Chapter 15
Invasive Insects in Plant Biosecurity: Case Study – Mediterranean Fruit Fly

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15.1 Introduction

Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) ("Medfly"), is one of agriculture's most destructive and infamous insect pests (Jackson and Lee 1985). Economic analyses suggest that the establishment of Medfly in California alone could cost the state more than $1 billion USD annually (Siebert and Cooper 1995). Medflies are polyphagous, with over 200 types of fruit reported as hosts (Liquido et al. 1991). They readily infest diverse commodities in major cropping systems, including citrus, pome fruits and stone fruits. The list includes numerous tropical fruits that are traded in lower volume than those mentioned above, but are still of regulatory concern. Examples include papaya (*Carica papaya* Linnaeus), loquat (*Eriobotrya japonica* Lindl.), guava (*Psidium* spp.), and litchi (*Litchi chinensis* Sonn. Mill.) among many others. Coffee (*Coffea arabica* Linnaeus) is considered as a possible ancestral host of the fly (Prokopy et al. 1997), and Medfly can build to very high numbers in coffee-growing areas. Still, the insect is not considered a major coffee pest because larvae feed primarily on the pulp of the fruit rather than the bean.

The current geographic range of the medfly includes Africa, southern Europe, Central America, Hawaii, portions of South America, and Western Australia (Diamantidis et al. 2008). The species may have evolved in Sub-Saharan Africa and spread through the remainder of its range within the past two centuries, largely

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Fig. 15.1 Mating pairs of the Mediterranean fruit fly "medfly," *Ceratitis capitata* (Image courtesy David Lance CPHST, APHIS, USDA)

via human transport of fruit (Bonizzoni et al. 2004; Malacrida et al. 1998). Because of this demonstrated ability to colonize new areas, medfly is a serious agricultural pest in its own right and a regulatory pest that is quarantined by many uninfested countries such as China, Japan and the USA (Bergsten et al. 1999). These countries regularly intercept incoming medflies, as well as other pest tephritids (Li et al. 2009; Liebhold et al. 2006). Despite these efforts, incipient populations of Medfly periodically appear and extensive programmes are required to ensure that populations are detected quickly and eradicated effectively (Bergsten et al. 1999).

Female medflies deposit eggs within fruit, and the larvae (maggots) feed on the fruit, creating tunneling damage and promoting rotting. Mature larvae leave the fruit to pupate in the litter. The adult flies, slightly smaller than a typical housefly, are multi-coloured with patterned wings (Fig. 15.1). The entire lifecycle can be completed in less than a month depending on temperature and available host plants. Medfly is a member of the Family Tephritidae (true fruit flies), which includes many other serious agricultural pests such as Oriental Fruit Fly (*Bactrocera dorsalis* (Hendel)), Mexican Fruit Fly (*Anastrepha ludens* Loew), and Apple Maggot (*Rhagoletis pomonella* (Walsh)). Medfly cannot survive harsh winters and consequently is restricted to tropical through warmer temperate regions (De Meyer et al. 2008; Papadopoulos et al. 2001a).

Fig. 15.2 Male Medfly releasing sex/aggregation pheromone from the tip of its abdomen (Image courtesy David Lance CPHST, APHIS, USDA)

15.2 Behaviour and Chemical Ecology

Adult Medflies exhibit a rich behavioural repertoire, with several traits that have lent themselves to the development of management tools. Those traits involve the flies' methods of locating mates, food, and egg-laying (oviposition) sites, and have been exploited in the development of monitoring tools (e.g., traps and lures), chemical control methods (bait sprays and other attract-and-kill methods) and the Sterile Insect Technique (SIT).

15.2.1 Mating Behaviour

Mating behaviour of Mediterranean Fruit Flies has been studied extensively, largely in relation to the SIT (discussed in detail below). Briefly, male medflies roost, typically on undersides of leaves, in small, loose aggregations that many authors have equated to the lek behaviour of some vertebrates (Prokopy and Hendrichs 1979). Males produce and release an odour (Jang et al. 1989) that functions as a sex-attractant pheromone (for virgin females) and presumably an aggregation pheromone (for males) (Fig. 15.2). When a female approaches a male, the male initiates a complex courtship ritual that involves rapid "head shaking" and pulsed wing-fanning in addition to continued release of pheromone. If the female remains attentive, then the male attempts mounting and may or may not subsequently succeed in mating. Females, then, actively choose mates and (more often than not) reject suitors by leaving at some point during the courtship process

(Lance et al. 2000). Following mating, females become relatively unresponsive to males and shift their focus to finding fruit for egg-laying (Jang et al. 1999).

Pheromone-related behaviour of medflies remains poorly understood, which is perhaps surprising given the volume of research on this species and the theoretical potential to use a male-produced sex attractant to suppress populations by trapping females. The complexity of the male-produced odour, which includes over 100 compounds (Jang et al. 1989; Mavraganis et al. 2008), has been a deterrent to fully characterizing the pheromone components and their behavioural effects. Blends of multiple components will attract females in an olfactometre (Landolt et al. 1992; Light et al. 1999) and show some ability to enhance trap catch (Baker et al. 1990; Heath et al. 1991); extracts of males have been shown to enhance capture of females and males in traps (Mavraganis et al. 2008). Practical applications of the pheromone, however, have not been developed.

15.2.2 Feeding and Food-Related Attractants

Adult Medflies require food for energy and egg production, and feed on a variety of substrates such as fruit juices, bird droppings, and other materials on leaf surfaces (Hendrichs and Hendrichs 1990; Hendrichs et al. 1991). Proteinaceous liquids such as nulure (a corn hydrolysate) and suspensions of torula yeast have long been known to attract medflies as well as many other tephritids (Heath et al. 1994), presumably because they approximate odours from potential sources of adult food. The attractive qualities of proteinaceous liquids have been exploited by using them to bait traps and by mixing them with insecticides to form "bait sprays". While much of the feeding attractant work has been based on odours from adult food (Heath et al. 1994, 1995b), medflies also appear to respond to visual and odour cues of larval food (i.e., egg-laying sites). Mated females will seek fruit models that emanate fruit odours (Jang et al. 1999). Responses to food and host odours can be influenced by colour, with yellows and greens preferred to blues and white (Epsky et al. 1996; Katsoyannos 1987). Odours of coffee berries, especially crushed berries, attract female medflies, though it could be argued that they provide a potential source of adult, as well as larval, food (Prokopy et al. 1997; Warthen et al. 1997b).

15.2.3 Male Attractants

In some groups of tephritids flies, males can be attracted using specific compounds that, at least when discovered, had no obvious relation to the insect's biology. More specifically, these compounds were not produced by the insect and were not obviously related to food or oviposition hosts, although some are plant-derived or

structurally similar to plant-derived compounds (Metcalf 1990). These attractants have been referred to as "parapheromones" although some authors reserve the term to refer to compounds that are strictly anthropogenic (i.e., do not occur in nature) and, in many cases, are analogs of components of the insect's actual pheromone (Renou and Guerrero 2000). Among tephritids, several male lures now appear to be pheromone components, precursors of pheromone components, or otherwise involved in enhancing mating competitiveness of males (Hee and Tan 1998; Nishida et al. 1997; Shelly 1999, 2001).

Known male attractants for Medfly include Trimedlure and related compounds, and α-copaene and similar compounds (Flath et al. 1994a, b). Trimedlure, tert-butyl 4- (and 5-) chloro-trans-2-methylcyclohexane-1-carboxylate, remains the standard attractant for medfly-specific (actually, *Ceratitis*-specific) trapping (Warthen et al. 1995). An iodo-analog, ceralure, is a more potent attractant but cost-effective methods for producing it have not been forthcoming (Avery et al. 1994; Leonhardt et al. 1996). The sesquiterpene α-copaene is also highly attractive but is very difficult (expensive) to synthesize, and it is more practical to obtain it from natural sources such as essential oils from ginger or *Angelica* (Nishida et al. 2000; Shelly 2001; Warthen and McInnis 1989).

Behaviours elicited by the various males' lures can differ somewhat; for example, male medflies will form aggregations and become relatively sedentary at sources of α-copaene, a behaviour that is not elicited by trimedlure (Shelly and Villalobos 2004). An increasing body of evidence also suggests that these or similar compounds may play a role in medfly mating, as the ability of males to acquire mates increases after males are exposed to either trimedlure or α-copaene (Shelly 2001; Shelly et al. 1996).

15.3 Management Tools

Activities aimed at protecting an area from incursions of an exotic invasive pest can be categorised, as elsewhere in this text, as pre-border (Chap. 5), border (Chap. 6), and post-border (Chap. 7), or functionally, as exclusion (typically pre-border and border), detection, and mitigation.

15.3.1 Exclusion Tools

Human transport of infested fruit is the most common manner by which tephritid flies are carried to areas outside of their geographic range (Reid and Malumphy 2009). Infestations of tephritids in fruit are not always apparent, so a common strategy for uninfested countries is simply to forbid importation of any host materials from infested areas (Chap. 6). Because this would block international trade of many commodities, several methods for ensuring that these materials are pest-free

(or nearly so) have been developed (reviewed by Follett and Neven 2006, and Chap. 5). In the case of fruit pests, the most basic is simply treating fruit before shipment to eliminate any pests that may be present. Approval of treatments for elimination of fruit flies has historically required post-treatment survival rates of 3.2×10^{-5} (Probit 9) or less, but this requirement can be relaxed under specific conditions (see *Systems approaches*, below).

