5. Handling, emergence and holding at release centre

STEP III OF PROCESS IN FLOW CHART IN APPENDIX 2

5.1. RECEPTION AND UNPACKING OF PUPAE

STEP III-a OF PROCESS IN FLOW CHART IN APPENDIX 2

Upon arrival at the release facility, the containers (boxes or bottles) are first examined for damage and then opened individually. The plastic bags in boxes are then inspected by the designated personnel and temperatures of specified bags are checked (FAO/IAEA/USDA 2003).

Each bag is examined for exposure to radiation by checking the colour change in the irradiation-indicators. If the colour change in the indicator is in question, the bag is not opened and the supervisor is immediately notified. The unopened bag of pupae is then double bagged and placed into a freezer for a minimum of 48 hours to destroy the contents. The examination procedure has to be applied to each numbered box of pupae before the next box is opened.

Once it has been determined that a bag of pupae has been properly irradiated, the bag is opened and the pupae poured into a collection container. A very small sample (ca. 5 ml) of each bag of pupae is collected for quality control testing purposes (see Section 13).

Radiation indicators are removed after all boxes/bags have been emptied; radiation indicators are counted and stored for a period of one year or as prescribed in the programme operating procedures.

In summary, upon arrival at the release centre the following steps should be carried out:

- Check the shipment documentation (see Section 4.2.5).
- Verify correct change in colour of irradiation indicator and required doses following Manual for Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies (FAO/IAEA/USDA 2003).
- Make sure that the holding room is set at the proper temperature (24 ± 1°C).
- Verify that the temperature of the pupae is in a range of 15 – 20°C (see Section 2.2).
- Open the container and sample for quality control (see Section 13).

TABLE 5.1
Amounts of pupae used in PARC boxes and towers in current operational programmes

Fruit fly species	PARC Boxes		Tower Trays	
Medfly *(C. capitata)*	6	Bags	50 – 80	Trays
	≦45,000	Pupae/Box	≦24,000	Pupae/tray
	660	ml/ Box	350-400	ml/ tray
	NA	NA	1.2 – 1.92	Million Pupae/tower
Mexfly *(A. ludens)*	6	Bags	50 – 80	Trays
	<24,000	Pupae/Box	≦13,000	Pupae/tray
	660	ml/Box	400 – 440	ml/ tray
	NA	NA	0. 65 – 1.04	Million Pupae/tower
West Indies fruit fly (*A. obliqua*)	2	Bags	NA	NA
	40,000	Pupae/Box	NA	NA
	500-560	ml/Box	NA	NA
	NA	NA	NA	NA

5.2 PROCEDURES FOR CHILLED ADULTS

5.2.1 Setting up for fly emergence

STEP III-b OF PROCESS IN FLOW CHART IN APPENDIX 2

At the present time there are two basic systems used in which to emerge sterile flies for a chilled release: The Plastic Adult Rearing Container (PARC) and the tower. The PARC consists of dispensing measured amounts of pupae into paper bags (2–6 bags depending on their size) that are then placed into the PARC. Pupae are volumetrically dispensed into bags (PARCs) or trays (towers) (**Table 5.1**). Each bag is stapled approximately 2.5 cm from each corner at the top of the bags (this is done to allow flies to emerge and to keep the waste and un-emerged flies in the bag and prevent emerged flies from re-entering the bags).

In the towers, pupae are placed into a hopper that dispenses measured amounts onto each individual screen tray. These trays are then stacked into towers (**Figure 5.1**). For amounts of pupae used in programmes utilizing these two emergence systems see **Table 5.1**.

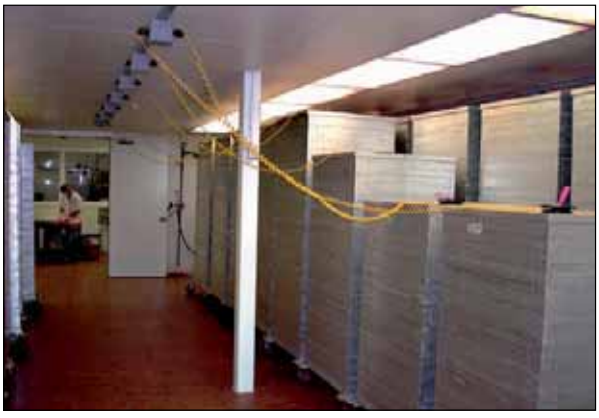

FIGURE 5.1
Towers used to emerge and hold sterile flies

FIGURE 5.2
PARCs used to emerge and hold sterile flies

In the PARC system, the lid is placed on each of the containers. Lids should be inspected to ensure the foam seal is intact to avoid flies escaping. PARC containers and lids require ongoing maintenance or replacement as needed (**Figure 5.2**).

