to develop a male only strain of Qfly for use in area wide management and eradication. The development of a male-only strain will avoid the production of both sexes, remove sterile to sterile mating and thus improve male SIT over-flooding ratios. The cost comparison between the bi-sex strain and the male-only strain was not available at the time of writing this manuscript and hence the potential adoption by growers is unclear. In lieu of an effective female attractant for Qfly, lure and infect or lure and sterilise in combination with SIT could improve management outcomes. During 2016 at Young (NSW), MAT was deployed throughout the township and followed up with SIT in summer. This strategy prevented urban wild flies from reaching the surrounding orchards (Dominiak *pers. com.* 2016).

A combination of lure and infect and lure and sterilize methods would require inoculating sterile male Qfly or Medfly with *Metarhizium* or feeding them with lufenuron prior to release. Whether or not this technique is viable depends on the field survival of sterile flies (Toledo *et al.* 2006). If sterile flies only live for 4-5 days after release, it may not be effective to release infected sterile flies. *Metarhizium* isolate 69 for example, takes 2 - 5 days to kill. If flies die before they mature, they will not pass the infection to female flies during mating, making the method ineffective.

DIRECTIONS FOR THE FUTURE

Mass trapping, lure and sterilize, and lure and infect are the first novel fruit fly management tools to become available in decades and will support the long standing lure and kill (MAT and bait spraying). Commercially available systems are only currently available for Medfly, though MAT is available for Qfly. Both need to be combined with other controls such as bait spraying to achieve suppression. SIT has been available for Medfly and Qfly and used in small programs. The SITplus Qfly facility in South Australia is scheduled to be operational in 2017 and will provide sterile Qfly in much larger numbers than previously available.

A do-it-yourself system could be developed locally by testing a range of trap types and lures. For example, the Maxi® trap has been combined with synthetic female attractants in the Perth Hills. Further application of these techniques may include area-wide management and eradication programs, and replacement of protein bait spraying as part of population suppression programs in urban and peri-urban areas. In particular, chemical sterilants such as lufenuron and pathogenic fungi may be compatible with SIT.

Trials evaluating mass trapping in combination with other management techniques are continuing in south-eastern Australia and should be finalised in 2016. Research results will provide guidance for the optimization of mass trapping as part of the overall suite of management options available to control Australian fruit flies. The development and adoption of mass trapping and similar strategies offers to compensate in part for the loss of dimethoate and fenthion.

Table 1. List of products and their manufacturers.

Product	Manufacturer
Adress®	Syngenta Agro S.A.
Amulet C-L®	BASF, Australia
BioLure®	Suterra LLC, Bend, Oregon, USA
Ceratitis® Unipack	Probodelt™, Tarragona, Spain
Cone trap	Probodelt™, Tarragona, Spain
Globe trap	BioTrap, Australia
Magnet MED®	Suterra LLC, Bend, Oregon, USA
Maxi® trap	Probodelt™, Tarragona, Spain
Pot type trap	Fruit Fly Trap Pro, Bugs for Bugs, Mundubbera, Queensland
Tephri® trap	Sorygar, Spain

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