Several treatments are used and approved, in different settings, to kill immature stages of medfly in fruit. For example, the USA specifies, depending on the type and origin of the fruit, fumigation with Methyl Bromide, heat treatment (water immersion, forced hot air, or vapour), cold treatment, a combination of cold and fumigation, and/or radiation (USDA-APHIS-PPQ 2010). In most cases, treatment schedules specify the required duration of a treatment at given intensity (e.g., temperature, concentration of fumigant) for specific combinations of commodity and pest. Other treatments are more generic; for example, 150 Gy of ionizing radiation is an acceptable treatment for any tephritid in any commodity. Acceptable treatments will not only be lethal to medfly, but also must not substantially degrade the quality of the fruit (Armstrong 1990; Obenland et al. 1999; Schirra et al. 2006). Research continues to provide treatment schedules for additional commodities and other treatment methods involving alternate fumigants, heating mechanisms (e.g., microwave, radiofrequency waves), or other physiological mechanisms such as modified atmosphere (Alonso et al. 2005; Armstrong and Follett 2007; Powell 2003; Torres-Rivera and Hallman 2007).

Regulations that forbid importation of untreated fruit are typically enforced through border checks and inspections. These efforts may be augmented through several off-shore or post-entry measures. In addition, new technologies in electronic imaging and chemical sensing are being adapted to detect regulated materials in baggage and cargo. Old "technologies" including dogs are also increasing in use. Quarantine and inspection efforts are a front line in the defense against medfly introductions, but not discussed here as they are not specific to medfly.

15.3.2 Detection and Survey Tools

Attractant-baited traps for adult flies are the most commonly used tools to survey medfly populations. Programmes at times will sample fruit, either by systematically cutting fruit to look for larvae and eggs or by holding fruit to allow larvae to mature, exit, and pupate (USDA-APHIS-PPQ 2003). Fruit sampling may be implemented for confirming the presence of locally breeding populations or as an adjunct sampling method. However, compared with trapping, sampling is a much less sensitive and more labour-intensive method. Several types of traps are used for medfly.

Traps based on male lures. The most commonly used trap and lure for medfly detection is a small trimedlure-baited "delta"-type trap known as a Jackson Trap (Fig. 15.3a). The trap is made of plastic-coated cardboard with a wire hanger; it is inexpensive and easy to deploy (FAO/IAEA 2003; USDA-APHIS-PPQ 2003).

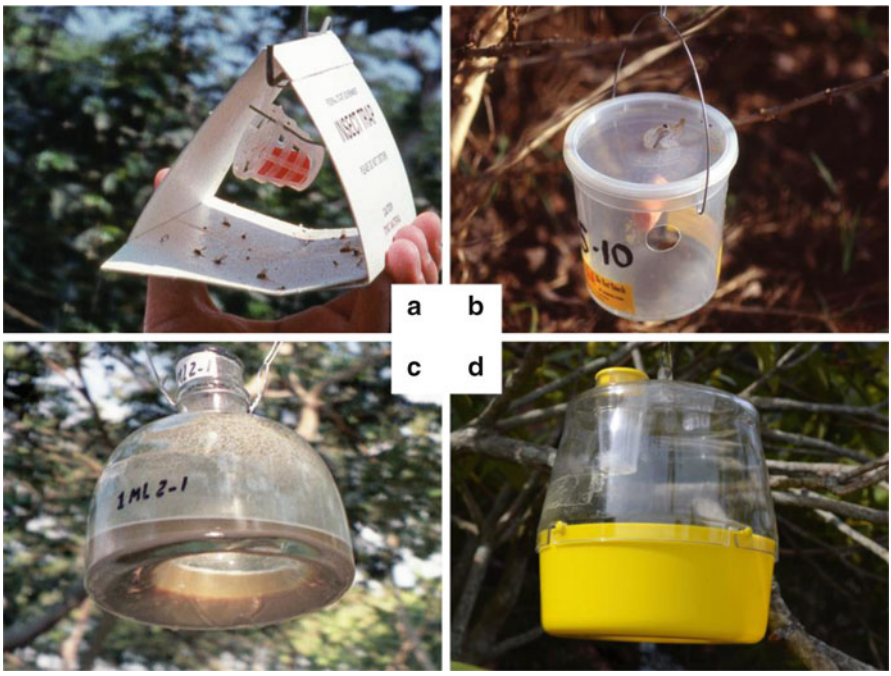

Fig. 15.3 Examples of medfly traps (**a**) *Jackson Trap*, with sticky bottom insert and basket containing a polymer plug that releases the attractant trimedlure. (**b**) *Bucket Trap*. These traps can vary regionally as to their size and shape as well as the number and diametre of entry holes. In some models, entry holes are invaginated to impede flies from leaving. (**c**) *McPhail Trap*, hand-blown glass with an invaginated bottom, used with proteinaceous liquids that act as food-odour lures. (**d**) *Multi-Lure Trap*, one of several plastic versions of the McPhail that can be used with synthetic as well as traditional food lures. A variety of other traps are also used for medfly (FAO/IAEA 2003) (Image courtesy David Lance CPHST, APHIS, USDA)

Trimedlure (typically 2 g) is often formulated into a polymer plug that is hung in a plastic basket inside the trap; some programmes may still use Trimedlure-soaked cotton dental wicks as lures. Flies enter the trap and are caught on the sticky surface of the replaceable insert that covers the trap bottom.

Other traps are also used in conjunction with male lures. Bucket Traps (Fig. 15.3b) can perform at least comparably to Jackson Traps and are preferable for monitoring high-density populations where the sticky surfaces of Jackson Traps can become saturated with flies (Cowley et al. 1990; Katsoyannos 1994). These traps are typically made from plastic containers (with lids) and have several holes around their upper perimetres; various versions have been referred to as Nadel, Lynfield, or Harris Traps, among other names. Another style is the Steiner Trap – a horizontal plastic cylinder with entry ports in the caps at both ends (Nakagawa et al. 1978). Flies that enter these types of traps are killed by an insecticide with fumigant action (typically DDVP or Naled (trade names Bromex, Dibrom). Tephri Traps incorporate both the side ports of the bucket traps and the invaginated bottom of McPhail Traps (see *Traps with food-based lures*) and can be used with either an

insecticide killing agent or a liquid to entrap and drown flies (FAO/IAEA 2003). Yellow panels can also be used – they can be more efficient than the Jackson Trap but are somewhat more difficult to handle and tend to catch more non-target organisms. In simplest form, a panel trap is a sheet of plastic-coated cardboard that comes folded in half with sticky material between the halves. For deployment, the trap is folded back along the crease to form a panel with sticky material on both sides. For years, Trimedlure was mixed directly into the stickem, but, more recently, the cardboard panel has been perforated and a wafer containing the attractant is placed between the two sides of the trap ("ChamP" Trap). Panel traps with other configurations have also been evaluated, such as the "C&C" trap, which incorporates two sticky panels on either side of a large polymer-panel release device (FAO/IAEA 2003; Leonhardt et al. 1994; Warthen et al. 1997a).

Traps with food-based lures. Food-related odours have been used extensively for trapping tephritid flies including medfly. Historically, food-odour baits have been proteinaceous liquids that are held in the reservoir of McPhail Traps (Nakagawa et al. 1971). These are bell-shaped traps with an invaginated opening in the bottom (Fig. 15.3c). The original McPhail Traps are hand-blown glass; plastic facsimiles are also available (USDA-APHIS-PPQ 2003). Flies that are drawn to the lure enter the opening and drown in the liquid. McPhail Traps, including the glass version baited with liquid, are still used in many programmes for general survey of tephritids and in some programmes are the primary trap type used for detection of *Anastrepha* spp. For Medfly, they have the advantage of attracting females as well as males. The most commonly used bait liquids are Nulure (or PIB-7; protein insect bait no. 7, a corn product) and a suspension of torula yeast (FAO/IAEA 2003). The yeast baits typically come as large pellets that are added, along with water, directly to traps (USDA-APHIS-PPQ 2003). These pellets also contain borax to improve field life of the liquid (and of captured flies) and to maintain appropriate pH, which can have a pronounced effect of attractiveness of proteinaceous baits (Heath et al. 1994). Several other commercial hydrolysates, with trade names such as Buminal, Pinnacle, and Solbait, have also been used (Fabre et al. 2003).