5.2.2 Food preparation and feeding

STEP III-c OF PROCESS IN FLOW CHART IN APPENDIX 2

The food medium consists of agar, water, sugar and preservative (**Table 5.2**). The food medium is normally prepared no more than 24 hours in advance. A 227 litre (60 U.S. gallon) steam kettle is commonly used to prepare the food. Agar and preservative are added to cold water. If the water is too warm when the agar and preservative is added, the agar will clump together. The mixture is then brought to a rolling boil. Upon boiling, granulated sugar is added and the mixture is again brought to a boil, stirring as needed. At this point the steam kettle is turned off. The sugar must be completely dissolved in the mixture to prevent breakdown of the agar.

The recipe for the amount of agar prepared can be altered by changing the measurements of the ingredients proportionally. In addition to changing the quantities, it may be necessary to modify the agar for firmness or if a breakdown problem occurs. These problems may be addressed by increasing or decreasing the amount of agar added to the mixture. More agar will firm up or tighten the agar; less agar will have the opposite effect. Agar that is too "tight" will not allow the flies to obtain the necessary moisture and sugar out of the gel. The ingredients and proportion used to prepare the mixture are the following:

Thus, for example, to prepare 10 litres of mixture, 8.4 litres water, 1.5 kg sugar, 0.08 kg agar (80 gr) and 0.001 kg preservative (1 gr) are required.

The liquid is carefully poured into fiberglass trays (41 cm width × 77 cm length × 5–7.5 cm deep) (16" × 30" × 3"). The agar slab will be approximately 1.9 cm (3/4") thick. One agar square is placed on top of the screen of PARC and tower trays. If the agar squares are too thick they will be squeezed through the screens of the PARC/tower trays. If they are too thin they will dry out too quickly and not allow the adult flies to feed from the moisture in the agar. A stainless steel blade is used to cut the agar, one tray at a time, into ten equally sized pieces for PARCs and twenty equally sized pieces for each tower tray.

After a piece of the diet is placed on top of each PARC they can be stacked and held together. This is to facilitate movement and prevent excess fly escapes. For tower trays, a piece of diet material is placed on each of the trays after they are loaded with pupae and then the trays are stacked on carts. An empty tray is placed on the top of each tower unit to prevent emerging flies from escaping through the ventilator fans that are placed on top of each tower. PARCs and/or towers are then transported into the emergence areas for holding for 4 to 7 days depending on species. The fans that operate on top of each tower pull air from the bottom and

TABLE 5.2
Diet preparation

INGREDIENT	PROPORTION (%)
Water	83.79
Sugar	15.40
Agar	0.80
Preservative (Methyl Paraben)	0.01

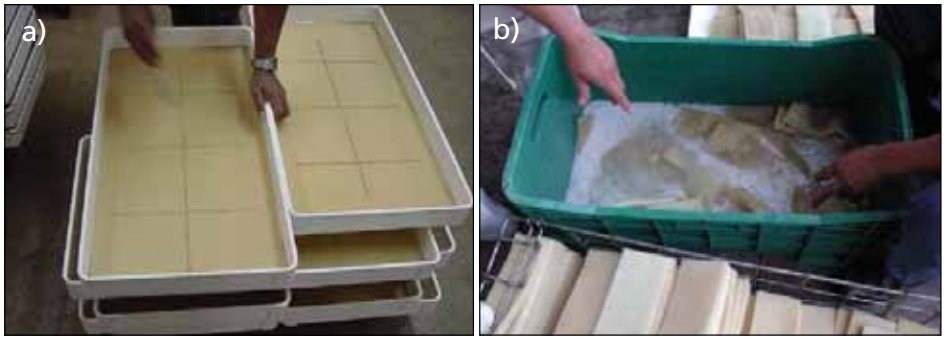

FIGURE 5.3
a) "Mubarqui" solid powder food and b) Water pillows (20 × 20 cm)

the water in the diet materials on the top and bottom trays evaporates rather quickly. To overcome this problem the food is doubled on the top and bottom 3 to 4 trays.

For the *Anastrepha* species (*A. ludens* and *A. obliqua*) a different type of food called "Mubarqui" is now in use which is based on natural protein, lipids, carbohydrates, antioxidants and fat. Ingredients are: amaranto, glasé sugar, peanut and egg. It is in a solid fine powder with a clear brown alabastro colour, according to Pantone colour guide. This food is in use in both PARC boxes and tower trays. Water supply in these emergence systems is also different, providing the flies with water in a special fabric device called "pillow" which holds the water without leaking (**Figure 5.3a and 5.3b**).

Preparation of adult food "Mubarqui" (Leal Mubarqui 2005):

- after peeling and toasting, the peanut is crushed to get granulated powder
- the peanut is incorporated to the amaranth grain and mixed for 15 minutes
- previously stirred egg is then slowly incorporated to the mixture and mixed for 20 minutes
- after 15 minutes of resting, the mixture is placed on a tray to be cooked at 220°C for 20 minutes
- the mixture is finally ground to obtain fine powder

5.2.3 Emergence and holding

Conditions for holding during development to adult emergence will vary depending on species and strain. For example, the Medfly bisexual strain is often held in darkness to lessen the mating between the early maturing flies in the PARCs. This is not necessary with the only male *tsl* strains since there are few if any females present. The length of

TABLE 5.3
Environmental conditions and periods required for holding of sterile adults in PARCs and towers

Factor	PARCs			Towers	
	West Indies fruit fly	Medfly	Mexfly	Medfly	Mexfly
Adult holding period (days)	5	4–7	5–7	4–7	5–7
Temperature range:					
(°C)	23–24	23–24	25–27	18–24	23–26
(°F)	73–75	73–75	75–80	65–75	73–78
Humidity range (%)	60–70	60–70	65–80	55–65	65–75

time required for holding varies between species (minimum of 4 days for Medfly, from 5 to 7 days for Mexfly and 5 days for West Indies fruit fly) (**Table 5.3**) (SAGARPA 1999, Tirado and Gomez-Escobar 2005).

After emergence, holding time is critical as, ideally, sterile flies should be released when they are close to reaching sexual maturity. In this way sterile males will be ready to mate immediately after release thus the use of the sterile flies is optimized. In some species such as Queensland fruit fly, reaching sexual maturity may take seven days and holding flies this long is not recommended (Meats *et al.* 2003). The number of days that the sterile flies are held before release needs to be balanced against mortality in the holding containers and in the field and mating in containers in case of the bisexual strains. Life expectancy of sterile flies in open field is known to be quite short due to predation, availability of food and other abiotic factors and also due to the fact that mass rearing conditions often inadvertently select for short-lived individuals (Cayol 2000, Hendrichs *et al.*1993 and Vreysen 2005).

5. 3 PROCEDURES FOR ADULTS PACKED IN PAPER BAGS

STEP III-b OF PROCESS IN FLOW CHART IN APPENDIX 2

5.3.1 Bagging procedures

After the shipment reaches the release centre the pupae container (e.g. plastic tray, plastic bottle, plastic bag) should be opened to break the hypoxia. The material is transferred to plastic containers and transported to the emergence/holding room. There all the paper bags have previously been prepared to receive the pupae (see Section 5.3.2).

The bags are regular Kraft paper, specifications of paper weight are usually 50g/m^2. Additional features can be added to the bag, such as containers for the pupae and structures for resting to allow emerging flies to expand their wings.

The pupae are measured volumetrically, in accordance with the amount of pupae to be poured into each bag. Since the volume is related to the pupae size and a fixed number of pupae per bag is required, confirmation must be done to assure the correct amount of pupae (FAO/IAEA/USDA 2003).

To establish the amount of pupae per bag the following must be considered:

- The capacity of the paper bag
- The historic updated QC data for emergence and fly ability
- The estimated percentage of females in case of genetic sexing strains

For example, in Argentina, the maximum volume of medfly pupae per bag is 60cc (ca. 3900 pupae per bag). The paper bags used are 40 cm height × 37.5 cm width. Inside the bag additional paper is placed which provides support to the bag and serves as resting area for the adult flies. The total surface of the paper bag is 2200 cm^2 thus the amount of pupae per cm^2 is 1.8 and roughly 1.3 adults per cm^2. In Chile, the maximum volume of Medfly pupae per bag is 65 cc (ca. 4000 pupae per bag). The total surface of the paper bags is 4085 cm^2 thus the amount of pupae per cm^2 is 1 and roughly 0.8 adults per cm^2.