During the past two decades, active components in proteinaceous baits have been isolated and identified, allowing the development of synthetic food-based lures (Epsky et al. 1999; Heath et al. 1995a). These lures have typically included ammonium acetate and putrescine; a third component, trimethylamine, may be added and typically enhances medfly capture (Epsky et al. 1999). Overall, the relative effectiveness of the two- versus three-component lure, as well as the synthetic lures versus proteinaceous liquids, has varied from test to test and among tephritids species. The synthetic lures are used in 2-piece, plastic McPhail-like traps (Fig. 15.3c), Tephri Traps, or open-bottom traps with sticky inserts (Epsky et al. 1999; Heath et al. 1996; FAO/IAEA 2003). The resulting trap/lure systems are easier to handle in the field and more standardised in terms of attractant release than the standard liquid-baited McPhail Trap. Depending on the programme, the traps may be deployed dry, using a controlled-release strip with DDVP to kill flies, or wet, where flies drown in an aqueous solution. The solutions typically include a few drops of a non-foaming detergent as a wetting agent plus low-toxicity antifreeze (propylene glycol; typically

5–10 %), which retards both evaporation and decomposition of captured insects. Food-lure-baited traps, in most cases, capture fewer total medflies over time when compared to Trimedlure Traps, but the relative effectiveness of the two trapping methods for various purposes remains a subject of debate (e.g., (Broughton and de Lima 2002; Papadopoulos et al. 2001b).

15.3.3 Suppression Tools

Many suppression tools are available to regulatory programmes, including:

Chemical control methods. Historically, the most commonly used method of suppressing medfly populations has been bait sprays. A liquid feeding attractant, typically one of the protein hydrolysates discussed in *Traps with food-bait lures*, is combined with insecticide and often with feeding stimulants (e.g., sugars) and other appropriate adjuvants. When the material is sprayed on foliage, flies are attracted to and feed on the dried droplets, making the sprays efficacious even when very low application rates of active ingredient are used. Organophosphates (Malathion in particular) were used in bait sprays for several decades, but in recent years environmentally "softer" insecticides, such as Spinosads, are becoming the active ingredients of choice (Mangan et al. 2006; Vargas et al. 2001). Bait sprays are applied using conventional spray equipment ranging from hand-held sprayers to spray planes and have been key components of both IPM and regulatory (eradicative) Medfly management efforts (Braham et al. 2007; Jackson and Lee 1985; Leza et al. 2008; McQuate et al. 2005; Vargas 2004).

In medfly management areas with high population pressure, more conventional insecticide sprays, often referred to as "cover sprays" may be used. To date, these sprays have typically consisted of organophosphate insecticides (discussed in *The Medfly in Australia*, below).

In eradication projects, soil drenches are sometimes used as a supplemental control method (USDA-APHIS-PPQ 2003). Areas under host trees are sprayed, from tree base to drip line, with a broad-spectrum and relatively persistent insecticide such as diazinon. These treatments are typically applied only in the immediate vicinity of areas where medflies were known to occur and, in particular, under trees that held fruit in which immature medflies were found.

Fruit stripping. The sanitation method of fruit stripping is sometimes used during eradication to eliminate egg and larval stage medflies in the immediate areas surrounding locations where detections occurred (USDA-APHIS-PPQ 2003). Cutting all or a portion of this fruit to look for immature medflies can be used to supplement trapping for delimiting or monitoring the population. Areas stripped are typically limited in size due to concerns that wider-area fruit stripping will encourage female flies to disperse long distances in search of oviposition sites. Stripped fruit is fumigated and/or deeply buried (USDA-APHIS-PPQ 2003).

Attract-and-kill methods. Several mass-trapping and bait station methods have been considered and evaluated for medfly suppression. This strategy is similar to bait

sprays in that flies are eliminated from the population when they come to sources of attractant; the distinction between the two is that bait sprays are broadcast applications whereas attract-and-kill methods rely on fewer but more concentrated sources of attractant that are distributed throughout the environment individually. When male lures are used, the technique is typically called "male annihilation" (MA) and functions by reducing mating rather than directly reducing the population. Because one male can mate with many females, success of MA requires that very high proportions of males are removed by the time of, or soon after reaching, sexual maturity. MA has been used successfully against *Bactrocera* flies (especially *B. dorsalis* – the Oriental Fruit Fly) since the 1960s (Knight 2003; Steiner et al. 1965). The effectiveness is due to methyl eugenol's potency as an attractant for *B. dorsalis* males, which become responsive before reaching sexual maturity (Steiner and Lee 1955). In contrast, while MA has been suggested as a possible medfly control tactic (Avery et al. 1994), it has not been shown to be effective enough for use for population suppression or eradication.

The availability of improved food-based attractants has led to development of attract-and-kill methods that target both sexes of medfly. Mass-trapping has most commonly involved traps and lures described, or similar to those described, in *Traps with food-based lures* (Navarro-Llopis et al. 2008). Population suppression has been sufficient to allow incorporation of mass-trapping into IPM schemes for medfly in the Mediterranean region (Cohen and Yuval 2000; Leza et al. 2008), although supplemental control may be needed in areas with high pressure of flies emigrating from outside of managed areas (McQuate et al. 2005). An alternative approach to simply trapping flies is to lure them to sources of Insect Growth Regulators such as Lufenuron, which is safe for vertebrates but functions as an insect chemosterilant (Bachrouch et al. 2008; Navarro-Llopis et al. 2007). To date, food-lure-based attract-and-kill methods apparently have not been evaluated for potential use in medfly eradication.

Sterile Insect Technique (SIT). The Sterile Insect Technique involves the release of large numbers of reproductively sterile insects such that the released males will mate with, and thus block reproduction of, wild females in the target population. SIT is probably the most complex insect suppression method in use today, because it involves large-scale rearing of insects, sterilization, and release methods, as well as monitoring of insect quality and programme effectiveness. Components of insect behaviour, physiology, genetics, and ecology come into play, along with a variety of additional disciplines as diverse as microbiology, food technology, nuclear physics, avionics, and public relations (Dyck et al. 2005). As a control method, however, it has become increasingly favored for area-wide Medfly programmes as it is species-specific and does not involve use of toxins or biological control agents that could have potential non-target effects. Currently, SIT programmes targeting medfly probably exceed, in scope and resources expended, those directed at any other insect.

Sterile medflies are produced in large, factory-like facilities. Eggs or neonate larvae are infested onto trays of a semi-solid diet that typically consists of a plant-based bulking agent (e.g., sugar cane bagasse, wheat middlings or corn cob grits)

Fig. 15.4 Mass-rearing medflies Facility manager Stuart Stein (*left*) and USDA-ARS Entomologist Eric Jang (*right*) discuss a tray of larval diet at the former Hawaii Fruit Fly Rearing Facility in Waimanalo (Image courtesy Scott Bauer ARS USDA)

mixed with nutrient sources (e.g., yeasts, sugars, supplemental ingredients), water, and agents to adjust pH and control microbial growth. Mature larvae migrate from the diet in 7–11 days and are placed in a container with sand, fine vermiculite, or other medium that encourages pupation. A few days before adult emergence, the pupae are sifted from the medium; at 1–2 days before emergence, they are irradiated to make them reproductively sterile. Before irradiation, pupae are treated with Day-Glo powder.

Higher dipterans use their ptilinum (a fluid-filled sack on their head) to break out of their puparium. The ptilinum retracts back into the head following emergence, and carries with it some of the Day-Glo powder to create a permanent mark that is used to distinguish sterile flies from wild flies. During irradiation and subsequent shipment to the emergence facility, pupae are kept under hypoxic conditions. Hypoxia is achieved by back-flushing their holding containers with nitrogen and/or sealing the flies in air-tight containers such as plastic bags. Hypoxia helps improve sterile fly quality by minimizing damage to somatic tissues during radiation, and it delays development so that flies don't emerge en route to the emergence facility, which may be hundreds or thousands of kilometres from the rearing factory (Bakri et al. 2005).

Irradiation is accomplished using isotopic sources (^{60}Co or ^{137}Cs) at most SIT facilities. Low-energy x-ray systems are being developed for this purpose (Mehta 2008; ISO/ASTM 2013). Doses for sterilizing medflies have typically been 145–160 Gy and expressed as a minimum required or central target (e.g., median) dose, with the former being the standard (see ISO/ASTM 2013). When using genetic sexing strains (and depending on the level of security required) somewhat lower doses may be used to improve sterile insect performance (Robinson 2002) (Fig. 15.4).

At emergence and release facilities, sterile insects are prepared for distribution into the field. A simple release method involves placing containers of pupae throughout the treatment area and allowing flies to emerge directly into the environment. More often, sterile medflies emerge at a central facility and are then released as adults. Pupae are distributed into emergence containers, such as PARC boxes (pupa-adult rearing container), or stacks of screen trays and provided with a source of moisture and sugar (Shelly et al. 2006b). When most adult flies are 2 days old, they are immobilized (chilled) and transferred to release devices. Most larger-scale programmes release flies from aircraft: The adults are put into refrigerated boxes from which they are metred out of the plane using a conveyor or auger system. Similar systems can also be truck-mounted for ground release. For aerial releases, GPS tracking systems are used to guide flights, record flight data, and monitor release of flies.

In earlier medfly SIT projects, flies of both sexes were released, but the development of genetic sexing strains in the 1990s now allows the release of only males. There are several advantages to males-only releases. First, field trial data indicate that males-only releases of sterile medflies are more effective than releasing the same number of males in bisexual releases (McInnis et al. 1994; Rendón et al. 2004). Second, sterile females don't produce eggs but will still "sting" fruit, creating blemishes and possible routes of entry for pathogens (Hendrichs et al. 1995). Third, males-only should be, potentially at least, more cost-effective, as fewer insects need to be reared, irradiated, shipped, and released. The current world standard for genetic sexing of medflies is a sex-linked temperature-sensitive lethal ("tsl") trait that allows facilities to kill off females by incubating eggs at 34°C for 24 h before they are seeded onto diet (Franz 2005; Hendrichs et al. 1995). A specialised colony maintenance procedure, known as a "filter," had to be worked out to maintain the sex-linked *tsl* trait under pressures of mass rearing (Caceres 2002; Fisher and Caceres 2000) (Fig. 15.5).