The bagging process can be done either manually or mechanically. The latter is recommended for high volumes. The general bagging process consists of the following (Castellanos 1997, Reyes *et al.* 1986, SAGARPA 1999, SAG 1984, Tirado and Gomez-Escobar 2005):

- Paper bags are placed on the floor if pupal loading is done manually or on a conveyor if pupal loading is done mechanically, giving a minimum distance of 20 cm between each bag.
- Pupal loading in the paper bags is done by means of a volumetrically measured cup.
- Additional features can be used, such as containers for the pupae, structures or piece of paper to provide resting area for adults in the bag.
- Place the food mat inside the bag.
- Once the pupae are placed inside the bag, and to avoid flies escaping, bags are closed by folding and stapling the opening, taking care not to damage the material

5.3.2. Setting up for emergence

Before storing the paper bags the following conditions should be met (based on requirements for Medfly and Mexfly):

- The temperature of the emergence and holding room ranges from 20 to 24°C (± 2).
- The minimum relative humidity is 65% and should not exceed 85%.
- The emergence and holding room must be kept dark, in order to allow the flies to rest and avoid wasting energy.

For emergence and release paper bags should be handled as follows:

- Paper bags should be held in the emergence and holding room before release. In cases where there is no water provision, bags should not be kept under those conditions for more than 3 days in the case of Medflies and Queensland fruit fly. Meats *et al.* (2003) reported that holding Queensland fruit fly for 7 days resulted in low recapture rates. In the case of *Anastrepha* species (*A. ludens* and *A. obliqua*) paper bags are held for 5 days due to the longer sexual maturation period.
- Place the bags in shelves or other structures, avoid direct contact with the floor.
- Mark every bag with the date and other specification to distinguish different traits. It is recommended to mark the bags with distinctive logos and general messages for the public.
- Samples of pupae held separately are evaluated to determine the moment of the desired emergence or maturation level.
- Once the required level is reached, the bags are shipped for aerial/ground release.
- Quality control tests for recently emerged adults are conducted, including flight ability and longevity under stress.
- Coordination with the release staff is required to assure that the material is delivered when the environmental conditions for release are met.

5.3.3 Food preparation and feeding in paper bags

STEP III-c OF PROCESS IN FLOW CHART IN APPENDIX 2

Feeding the emerged adults is critical for survival and to improve competitiveness. After release sterile insects must find a food source or a host to replenish their limited energy reserves (Jacome et al 1995). In the absence of food, their life expectancy

is determined by the available initial energy reserve (Hendrichs *et al.* 1993b, Hendrichs *et al.* 1993c, Hendrichs *et al.* 1993d, Jacome *et al.* 1999). Commonly, water, sugar (for energy) and protein (to assist maturation of both sexes) are components of a food source. A wet mixture is better than dry mixtures for several reasons. Dry mixtures may contribute to dehydration of adults and decrease survival. Dry compounds also are less likely to be aromatic and less attractive to flies. Adult flies may leave the feeding areas without recognising that food is available.

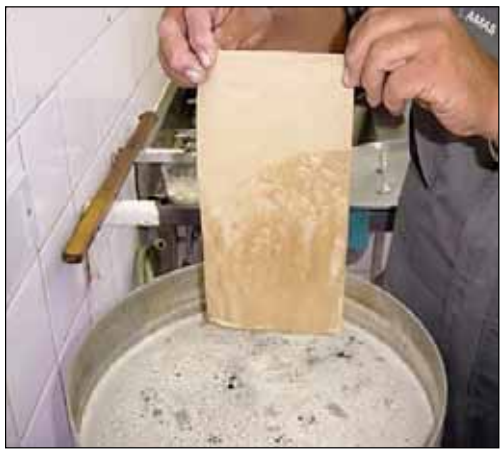

FIGURE 5.4
A food mat being prepared. This food mat is dipped into a sugar and agar solution, allowed to dry and placed in paper bags. Other systems use water and sugar, and may be painted onto the food mat. Other mechanism may be used to provide water, sugar or protein to freshly emerged adults.

Water alone may be provided by a wettex (thick cleaning cloth or similar) or together with agar. Free water often results in flies drowning and this method is discouraged. In Argentina, paper bags are gently sprayed with water one day before release (release is done 5 days after pupae have been packed). Paper bags are 55 g thus they are thick enough not to rip when sprayed with water. Sugar alone may be provided as crystal or cubes, however, crystallized sugar is likely to contribute to dehydration and is not ideal. In Chile, 2 g of wheat flour is added to 1 kg of sugar and water to provide an additional source of carbohydrates (see preparation of diet in page 26). Protein alone may be provided as autolyzed or hydrolysed protein, or yeast; other protein forms are rarely used. Autolysed protein is less attractive than hydrolysed protein, however, low pH of the mixture may alter the attractiveness (see Section 5.4.1 Nutritional supplements).

According to the currently used diet formulations, a kettle or pan is used to prepare the required diet. Either dry or "gel" diets are commonly used.

In the case of the dry diet a paper "food" mat (i.e. piece of paper impregnated with adult food) is dipped or painted in a thick sugar water solution and allowed to dry. This is placed in the bag and the emerged adults then feed on the dried sugar on the paper mat. This also increases the area for flies to stand and spread their wings (**Figure 5.4**).

In some cases paper mats are much smaller since the only function is to provide food to the newly emerged adult flies and do not function as resting area. For example, a 10 by 10 cm piece of paper food mat is used for a bag holding 2,500 adult medflies.

The materials required are:

- Paper type Kraft (not plasticized)
- Paintbrush (10 cm in width)
- Kettles/pans
- Heating unit
- Safety equipment
- Gel (agar), water, sugar, sodium benzoate

The preparation of a simple diet based on water and sugar is as follows:

- Place in a 15 litre kettle 2 parts of sugar and 1 part water boiling and stirring continuously for a few minutes. With a 15 litre capacity kettle 20 kg of sugar and 10 litres of water are used.
- With a paintbrush the liquid food is brushed on pieces of paper (2 meters length and 40 cm in width). This allows for preparation of 80 pieces of paper with food (10 cm length × 10 cm width).
- Paper with food is left to dry before it is placed on the paper bags.

The preparation of a diet based on water, sugar and agar is as follows:

"Gel" diets prepared with agar is used to provide water to the flies. Protein and energy supplements can also be added (see 5.2.4). A commonly used formulation is the following:

Water (85%)
Sugar (13.4%)
Agar (1.6%)

To prepare 50 litres of mixture the following amounts are required: water (42.5 litres), sugar (6.7 kg) and agar (0.8 kg)

Agar is added to the cold water and when completely dissolved sugar is added. The mixture is stirred and heated until boiling point and left boiling for one minute before the kettle is turned off. The sugar must be completely dissolved and the mixture most be transparent. The mixture is left to cool-down and ¾ of the piece of paper is submerged in the mixture and left to dry. The paper food mats are then placed inside the paper bags in a vertical position.

When the paper bags are closed by using staples or rubber bands, the paper food mats are fixed to the top of the bags with the staples or the rubber bands. The paper mat containing the diet should be prepared 24 hours before, to make sure it is not sticky (Castellanos 1997, Reyes *et al.* 1986, SAGARPA 1999, SAG 1994, Tirado and Gomez-Escobar 2005).

5.4 ENHANCING PERFORMANCE OF RELEASE STERILE MALES

Recent research has identified the post-production period before release, at the emergence and release facility, as suitable for manipulating sterile flies in a manner that will significantly improve their mating success in the field following release. There are three types of supplements that have been evaluated:

5.4.1 NUTRITIONAL SUPPLEMENTS

Both male and female tephritids are anautogenous, emerge as adults with undeveloped gonads, and relying on foraging during adult life to provide the proteins needed for gonadal and accessory gland development (Drew and Yuval, 2000). In addition to protein, carbohydrates must be frequently ingested to fuel metabolic activities.

Recent studies on species from several tephritid genera (*Anastrepha, Bactrocera Rhagoletis* and *Ceratitis*) indicate that providing protein nutrition to males in the days following eclosion can enhance male reproductive success. These studies have been extended to sterile male Medflies, *Ceratitis capitata*, establishing the potential for including protein

in the diet offered to sterile males in the release facility (Kaspi and Yuval, 2000), although the optimal dosage and form of presentation still needs to be established (Papadopoulos *et al.*, 1998; Shelly and Kennelly, 2002). Furthermore, recent studies indicate that several species of bacteria are common residents in the tephritid gut, and may make a significant contribution to fly fitness (Drew and Yuval, 2000; Lauzon *et al.*, 2000).

Currently, sterile males of most species are usually offered a pre-released diet of highly concentrated sucrose, presented in an agar block (Teal *et al.* 2005). The formulation and testing of optimal pre-release diets, containing sugar, protein and bacteria (and possibly other ingredients) in proportion that will result in enhanced sterile male performance in the field, are being studied and developed through the research programme of the Joint FAO/IAEA Programme.

There is some indication that protein feeding during the post teneral stage enhances male sexual competitiveness but may shorten longevity (Kaspi and Yuval 2000, Levy *et al.* in press). Additionally the ratio of sugar to protein may affect adults, however, there are no clear guidances currently available (Blay and Yuval 1997, Shelly and Kennelly 2002, Shelly and McInnis 2003). Managers should evaluate this aspect for their fruit fly species and decide on the most appropriate feeding regime for their programme.