For a successful SIT effort, the sterile male flies must compete for mates against the wild flies in target populations. The factory environment and artificial diets can result in insects that differ behaviourally and physiologically from wild insects, both through direct effects on phenotype and due to genetic changes in the colony as it adapts to the factory setting (Briceño and Eberhard 2000; Lance and McInnis 2005). In addition, sterilization, shipping, and release procedures can degrade the quality of sterile insects. As a result, sterile medflies are routinely monitored for such traits as size, percent emergence, survival, flight ability, and ability to mate, including mating with wild-type females (Calkins and Parker 2005; IAEA 2003).

The issue of mating competitiveness is especially critical for sterile medflies because of the combination of a female-choice mating system and their complex male mating behaviour: Wild females could potentially tend to reject sterile males if their mating behaviour becomes altered even slightly by production processes (Hendrichs et al. 2002). Indeed, wild female medflies have typically been found to accept sterile males several-fold less readily than they accept males from their own population, which reduces effectiveness of releases (Lance et al. 2000; Rendón et al. 2004). In one instance, the ability of wild medfly females to select wild over sterile males led to the evolution of resistance to SIT following an extended period of releases (McInnis et al. 1996).

Fig. 15.5 Mass-rearing medflies Medfly larvae are reared in large diet-filled trays that are stacked on carts or trolleys (*on right*) and held in large environmentally controlled rooms. Mature larvae migrate from the diet (some are visible on the sides of trays at *left*) and, in most rearing systems, drop into water. The water stops migration and stalls development, which improves synchronization of pupation following harvest (Image courtesy David Lance CPHST, APHIS, USDA)

In practise, medfly SIT programmes often target "overflooding" (sterile: wild) ratios of 100:1 or greater (Jackson and Lee 1985), but that still may not be sufficient to induce high levels of sterility into the wild population under some conditions (Rendón et al. 2004). Along with a switch to males-only strains, several approaches have been tried in an effort to improve competitiveness of sterile medflies, including improved adult diets (Shelly et al. 2006a; Yuval et al. 2007), altered microbial associations (Niyazi et al. 2004), selective breeding of flies with high mating competitiveness or survival (McInnis et al. 2002), and exposing males to attractants (or "aromatherapy") (Shelly 1999, 2001). Of those, aromatherapy, especially with oils containing α-copaene, has consistently enhanced mating success and has been incorporated into medfly SIT programmes (Shelly et al. 2007).

15.4 Management Strategies for Regulatory Programmes

The destructive nature of medfly has prompted uninfested countries to develop a diversity of programmes designed to exclude the insect. Most typically, quarantines are imposed on host fruit from infested areas, and regulatory agencies enforce the

quarantines by inspecting incoming materials such as cargo and passenger baggage for medfly host material (See Sect. 5.2). Additional programmes are often put in place to detect and (typically) eradicate incipient populations that can occasionally arise when infested fruit make their way past agricultural inspection. Diverse strategies and tactics are used in the delivery and execution of these regulatory programmes.

15.4.1 Reactive (Detect and Eradicate) Strategies

Reactive programmes are based on extensive detection trapping efforts (see *Design of medfly trapping programmes*, below). Capture of a medfly leads to intensified "delimitation" trapping in the area of initial captures to confirm the presence of a population and determine its extent and size. If one or more "triggers" are met, then the area is considered infested and regulatory and eradication measures are started (USDA-APHIS-PPQ 2003). Typical triggers include the discovery of a mated female or immature stages breeding in the environment, or multiple captures of males within a specified time and area. The mitigation (typically eradication) methods employed will depend on several factors, including availability, funding, environmental constraints, and size of the population at detection, as well as social, political, and land-use characteristics in the programme area. Due to public concerns about the use of insecticides and other chemicals, mitigation programmes have become increasingly reliant on the use of sterile insects over the past two decades.

Regulatory measures are imposed to reduce the risk of local spread of the population through movement of infested fruit and to stop infested fruit from entering intra- and international commerce. Typically, quarantines are put in place to forbid transport of untreated host fruit out of the programme area. In the USA, fruit wholesalers, retailers, and processors are put under compliance agreements that require them to take measures to safeguard against marketing or moving infested items (USDA-APHIS-PPQ 2003).

15.4.2 Preventative Release Programs (PRP)

In areas at high risk of introduction and establishment of medfly, a Preventative Release Program (PRP) is an alternative to reactive detect-and-eradicate programs. PRP is an area-wide tactic in which sterile flies are released continuously over the at-risk area during periods when adult flies could be active (in most cases, year-around). The theory is that newly introduced female medflies will mate with the sterile males that are present, squelching most infestations before they reach a size where they are likely to be detected (Dowell et al. 2000; Dowell et al. 1999). Release rates (sterile flies per unit area) under PRP are typically half or less compared with those used in conventional SIT eradication efforts. PRP has been used for medfly

Fig. 15.6 Reproductive sterilization by radiation Bags of medfly pupae are being loaded into the "drawer" of a self-contained dry-storage irradiator in Guatemala. The drawer lowers to the center of an annular array of vertical rods that contain Cobalt-60, which exposes pupae to a highly uniform dose of gamma radiation. The irradiator weighs >4 metric tons because of the lead shielding required to protect operators from radiation. The red color of the pupae (bags on cart at *left*) is from Day-Glo powder used to mark sterile flies (Image courtesy David Lance CPHST, APHIS, USDA)

management in the U.S. (California and Florida) and in South Australia (Dowell et al. 1999; Shelly et al. 2006c; Smallridge and Hopkins 2004) (Fig. 15.6).

Historical evidence suggests that occasional medfly infestations will still arise under PRP, but the number and scale of eradication efforts will be far less than what would be experienced in the same area under a reactive management strategy (Dowell et al. 1999). Infestations that are detected under PRP can typically be eliminated with a temporary increase in sterile fly release rate around the infestation. The SIT in these cases is often augmented with highly localized use of bait sprays at the points of detection.

The decision to switch from a reactive to proactive (PRP) strategy is based at least in part on economics. When medfly detections occur frequently, the reduction in eradication efforts under PRP can be substantial enough to make the strategy less expensive over time than a conventional reactive medfly program, despite the cost of continuously rearing, releasing, and identifying (in trap catch) the sterile flies. Policy makers and politicians tend to appreciate the reduced eradication activity with PRP because large-scale eradication programs are becoming less popular with the public in many areas. In addition, frequent or ongoing eradication can erode the confidence of trading partners and the public in the effectiveness of the overall

biosecurity program. Management of rearing facilities is also simplified under PRP, as the demand for flies is relatively constant in comparison with reactive detect-and-eradicate programs, where production is intermittently scaled up and down (along with labour forces, supply needs, etc.) to meet the broad changes in demand for sterile insects.

15.5 Design of Medfly Trapping Programs

Regulatory medfly trapping programs are tailored to several different goals which may include: (1) Detecting incipient populations in an area that is not known to be infested, (2) delimiting (determining extent and size of) a newly detected population, (3) monitoring wild or sterile fly populations, (4) "confirming" eradication, and/or (5) providing data in support of a systems or fly-free-zone approach to quarantine security.

15.5.1 Detection

Detection trapping is conducted in at-risk areas where medfly is not known to occur and includes some of the most extensive and expensive of all insect surveillance efforts. Detection programs should be designed to find an incipient population when it is at a size that allows the program to carry out the desired mitigation measures. In the case of medfly, the desired mitigation often is eradication. Theoretically, as more effort (=cost) is put into detection, incipient populations will be discovered at an earlier time and smaller stage of development. This will make them easier and less expensive to eradicate. However, highly intensive trapping programs, like large eradication programs, are very expensive. Ideally, we strive to balance costs of detection trapping against the expected average annual cost of eradicating these populations such that the combined overall program costs are minimised. Detection trapping is probabilistic, meaning that the actual size of a population at detection can vary quite a bit just due to chance (Lance and Gates 1994). Medfly detection systems, then, should also be designed so that, even at the maximum expected size of a population at detection, eradication will still be a feasible and prudent option. The likelihood that a population will spread to additional sites prior to detection (including by human transport) is another consideration.

Many factors influence the balance of costs between detection trapping and the resulting mitigation efforts. For example, the risk (frequency) of a population being introduced and establishing will directly affect the average annual cost of eradication. With many invasive species, risk of introduction and initial establishment is difficult to estimate because these events are relatively rare. However, reasonably accurate figures can be developed for medfly, at least for higher-risk areas using historic program data.