5.4.2 Semiochemical supplements

In recent years it has been demonstrated that exposure to certain essential oils, in particular ginger root oil (GRO) and citrus peel oils, dramatically increases the mating success of male Medflies, (Barry *et al.* 2003; Katsoyanos *et al.* 2004; Katsoyanos *et al.*, 1997; McInnis *et al.* 2002; Papadopoulus *et al.*, 2001; Shelly 2001a; Shelly and McInnis 2001; Shelly *et al.* 2002, 2003). GRO exposure, which is a simple and inexpensive technique, can significantly increase the relative mating frequency of mass-reared males. This technique is being used at the Florida eclosion facility in Sarasota and at Los Alamitos in Los Angeles, site of the CDFA-USDA Medfly eclosion facility. The most effective way of applying this technique for the Medfly emergence using the tower system is to place 1 ml of GRO on a cotton wick in a small glass container (the oil eats plastic) under the tower, 24 hours prior to release (Shelly *et al.* 2004).

Ingestion of methyl eugenol (ME) by Oriental fruit fly (*Bactrocera dorsalis*) improves the mating competitiveness of males by at least three fold when compared with ME deprived males (Shelly 2001b, Tan 2000). It is envisaged that providing sterile males with a source of ME to feed on before release will place them on at least an even playing field against wild males, thereby potentially reducing the number or frequency of sterile males released. Feeding on ME significantly reduces male response to ME in male annihilation traps, thus potentially allowing simultaneous application of the SIT and male annihilation methods.

Similarly, it has been demonstrated that exposure of Oriental fruit fly (*Bactrocera dorsalis*) to artificial or natural sources of methyl eugenol, enhances male competitiveness. However, this technique has not yet been routinely applied in large-scale operational programmes.

5.4.3 Hormonal supplements

Age is a significant factor affecting sexual signalling and reproduction in numerous tephritid species. For example, members of the *Anastrepha* genus typically require

between two and three weeks to become sexually mature. Although mass rearing results in selection of strains which become sexually mature much earlier than wild flies, the most rapidly developing strains of *A. suspensa* and *A. ludens* still require more than 7 days to become sexually mature. This delay between adult emergence and sexual maturity poses a significant problem for SIT programmes because males must be held for a longer period of time prior to release, or have to be released before becoming sexually mature, resulting in fewer surviving to maturity and copulation.

Clearly, development of cost effective methods to accelerate sexual maturity in released flies would have a significant positive impact on the efficacy of the SIT. Effects of juvenile hormones (JH) on the reproductive behaviour of some species of fruit flies including Mexfly and Medfly have been studied (Teal *et al.* 2000). Mimics of JH including fenoxicarb and methoprene accelerate the reproductive behaviour of treated males by beginning the calling and mating behaviour four days before untreated flies. The reduction of the time for the beginning of sexual calling behaviour in released sterile flies allows the released sterile males to be ready to copulate at the moment of the release. Females mated with JH treated males produce the same quality and quantity of eggs as females mated with untreated males (Teal and Gomez-Simuta 2002, Gomez-Simuta and Teal, in preparation). It has been shown that effects of methoprene are optimal when as little as 0.05% (active ingredient) is incorporated in the adult diet. This coupled with the relatively low cost of methoprene in a water-soluble formulation, indicates that incorporation of hormone supplements into adult emergence procedures may be a cost-effective way to improve the efficiency of SIT. Currently, work is focusing on evaluating methodologies for practical use of these products in large-scale operational programmes.

5.5 REFERENCES CITED

Barry, J.D., T.E. Shelly, D.O. McInnis, and J. G. Morse. 2003. Potential for reducing overflooding ratios of sterile Mediterranean fruit flies (Diptera: Tephritidae) with the use of ginger root oil. Florida Entomologist 86: 29–33.

Blay, S., and Yuval, B. 1997. Nutritional correlates to reproductive success of male Mediterranean fruit flies. Animal Behaviour 54:59–66.

Castellanos, D. 1997. Aplicación del combate autocida contra mosca del mediterráneo (*Ceratitis capitata* Wied.), 425–450 pp. *En* Memorias del Curso Regional Sobre Moscas de la Fruta y su Control en Areas Grandes con Enfasis en la Técnica del Insecto Estéril. Programa Mosca de Mediterráneo-SAGAR y Organismo Internacional de Energía Atómica. Metapa de Dominguez, Chiapas, México.

Cayol, J. P. 2000. Changes in sexual behaviour and life history traits of tephritid species caused by mass rearing processes, pp. 843–860. *In* M. Aluja and A.L. Norbom (eds.), Fruit flies (Tephritidae): Phylogeny and Evolution of Behaviour. CRC Press, Boca Raton, FL, USA.

Drew, R. A. I., and B. Yuval. 2000. The evolution of fruit fly feeding behaviour, pp. 731–749. *In* M. Aluja and A. L. Norrbom (eds), Fruit Flies (Tephritidae): Phylogeny and Evolution of Behaviour. CRC Press, Boca Raton, FL, USA.

FAO/IAEA/USDA. 2003. Manual for product quality control and shipping procedures for sterile mass-reared tephritid fruit flies, Version 5.0. International Atomic Energy Agency. Vienna, Austria. 85 pp.

Gomez-Simuta, Y., and P. Teal. Incorporating juvenile hormones therapy in the fruit fly mass rearing process to accelerate reproductive development of mass rearing insect to enhancing SIT effectiveness. (in preparation)

Hendrichs, J., V. Wornoayporn, B. I. Katsoyanos, and K. Gaggl. 1993. First field assessment of the dispersal and survival of mass reared sterile Mediterranean fruit

fly of an embryonal temperature sensitive genetic sexing strain, pp. 453–462. *In* proceedings: management of Insect Pest: Nuclear and related Molecular and Genetic Techniques. FAO/IAEA International Symposium, 19–23 October 1992, Vienna, Austria. STI/PUB/909. IAEA, Vienna, Austria.

Jacome, I., M. Aluja, and P. Liedo. 1999. Impact of adult diet on demographic and population parameters of the tropical fruit fly *Anastrepha serpentina* (Diptera: Tephritidae). Bulletin of Entomological Research, 89: 165–175.

Jacome, I., M. Aluja, P. Liedo, and D. Nestel. 1995. The influence of adult diet and age on lipid reserves in the tropical fruit fly *Anastrepha serpentina* (Diptera: Tephritidae). Journal of Insect Physiology 41: 1079–1086.

Kaspi, R., and Yuval, B. 2000. Post-teneral protein feeding improves sexual competitiveness but reduced longevity of mass-reared male Mediterranean fruit flies (Diptera: Tephritidae). Annals of the Entomological Society of America 93: 949–955.

Katsoyanos B. I., N.T. Papadopoulos, N. A. Kouloussis, and J. Hendrichs. 2004. Effect of citrus peel substances on male Mediterranean fruit fly behaviour. pp 13–17. *In* Proceeding of 6th International Fruit Fly Symposium 6–10 May 2002, Stellenbosch, South Africa Book.

Katsoyanos B. I., N. A. Kouloussis, and N.T. Papadopoulos. 1997. Response of *Ceratitis capitata* to citrus chemicals under semi-natural conditions. Entomologia Experimentalis et Applicata 82: 181–188.

Leal Mubarqui, R. 2005. Manual de operación del método "MUBARQUI". Mexico.

Levy, K., T. E. Shelly, and B. Yuval. 2005. Effects of the olfactory environment and nutrition on the ability of male Mediterranean fruit flies to endure starvation. Journal of Economic Entomology 98: 61–65.

Lauzon, C.R., R.E. Sjogren, and R. J. Prokopy. 2000. Enzymatic capabilities of bacteria associated with apple maggot flies: A postulated role in attraction. Journal of Chemical Ecology 26: 953–967.

Meats, A. W., R. Duthie, A. D. Clift, and B. C. Dominiak. 2003. Trials on variants of the Sterile Insect Technique (SIT) for suppression of populations of the Queensland Fruit fly in small towns neighbouring a quarantine (exclusion) zone. Australian Journal of Experimental Agriculture 43: 389–395.

McInnis, D.O., T.E. Shelly, and J. Komatsu. 2002. Improving male mating competitveness and survival in the field for medfly, *Ceratitis capitata* (Diptera: Tephritidae) SIT programs. Genetica 116: 117–124.

Papadopoulus N.T., B.I. Katsoyanos, N.A. Kouloussis, A. P. Economopoulos, and J. R. Carey. 1998. Effect of adult age, food and time of day on sexual calling incidence of wild and mass-reared *Ceratitis capitata* males. Entomologia Experimentalis et Applicata 89: 175–182.

Papadopoulus N.T., B.I. Katsoyanos, N.A. Kouloussis, and J. Hendrichs. 2001. Effect of organge peel substances on mating competitiveness of male *Ceratitis capitata*. Entomologia Experimentalis et Applicata 99: 25–261.

Programa Regional Moscamed Guatemala-Mexico-Estados Unidos. 2002. Manual de control autocida de la mosca del mediterraneo esteril por el sistema de adulto frio. Guatemala, Guatemala.

Reyes J., A. Villaseñor, G. Ortiz, and P. Liedo. 1986. Manual de las operaciones de campo en una campaña de erradicación de la mosca del mediterráneo en regiones tropicales y subtropicales, utilizando la técnica del insecto estéril. Moscamed Programme SAGARPA-USDA.