Tools that are available for suppressing a medfly population also influence the cost and feasibility of eradication. Medflies typically are moved into uninfested areas by people transporting fruit, and, accordingly, most detections of incipient populations occur in areas with high human population densities (cities and suburbs). Due to public concerns, programs in urbanized areas have been relying increasingly on SIT rather than insecticides as the primary eradication tactic. This places an additional premium on detecting the population at an early stage. As a result, medfly detection programs often deploy traps at 2–4 sites per km^2 in urbanized areas (FAO/IAEA 2003).

The traps usually include a combination of male-lure- (typically Trimedlure) and food-lure-baited traps (USDA-APHIS-PPQ 2003). Risk of medfly establishment is relatively lower in areas where medfly hosts are grown commercially, and detection traps in those areas may be as sparse as one per several km^2 (USDA-APHIS-PPQ 2006b). This at first may seem counter-intuitive (commercial production is what the program is ultimately protecting), but, in rural agricultural areas, introduction rates of medfly will be low (fewer people to carry them in) and use of tactics for knocking down higher-density medfly populations, such as aerial application of bait sprays, may still be feasible.

In practise, detection programs typically distribute traps more-or-less uniformly throughout an area to be surveyed. For example, in urban areas of California each 2.6 km^2 block (1 mile by 1 mile) is divided into five sections of equal area. For areas under PRP, a trimedlure-baited trap is deployed in each section. This strategy ensures that no areas in the landscape are more than several hundred metres from a trap. The probability of catching a fly in a trap is highly dependent on the distance between the fly and the trap. Populations that are centred hundreds of metres from traps can potentially build to large sizes before being detected (i.e., one or more flies are trapped) (Lance and Gates 1994).

Fruit fly detection programs can also minimise the maximum distance from fly population to trap over time by relocating traps on a scheduled basis (e.g., every 6 weeks). Computer simulations of detection trapping grids indicate that relocation does not affect the likelihood of detecting very small populations, but does reduce the chances that populations will grow to unacceptably large sizes before being discovered (DRL, unpublished). Relocation also provides the programs with an opportunity to keep traps in trees with ripe fruit whenever possible, which will keep traps in areas that flies are likely to frequent.

15.5.2 Delimitation

After a fly is captured in a detection trap, a much denser grid is deployed in the surrounding area to confirm the presence of an infestation, and to provide the higher-resolution information needed to determine the population's size and the area it occupies. This information is critical for effectively employing mitigation measures. In areas within ca 1 km of the initial find, delimitation, traps are typically placed at 20–50 traps per km^2 (FAO/IAEA 2003; USDA-APHIS-PPQ 2003).

Arrays as dense as 400 traps per km^2 have been used (Lance and Gates 1994). Delimitation trapping typically continues for several km beyond the core area, often decreasing in density with increasing distance, to help ensure that the initial capture was not a fly that had dispersed or been carried a mile or more from its source population (USDA-APHIS-PPQ 2003). Following eradication efforts, grids comparable in density to delimitation protocols are often deployed for several generations to help assure that no breeding population remains in the program area.

15.5.3 Systems Approach to Medfly Exclusion

A Systems Approach to quarantine security consists of a series of steps designed to safeguard against movement of a pest to an uninfested area. None of those steps individually may provide adequate protection, but, taken together, their cumulative effect is to provide a high degree of assurance that the pest will be excluded. A Systems Approach typically is developed through cooperation between exporting and importing countries.

An example of a Systems Approach to Medfly exclusion is the agreement between Spain and the USA allowing commerce in clementines (a variety of mandarin orange, *Citrus reticulata* Blanco) and summarized by Livingston et al. 2008. Spanish growers who wish to export their crop to the USA must trap for medfly according to a specified protocol, starting at least 6 weeks before harvest. If medfly catch exceeds an established threshold, then bait spray treatments are required for the orchard. Before shipment, a USDA-APHIS inspector randomly samples several hundred clementines based on hypergeometric sampling and size of the shipment for the presence of live medfly (all life stages), and discovery of a single insect will cause rejection of a shipment. During transit to the USA, shipments receive a cold treatment that will kill most Medflies that may have survived and gone undetected to that point. In the USA, APHIS inspectors examine the cold treatment data to confirm that specified time and temperature criteria were met and also re-sample fruit for the presence of live medfly life stages. A final level of security results from the fly's bisexual mode of reproduction – given that the numbers of live medflies in the fruit should be minimal, it would be very unlikely that two would survive and emerge near enough in space and time to mate and reproduce.

15.5.4 Fly-Free zones

Countries with established medfly populations may choose to keep one or more agricultural areas free of medfly, allowing fruit from that area to be exported freely, at least with regard to medfly. This strategy is arguably best suited for countries such as Chile or Australia (Gonzalez and Troncoso 2007); also see *Mediterranean Fruit Fly in Australia*, below), where specific production areas are geographically

Fig. 15.7 "El Pino" rearing facility The El Pino facility (Barbarena, Guatemala) is the world's largest fruit fly rearing facility. The four original rearing modules (at *left* in the picture) and two additional two-story modules provide ~10,000 m^2 of rearing area and capacity to produce >3 billion sterile male medflies per week (Image courtesy David Lance CPHST, APHIS, USDA)

remote from medfly-infested regions and/or are ecologically isolated by wide tracts of desert or other habitat unsuitable to the insect. The programs to keep the areas medfly-free will typically be similar to national programs of medfly-free countries, with quarantines, detection trapping, and protocols and systems in place to respond rapidly to detections in the pest-free zone. Reactive and/or preventative-release strategies may be employed (Gonzalez and Troncoso 2007).

15.5.5 Offshore Risk-Reduction Efforts

Uninfested countries, at times, will work proactively with exporting and/or neighbouring countries to reduce the risk of medfly introductions. A significant example is the Moscamed program, which has been a joint effort of the USA, Mexico, and Guatemala for over 30 years (USDA-APHIS-PPQ 2006a). The ultimate stated goal of the program is to eradicate medfly from Mexico and Guatemala, though shorter-term operational goals have been to reduce medfly populations in Guatemala and stop their spread northward into Mexico. Currently, the program conducts trapping to monitor populations in the Mexico-Guatemala border area and to detect populations in other parts of Mexico. The program's control efforts rely primarily on sterile insects; bait sprays and biological controls have also been used. Moscamed operates the El Pino (Guatemala) rearing facility, which is currently the world's largest fruit fly factory with a capacity of several billion pupae per week (USDA-APHIS-PPQ 2006a) (Fig. 15.5). El Pino provides sterile flies for Moscamed's control efforts and the PRP and emergency programs in the USA, among other users (USDA-APHIS-PPQ 2006a) (Fig. 15.7).

15.6 Mediterranean Fruit Fly Programs in the USA

15.6.1 History of Medfly in the USA

With the exception of Hawaii, the USA is not generally infested with medfly. However, numerous medfly infestations have been discovered in the continental USA since 1929. The resulting programs to keep the country medfly-free have led to eradication projects in Florida, Texas, and California. These programs have typically been operated jointly by the USDA and state agencies – in cases detailed here, either Florida Department of Agriculture and Consumer Services or California Department of Food and Agriculture (CDFA). These programs have included some very large, expensive, and contentious efforts, and have evolved over the years in response to changes in pest risk (introduction rates), available technology, environmental regulations, public attitude toward large-scale insect control projects, and experience gained in the previous eradication programs.

15.6.2 Florida 1929–1930

In April of 1929, the presence of maggots in grapefruit led to the discovery of a very large medfly infestation in central Florida. The ensuing eradication program eventually covered four million hectares and employed approximately 6,000 people (Ayers 1957; Clark and Weems 1989). Control actions consisted primarily of an effort to eliminate all fruit, even uprooting some plantings, within 1 mile of known infested areas in combination with crude bait sprays that were applied using ground-based equipment. The most commonly used bait was mixture of brown sugar, molasses, water, and lead arsenate – an insecticide that was originally developed for use against another invasive insect pest, the Gypsy Moth. Over 135,000 kgs of lead arsenate were reportedly applied in this effort (Clark and Weems 1989). McPhail traps (>12,000) were used along with fruit sampling to detect infested areas and to monitor program progress. The traps were baited with kerosene, which attracted male medflies (Clark and Weems 1989). In addition, regulatory measures, including roadblocks manned by the National Guard, were put in place to stop movement of fruit out of the program area (Rohwer 1958). The program concluded in late 1930 at a cost of over $7 million USD, and a medfly population wasn't seen again in Florida until 27 years later (Ayers 1957; Clark and Weems 1989).

15.6.3 Florida 1956–1958

The discovery of the second incursion of medfly in Florida occurred in the Miami area in April of 1956. By that time, technology for survey and control of the pest had

advanced substantially – and continued to advance during the program. Arsenate insecticides were supplanted by organophosphates (in particular, malathion), which were relatively more effective despite their lower application rates. Protein hydrolysates replaced less attractive molasses and sugar, and the baits were applied primarily by aircraft, which delivered them much more efficiently than ground-based equipment. In all, over 300,000 ha were treated – most multiple times – leading to a total aggregate treatment of ~2.5 million hectares (Clark and Weems 1989; Rohwer 1958). Along with this, the role of fruit removal was downplayed in comparison to the 1929 program (Ayers 1957; Clark and Weems 1989). Plastic "Steiner" traps largely replaced McPhails early in the program (Steiner et al. 1961). These were initially baited with *Angelica* seed oil, later shown to contain the male lure α-copaene (Jacobson et al. 1987), and subsequently with a synthetic attractant named "Siglure," which formed the basis of later structure-activity studies that led to the development of Trimedlure and Ceralure (Beroza et al. 1961; Warthen et al. 1994).