(SAGARPA) Secretaria de Agricultura, Ganaderia, Desarrollo Rural, Pesca y Alimentacion. 1999. Norma Oficial Mexicana NOM-023-FITO-1995. Apéndice técnico para las operaciones de campo de la campaña nacional contra moscas de la fruta. February 1999, Government of Mexico.

(SAG) Servicio Agrícola y Ganadero. 1984. Manual práctico de dispersión de moscas estériles del Mediterráneo TIE. SAG Proyecto 335 Moscas de da Fruta, I Región. Editado por: Rafael Mata Pereira Programa Moscamed, Guatemala y Jaime Godoy M., SAG, Chile. Arica, Chile.

Shelly, T. E. 2001a. Exposure to α-copaene and α-copaene-containing oils enhances mating success of male Mediterranean fruit flies (Diptera: Tephritidae). Annals of the Entomological Society of America 94: 497–502.

Shelly, T. E. 2001b. Feeding on Methyl Eugenol and *Fagraea berteriana* flowers increases long-range female attraction by males of the Oriental fruit fly (Diptera: Tephritidae). Florida Entomologist 84: 634–640.

Shelly, T. E., and D. O. McInnis. 2001. Exposure to ginger root oil enhances mating success of irradiated, mass-reared males of Mediterranean fruit fly (Diptera: Tephritidae). Journal of Economical Entomology 94: 1413–1418.

Shelly, T. E., and S. S. Kennelly. 2002. Starvation and the mating success of wild Mediterranean fruit flies (Diptera: Tephritidae). Journal of Insect Behaviour 16 (2): 171–179.

Shelly, T. E., A. S. Robinson, C. Caceres, V. Wornoayporn, and A. Islam. 2002. Exposure to ginger root oil enhances mating success of male Mediterranean fruit flies (Diptera: Tephritidae) from a genetic sexing strain. Florida Entomologist 85: 440–45.

Shelly, T. E., P. Rendon, E. Hernandez, S. Salgado, D. O. McInnis, E. Villalobos, and P. Liedo. 2003. Effects of diet, ginger root oil, and elevation on the mating competitiveness of male Mediterranean fruit flies (Diptera: Tephritidae) from a mass-reared, genetic sexing strain in Guatemala. Journal of Economical Entomology 96: 1132–1141.

Shelly, T. E., and D.O. McInnis. 2003. Influence of adult diet on the mating success and survival of male Mediterranean fruit flies (Diptera: Tephritidae) from two mass-rearing strains on field-caged host trees. Florida Entomologist 86: 340–344.

Shelly, T.E., D.O. McInnis, E. Pahio, and J. Edu. 2004. Aromatherapy in the Mediterranean fruit fly (Diptera: Tephritidae): sterile males exposed to ginger root oil in pre-release storage boxes display increased mating competitiveness in field-cage trials. Journal of Economical Entomology 97: 846–853.

Tan, K.H. 2000. Behaviour and chemical ecology of *Bactrocera* flies. pp. 647–656. *In* K. H. Tan (ed.), Area-Wide Control of Fruit Flies and Other Insect Pests. Universiti Sains Malaysia Press, Penang, Malaysia.

Teal P. E. A., Y. Gomez-Simuta, and A. T. Proveaux. 2000. Mating experience and juvenile hormone enhance sexual signalling and mating in male Caribbean fruit flies. Proceedings of the National Academy of Sciences of the United States of America 97: 3708–3712.

Teal P. E. A., and Y. Gomez-Simuta. 2002. Juvenile hormone: Action in regulation of sexual maturity in Caribbean fruit flies and potential use in improving efficacy of sterile insect control technique for Tephritid fruit flies, pp. 157–166. *In*: Witzgakll, P., Mazomenos, B. and katsoyanos (Ed.), Pheromone and Other Biological Techniques For Insect Control in Orchards and Vineyards. International Organization of Biological Control/West Palearctic Region Section. (IOBC WPRS) Bulletin.

Teal P. E. A., J. M. Gavilanez-Sloan and B. D. Dueben. 2004. Effects of sucrose in adult diet on mortality of males of the Caribbean fruit fly, Anastrepha suspense (Loew). Florida Entomologist 87: 487–491.

Tirado, P. L., and E. Gomez-Escobar. 2005. Prácticas de empaque y liberación de moscas y parasitoides (Anexo 3, 3 p), *En* Memorias del XVI Curso Internacional Sobre Moscas de la Fruta. Programa Mosca de Mediterráneo DGSV-SENASICA-SAGARPA. Metapa de Domínguez, Chiapas, México.