The second Florida medfly eradication project ended in February 1958 at a cost of ~$11 million USD (Clark and Weems 1989). Although the 1956 program was smaller in area than the 1929 effort, it was still massive by modern standards. From 1956 to 1958, the program's 54,000 traps caught nearly 12,000 medflies (Clark and Weems 1989) – a number far greater than total number of wild medflies captured in all of California's medfly programs (Carey 1996; K. Hoffman, CDFA, personal communication). One outcome of the program – and the reason that subsequent eradication programs have been smaller – is that intensive trapping programs were put in place to detect incipient populations of exotic fruit flies in Florida and other at-risk portions of the USA (Rohwer 1958).

15.6.4 California 1975–1982

Medfly was first detected in California during 1975, and the ensuing eradication effort marked the first time that sterile flies were used in medfly eradication (Cunningham et al. 1980). The program also included bait-sprays of host trees using ground-based equipment and limited fruit-stripping, but SIT was considered the primary control method (Jackson and Lee 1985). A total of 77 wild flies were captured in 1975, with no additional captures until 5 June 1980, when another, similar, eradication effort was launched against a small infestation (total of five flies captured) in southern California (Carey 1991).

Also on 5 June 1980, two male medflies were discovered in a trap in Santa Clara County, just south of San Francisco Bay (Jackson and Lee 1985). This detection led to the most contentious and controversial insect program in California's history. Trapping in the area had been minimal, and the program was slow to start. Control efforts consisted of SIT, ground-based bait sprays, soil drenches, and some fruit stripping, but weren't sufficient to get ahead of the population. By December 1980, the USDA had begun pressuring California's governor (Jerry Brown) to begin aerial bait spray applications (Jackson and Lee 1985). The idea of aerially applied

Malathion was extremely unpopular with the residents of Santa Clara County, and alternate strategies such as systematic fruit-stripping were substituted until June 1981. At that point, detections – which had been down early in the year – suddenly increased. Several scientists believed that the increase resulted from the release of a batch of Peruvian "sterile" flies that had not been properly irradiated (Marshall 1981) – a claim that was never conclusively proven or refuted. Regardless, on July 10, Governor Brown accepted the USDA's recommendation to allow aerial spraying. The detection trapping system was also substantially upgraded at that time, resulting in detections that pushed the area under regulation to almost 10,000 km^2 across seven counties at the peak of the program – with nearly 4,000 km^2 being treated with regularly scheduled bait sprays. The spraying phased down in the fall of 1981 but continued at some level until June 1982; the last area came out from under regulation that September. The entire program, including smaller eradication efforts in 1980 and 1981–82 in Los Angeles County, cost ~$100 million USD (Jackson and Lee 1985).

The Central California medfly program brought about several changes. Legislation was passed to improve the USDA's ability to respond to emergencies rapidly and to fund emergency activities. In addition, changes to the Federal Plant Pest Act made it possible for the Secretary to invoke emergency powers regarding regulation of intrastate movement of commodities, initiating eradication programs, and entering private property, though in practise these powers have rarely been used. The density of traps in detection grids in high-risk areas (i.e., residential neighbourhoods) was increased from 0.4 (or fewer) up to 2–4 traps per km^2 in hopes of catching incipient populations while they were small enough to easily and quickly remove (Jackson and Lee 1985).

15.6.5 California 1987 to Mid-1990s

After 1981, single medflies were captured in 1982, 1984, and 1986. However, starting in 1987, infestations cropped up annually, usually at multiple foci, in southern California. Each infestation was met with an eradication program, based increasingly on SIT with supplemental ground-based bait sprays and localized soil drenches under known infested trees. Despite this effort, the pattern and frequency of medfly discoveries led to controversy over their source: Was each infestation the result of a separate introduction, as proposed by USDA and CDFA, or was southern California generally infested with a medfly population that would occasionally increase in one area or another to the point where detection was likely? Either way, the resulting programs were expensive, and their extent and frequency was eroding confidence of the public, the states, and USA trading partners in the federal regulatory pest programs. In 1996, the medfly program in southern California switched from reactive to a proactive Preventative Release Program (Dowell et al. 1999). The program has

reduced the number of infestations detected from several per year to <1 per year (many of which have been outside the PRP zone) and reduced overall program costs (Dowell et al. 1999; Dowell et al. 2000).

15.6.6 Current Medfly Programs in the USA

The USA has active programs to keep all susceptible areas, with the exception of Hawaii, free of medfly. Exclusion efforts rely primarily on the country's broader phytosanitary program, which operates through a combination of regulations, inspections, pre-clearance measures, and penalties to either exclude host fruit or certify it as pest-free based on various measures (see Chaps. 5 and 6). In addition, several programs specific to excluding medfly, such as Moscamed in Guatemala and Mexico, and the Spanish Clementine program are detailed above.

The USDA continues to operate, in conjunction with state and, in some cases, county agencies, extensive trapping programs to detect incipient populations of medfly (USDA-APHIS-PPQ 2003, 2006b). Allocation of trapping effort is risk-based with 2–4 traps per km^2 being deployed in high-risk (residential and urbanized) areas. Captures of flies are followed by specific delimitation trapping protocols, and, if triggers are met, by an eradication effort. Most detections occur in populated areas, and eradication in those areas currently rely primarily on releases of sterile flies (USDA-APHIS-PPQ 2003, 2006a). The SIT efforts are supplemented by ground-based application of bait sprays near fly finds, and, in some instances, by soil drenches of insecticides under trees suspected of harbouring immature medflies. The organophosphates in the bait sprays have been replaced by "softer" insecticides, with Spinosad being the toxin of choice since the early 2000s.

As noted above, a large portion of Los Angeles County (~5,400 km^2) was put under a Preventative Release Program in 1996, and releases of sterile insects have continued to the time of this writing. The PRP area was increased to >6,400 km^2 in 2000 to cover portions of Orange and Riverside Counties where infestations occurred in 1998 (K. Hoffman, CDFA, personal communication). California has had approximately a dozen medfly eradication programs since 1996, with almost half of those resulting from finds within the PRP zone. Still, this represents a substantial drop in the frequency of eradication programs since the late 1980s and early 1990s (Dowell et al. 1999). The recent programs have typically been quite small; in all cases, fewer than 30 wild flies were captured, with the exception of Riverside County in 1998 (75), which was not in the PRP zone at the time.

In Florida, medflies were found in the Tampa area during 1998, and the subsequent delimitation determined that the population had grown undesirably large, both in area and in numbers of flies, before detection. Following an extensive eradication project, the infrastructure that was developed for emerging and releasing sterile flies was left in place (though it has subsequently been moved), and the project transitioned to a PRP program. Portions of South Florida (Miami area) were also put under PRP. Medflies were not detected again in Florida until 2010 in Boca Raton.

The South Florida PRP has since been expanded northward to incorporate that program area and other high-risk portions of Dade, Broward and Palm Beach counties. Other medfly-susceptible areas in the USA, including parts of Texas, Arizona and several other southern states, along with lower-risk portions of California and Florida, remain under reactive medfly programs.

15.6.7 Is Medfly Established in California?

During 1991, Dr. James Carey, an entomologist at University of California at Davis, published a paper in the journal *Science* proposing that medfly was established through much of the greater Los Angeles (LA) area (Carey 1991, 1996). This flew in the face of assertions by USDA and CDFA that each new infestation was being successfully eradicated. Carey's hypothesis was based on several factors: (1) Medfly populations had been detected annually since 1986 in southern California, usually at multiple locations; (2) the detections tended to be centred around, and spread outward from, locations where flies had been detected in previous years; (3) an examination of data on pests intercepted by USDA in measurable pathways (such as airline baggage) purportedly indicated that introduction rates were too low to account for the number of infestations found; (4) detections of Medfly in the LA area appeared disproportionally higher than in other parts of the USA with suitable Medfly habitat (as well as being disproportionately higher than those of other exotic tephritids); and (5) an assumption that populations could exist for extended periods at a level below the ability of the trapping system to detect them. The controversy spilled over into the popular press, reducing the public's already-eroding confidence in California's medfly program and causing consternation among the state's trading partners. At least two nations that imported California produce responded by sending staff to review U.S. fruit fly programs.

Since the publication of Carey's 1991 and 1996 papers, DNA analyses have provided evidence for multiple introductions of the insect into California (Meixner et al. 2002), and subsequent examinations of pest interception data suggest that Carey substantially underestimated the rate at which Medflies are being introduced into the USA (Liebhold et al. 2006). These realizations don't rule out the possibility of an established Medfly population in California, but they do leave the situation open to alternate explanations. In addition, data on the sensitivity of the Medfly detection trapping system (Lance and Gates 1994) make it difficult to envision the existence of numerous sub-populations that are large enough to be viable yet completely escape detection for multiple consecutive generations across southern California (for example, no Medflies were caught in California in 2000, 2003 or 2006; K. Hoffman, CDFA, personal communication). A more parsimonious explanation for observed patterns of Medfly discovery in California may be that commercial fruit smuggling plays a central role, given the levels of wholesale agricultural products that are being intercepted coming into the U.S. illegally (USDA-APHIS-PPQ 2006c). Regardless, the controversy continues (Carey 2010; Liebhold et al. 2010).

15.7 Mediterranean Fruit Fly in Australia

15.7.1 Origin and Spread of Medfly in Australia

Medfly established in Western Australia (WA) during the late 1890s (Sproul 2001), probably through infested citrus fruit. Around the same time medfly was found near Sydney and Tasmania where it was eradicated. In New South Wales (NSW), medfly established and remained an important pest until it died out during the 1940s, possibly as a result of the expansion in range of Queensland Fruit Fly (*Bactrocera tryoni* (Froggatt)) (Hely et al. 1982; Waterhouse and Sands 2001). Medfly also established in Victoria with the last outbreak in 1953 in Melbourne (R. Mapson, personal communication). Outbreaks at Alice Springs in the Northern Territory during 1976 and 1982 were eradicated (Allwood et al. 1979). In South Australia, which has a similar Mediterranean climate to WA, infestations have been detected every 2–3 years (Madge et al. 1997), and, during the last 10 years, outbreaks have occurred in Adelaide in 2000, 2002, 2006 and 2010. Medfly populations have not been reported in other states during the last 50 years. Medfly has not established in Queensland, possibly due to competition from the large endemic fruit fly fauna.

From the initial infestation in Perth, medfly spread on infested fruit and is now established in towns and growing areas from Esperance on the south coast to Derby in the subtropical north. Medfly is most pestiferous in growing areas surrounding the capital city Perth, where large populations develop in urban areas. In the colder Manjimup region, it is only a minor pest, and an area free of medfly is maintained in the Ord River Irrigation area near Kununurra in the far north. That area has an extreme tropical climate, with high temperatures and humidity in the wet season, which is unfavourable for Medfly survival.

In most of the WA bushland, there are no native or feral medfly hosts. A few feral hosts survive along river systems in the southwest while some native hosts grow in the tropical north but disjunctions in fruiting phenology means that medfly is unlikely to survive away from human habitation. The desert areas between western and eastern Australia form a natural barrier to its spread eastward.

Medfly is the only species of *Ceratitis* established in Australia. Medfly adults are readily distinguished from other pestiferous Australian tephritids, which are primarily *Bactrocera* species (White and Elson-Harris 1992). Larval keys are available (Dadour et al. 1992) and allozyme methods (M. Adams: personal communication) can be used to separate medfly larvae from those of Queensland Fruit Fly.

15.7.2 Host Range in Australia

In reviewing the WA literature, Sproul (2001) reported 69 hosts while (Hancock et al. 2000) listed 53 species in 23 plant families as hosts. Key commercial hosts include citrus, stone fruit, and pome fruit. Citrus is the main over-wintering host.

In urban areas loquats and kumquats (*Citrus, sensu lato*) are important hosts while table grapes (*Vitis vinifera* L.) and olives (*Olea europaea* L.) can also be attacked. At Carnarvon, 1,000 km north of Perth, medfly attacks citrus, mangoes (*Mangifera indica* L.) and infests overripe capsicums (*Capsicum anuum* L.) left after harvest. The host range in the town of Broome, 2,000 km north of Perth has been studied with fruits of 18 plants found to be hosts (Woods et al. 2005). Based on abundance, fruiting phenology and host suitability, the most important hosts were mango, kumquat, Barbados cherry (*Malpighia glabra* L.), orange jessamine (*Murraya paniculata* (L.) Jack), guava, Pacific almond (*Terminalia catappa* L.), "blackberry tree" (species not confirmed) and yellow oleander (*Cascabela thevetia* (L.) Lippold).

15.7.3 Eradication Programs in Australia

In 2001, the Department of Agriculture in Western Australia commissioned a benefit-cost analysis on the eradication of medfly from Australia (Mumford 2005). The analysis found that eradication over 6 years, using baiting followed by release of sterile insects, would cost US$35 million, had a high probability of success, and if the area planted for horticulture doubled over 20 years then net benefits at present value were likely to be positive.

15.7.4 Tools for Managing Medfly in Endemic Areas

Bait sprays. Before the advent of systemic organophosphate cover sprays in the late 1950s, baiting combined with good orchard hygiene was the mainstay of fruit fly control. Baiting is still an important control tool, especially in citrus orchards where weekly baiting can maintain good control. In high pressure areas with susceptible crops such as stone fruit, baiting must be supplemented by cover sprays.

Twice weekly bait applications are required if fly pressure is high. Protein hydrolysate baits are most widely used in combination with the insecticide Malathion (known in Australia as "Maldison") or Trichlorfon. Spinosad is an organically compatible alternative but is higher priced and rarely used except by organic growers and in some community baiting schemes in urban areas. In the latter cases, Spinosad's low toxicity and public relations benefits can justify the extra cost, although some community baiting schemes still rely on protein bait/organophosphate mixtures.

Community baiting schemes involve application of bait mixture to all fruiting trees in a town or adjacent horticulture area for 6–12 months of the year. If well managed, they can maintain Medfly populations at non-damaging levels. Ratepayers are generally levied fees based on the number of trees per property and shire involvement is essential for long-term sustainability. Maintaining these schemes over several years has proved a difficult task, with many schemes terminating due to funding or staffing issues.

Cover sprays. The introduction of the systemic cover sprays containing the organophosphate insecticides fenthion and dimethoate revolutionized fruit fly control in Western Australia (WA). They are still widely used, especially in high-susceptibility crops in high-pressure areas. These areas often abut urban areas where fly populations can reach very high levels. If maggots infest fruit, cover spraying is the only effective option left for growers. Restrictions on the use of these insecticides are likely in the near future, which may make fruit production uneconomical in some areas.

Mass trapping. As noted above, effective female lures have been available for at least 10 years (Heath et al. 1996), but growers in Australia have only recently begun experimenting with mass trapping. Mass trapping alone probably will not give effective control, especially in high-pressure areas where many small orchards are adjacent to urban areas or orchards with unmaintained fruit trees.

Biological Control. Parasitism of field-collected larvae in WA is very low with only the native *Bactrocera* parasitoid *Diachasmimorpha kraussii* (Fullaway) reared (I. Lacey: personal communication). Attempts to establish other parasites during the 1950s were not successful (Waterhouse and Sands 2001). *Fopius arisanus* (Sonan) is established in Queensland on *Bactrocera* species and could possibly be used for inundative release in conjunction with SIT, because it causes significant mortality to medfly in Hawaii (Rousse et al. 2005). *Fopius ceratitivorus* Wharton is the most promising candidate for classical biological control (Lopez et al. 2003) but must undergo specificity testing against native tephritids before approval would be given for its release into Australia.

Sterile Insect Technique (SIT). SIT has not been used for field control in WA. Orchards are generally small, may contain mixed varieties of fruit, and often abut urban areas, making area-wide management using SIT difficult. SIT, however, is a critical part of programs to keep South Australia free of medfly (see below).

Interstate movement. The Medfly Code of Practise (Anon 2008) describes responsibilities and procedures that apply to the management of medfly and lists phytosanitary requirements for trade between Australian states. The draft document is under constant review and treatments may differ from international standards. The Code lists requirements for pest free areas, areas of low pest prevalence, pest free places of production and infested areas. Surveillance procedures for establishment of area freedom (e.g. trap type, trap number; trap location and frequency of inspection) are listed. Risk management in terms of buffer zones, movement restrictions, and treatment of susceptible hosts is discussed. Thresholds for suspension and suspension areas are listed. Annexes list susceptibly of produce, approved disinfestation treatments, and re-instatement dates after loss of area freedom.

Quarantine barriers. Only one major paved road connects Perth with Adelaide in South Australia, and a 24-h quarantine checkpoint operates at Ceduna, which is near the South Australia border. All vehicles are stopped, manifests checked, and private vehicles inspected for fruit fly hosts.

Interstate Certification Assurance (ICA). This is a system of plant health certification based on quality management principles and is a national scheme administered by all Australian states and territories (Anon 2013). To be accredited, a business must

demonstrate it has effective in-house procedures in place that ensure produce consigned to intra- or interstate markets meets specified plant quarantine requirements. The ICA scheme seeks to provide a harmonized approach to the audit and accreditation of businesses throughout Australia. Under ICA protocols businesses can issue a plant health certificate enabling interstate trade rather than involving government officials at far greater cost. Businesses issuing ICA's are regularly audited by government authorities. ICA's of relevance to medfly include ICA-04: Fumigating with methyl bromide, ICA-07: Cold treatment, ICA-16: Certification of mature green condition of bananas, ICA-23: Area or property freedom, and ICA-30: Hard condition of avocado for Mediterranean Fruit Flies.

Disinfestation. The code of practise lists over 100 species that require disinfestation treatment to enter the other states. Fifteen of these are in the Family Rutaceae, e.g. *Citrus* spp., and 13 in the Family Roseaceae, *e.g. Prunus* spp. Avocado (*Persea americana* Mill.), banana (*Musa acuminata* Colla), lime (*Citrus* spp.), olives, papaya and strawberry (*Fragaria* spp.) must be treated unless harvested mature green. Durian (*Durio zibethinus* Murr.), lychee (also called litchi), mangosteen (*Garcinia mangostana* L.), passionfruit (*Passiflora edulis* f. *flavicarpa*), pomegranate (*Punica granatum* L.), and rambutan (*Nephelium lappaceum* L.) do not require treatment if the skin is unbroken. Of the solanaceous vegetables listed as hosts, capsicums can be attacked when overripe, tomatoes (*Lycopersicum esculentum* L.) are very rarely attacked even when overripe, and the host status for eggplant (*Solanum melongena* L.) needs to be confirmed.

The list of some of the produce from WA that requires disinfestation treatment for medfly includes: Apple (*Malus domestica* L.), apricot (*Prunus armeniaca* L.), blackberry (*Rubus fruticosus* L.), blueberry (*Vaccinium corymbosum* L.), calamondin (×*Citrofortunella mitis* (Bunge) Wijnands), capsicum, carambola (*Averrhoa carambola* L.), cherry (*Prunus avium* L.), chili (*Capsicum* spp.), citron (*Citrus medica* L.), eggplant, feijoa (*Acca sellowiana* (O. Berg) Burret), fig (*Ficus carica* L.), grape, grapefruit (*Citrus* × *paradisi* Macfad.), kiwifruit (*Actinidia* spp.), kumquat, lemon (*Citrus limon* (L.) Burm.), lime, loquat (*Eriobotrya japonica* (Thunberg) Lindl.), lychee, mandarin (*Citrus reticulata* Blanco), mango, mulberry (*Morus spp.*), nashi (*Pyrus pyrifolia* (Burm. Nak.)), nectarine (*Prunus persica* (L.) Batsch var. *nucipersica* (Suckow) C. K. Schneid), olive, orange (*Citrus* × *sinensis* (L.) Osbeck), papaya, peach (*Prunus persica* (L.) Batsch), pear (*Pyrus* spp.), persimmon (*Diospyros* spp.), plum (*Prunus domestica* L.), pomegranate, quince (*Cydonia oblonga* Mill.), raspberry (*Rubus* spp.), strawberry, Tamarillo, tangelo (*Citrus* × *tangelo* J. Ingram & H. E. Moore), and tomato.

Disinfestation methods. Despite the recognition of generic radiation dose for fruit fly disinfestation, there are no commercial irradiators in WA so this option is not available. Also, hot water treatment, high temperature forced air, and vapour heat are not used for medfly disinfestation in WA. Cold treatment is the most common disinfestation treatment and is widely used on temperate crops. Its effectiveness at different temperature on a wide range of commodities has been demonstrated in Western Australia (F. DeLima, personal communication). Fumigation with Methyl Bromide remains the treatment of last resort and is still widely

used despite the possibility of deregistration in the long term. Post-harvest dipping or flood spraying with insecticide was once widely used, but these treatments are likely to be withdrawn because of residue concerns.

Area-wide management and Systems Approaches. Currently, no Systems Approaches are certified for export of produce potentially infested with medfly (Jessup et al. 2007). A draft ICA for Systems Approaches is being developed. Some areas of Western Australia with low pest pressure due to unfavourable climate and/or lack of hosts (e.g., west Midlands, Manjimup) may be able to export fruit to eastern Australia under a protocol combining areas of low pest prevalence with pest free places of production. As yet no officially recognised areas of low pest prevalence exist in Western Australia.

15.7.4.1 Fly-Free Zones

Area freedom in Western Australia. In WA area freedom from medfly is maintained in the Ord River Irrigation Area (ORIA) in the north of the state. Approximately one hundred traps on a 400 m grid in the town of Kununurra and a 1 km grid in the growing area are checked weekly (April-October) when outbreaks are likely and fortnightly at other times. Traps are bucket-type Lynfield Traps (Wijesuriya and De Lima 1995) with dental wicks containing Capilure® (a commercial mixture of Trimedlure with extenders to increase field life (Hill 1987) and a small piece of Dichlorvos Pest Strip (1 cm^2) in the traps to kill the flies and pests such as ants. Traps are re-lured and pest strips changed every 2–3 months. Outbreaks in the past have been eradicated using a combination of bait spraying, fruit stripping and cover spraying. Future eradications are likely to use SIT with bait spraying and limited fruit removal.

Area freedom in South Australia. The state of South Australia is free of fruit flies and supports a large horticultural industry with export of citrus fruit to the USA from the inland Riverland region. Medfly outbreaks regularly occur in Adelaide and are eradicated. The eradication programs have evolved from those involving primarily fruit stripping, then organophosphate insecticide baits and cover sprays, to (since 2001 an integrated approach involving baiting with organic insecticide, minimal fruit stripping, and release of sterile flies.

Lynfield Traps (Cowley et al. 1990) are used to detect incursions with Capilure® and dichlorvos added to the dental wicks. These are placed on 400-m grids in urban areas and 1-km grids in growing areas. Traps are checked weekly in the season when outbreaks are likely and fortnightly at other times. Despite the intensive trapping, medfly infestations in this area are often found by the public reporting larvae in fruit before flies are found in traps (D. Cartwright, personal communication). Reporting of any maggot-infested fruit is encouraged through regular media campaigns. When an outbreak has been detected, supplementary traps are deployed. Both male and female traps are used. The female traps are McPhail-type using the three-component synthetic food lures (Broughton and de Lima 2002).

When an outbreak has been declared, release of sterile flies follows an initial 2–4 week period of baiting with Spinosad to lower fly numbers to negligible levels. Sterile fly release typically continues for 10 weeks after the baiting ceases. However, if winter weather encroaches into this period then sterile release may be suspended and resumed in spring.

Sterile fruit flies produced at the Department of Agriculture and Food Western Australia factory at South Perth, Western Australia are used to eradicate outbreaks in South Australia. Flies must meet or exceed international quality standards (IAEA 2003). The Vienna 7/99 "mix" strain has been in continuous production since 1999, with production levels maintained above 1.5 million per week and to up to 10 million per week during outbreaks. Vienna 7/99 is a *tsl* genetic sexing strain, and a filter rearing system is used to ensure that the level of deleterious recombinants are minimised (Fisher and Caceres 2000). Flies are reared on a bran-based diet using boiling water to minimise bacterial contamination. Larvae are collected into water, spread on vermiculite for pupation, and hand-sieved to maximize pupal quality and flight-ability. Two days before emergence, pupae are irradiated at a mid-point dose of 160 Gy in a Gamma cell 220 irradiator. Pupae are flushed with nitrogen for 10 min before irradiation and during irradiation. A radiation-sensitive indicator (Sterin badge) is placed on each canister to provide visual confirmation that the canister was irradiated (ISO/ASTM 2013).

Irradiated pupae are dyed, heat sealed in plastic bags and placed in foam vegetable boxes with Techni ice® for overnight shipment to South Australia. In South Australia 14.5 g of pupae are placed into 5 l cardboard buckets and held in an emergence room for 5 days. The buckets have two screen inserts in the side to allow room exposure to ginger root oil (GRO, a source of α-copaene) which improves mating competiveness (Shelly et al. 2007). On day five the flies are moved to a separate cool room for exposure to GRO from wicks hung from the roof using fans to circulate the air. Flies are released from a purpose-built release pod on the back of a utility vehicle; one bucket is released approximately every 100 m. Initial release rate is higher before dropping to a lower maintenance level once sufficient fly numbers have been reached in the area. Identification of wild or sterile flies is accomplished with an electro-florescent microscope and confirmation is established by dissection of the ptilinum.

Suspension zones These are based on analysis of outbreak data from South Australia (Meats et al. 2003). If a gravid female or larvae are found then an outbreak is declared with a 7.5 km radius from the outbreak centre. If one male is caught, then no further action is required. If two males are caught then a larval search is required and 16 supplementary male traps may be deployed within the 200 m zone. If supplementary traps are not deployed and three males are caught within 1 km and 14 days, then an outbreak must be declared. If supplementary traps are deployed the trigger moves to five flies before an outbreak is declared. However despite scientific evidence for a 7.5 km zone, Queensland only accepts a minimum 15 km zone and Tasmania a 80 km zone.

Reinstatement of pest freedom The pest free status of a suspended area, which is subject to eradication following a Medfly incursion can be reinstated providing no

wild Medfly is trapped in the surveillance program for a generation and 28 days, or 12 weeks after the last larva or wild Medfly is captured in the traps, whichever is the longer. Reinstatement dates for several centres and dates have been calculated from Australian government metrological data and are tabulated in an annex to the code of practise (Anon 2008).

15.8 Concluding Remarks

The Mediterranean Fruit Fly remains a feared agricultural pest throughout tropical to warmer temperate areas around the globe. While it can produce substantial in-field losses of many fruit crops, the insect's ability to move though commerce to uninfested areas has led to most of its infamy. The medfly is one of the most studied insects in the world, and efforts to detect and control the pest have led to broader innovations in areas such as insect attractants and detection, control methods, and regulatory procedures. The development of programs to combat and contain this insect has mirrored the broader development of regulatory programs worldwide, making medfly an excellent case study in regulatory entomology.

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