Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

# Guideline for packing, shipping, holding and release of sterile flies in area-wide fruit fly control programmes

Second edition

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Edited by

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# Foreword

The International Plant Protection Convention (IPPC) is the international treaty under which the International Standards for Phytosanitary Measures (ISPMs) are adopted. ISPM No. 3, "*Guidelines for the Export, Shipment, Import, and Release of Biological Control Agents and Other Beneficial Organisms*", adopted in 2005, includes sterile insects among *beneficial* organisms. This development and the increasing use and transboundary shipment of sterile insects led to the demand for harmonized and optimized technologies for all post-mass-rearing processes related to the application of the Sterile Insect Technique (SIT), from pupal shipment, to fly emergence through to insect release. This document is a compilation of the standardized processes currently used in most of the fruit fly SIT applications world-wide.

This 2nd edition of this guideline is an updated version of the original FAO/IAEA guideline published by FAO in 2007. This new version was also prepared by a group of experts most of which work in area-wide Integrated Pest Management programmes that incorporate an SIT component. The latest innovations resulting from research and development and validated during the practical application of SIT technology were all included in this updated guideline.

The review was thorough and included updates of most chapters and topics such as new methods to estimate sterile to fertile fly ratios and densities, novel packing, holding and release equipment and procedures, a much expanded GIS chapter, as well as updated figures, tables and references to recent relevant literature. The previous Chapter 11 (post-irradiation quality control) and 12 (identification of recaptured sterile and fertile flies) were deleted, since specific guidelines are available covering these topics.

The majority of the procedures described in this guideline were initially designed specifically for the Mediterranean fruit fly *Ceratitis capitata* (Wied.). Nevertheless, these can be applied with some modifications to other tephritid pest species of the genera *Anastrepha*, *Bactrocera* and *Dacus*. The guideline is designed to be a working document, subject to periodic reviews and updates based on new developments in SIT technology. Future editions will endeavour to include more specific recommendations for other species of fruit flies as the relevant data become available.

The procedures described will help ensure that released sterile fruit flies be of the best possible quality and that the sterile fly release densities are adequate to the needs of area-wide SIT programmes. This guideline is aimed at quickly identifying and correcting problems affecting the effectiveness of operational programmes, resulting from less than optimal emergence, handling, and release conditions that have negative impacts on the sterile fly quality.

This guideline is aimed at facilitating the transfer of harmonized and state of the art technology to FAO and IAEA Member States that want to embark on area-wide action programmes that use SIT. There is also increased interest by the private sector in investing in sterile insect production, packing and release. This harmonized guideline that cover the post-production phase will facilitate SIT application and foster its commercialisation.

The officers of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture responsible for this updated version were Walther R. Enkerlin, J. Reyes and J. Hendrichs.

# 1. INTRODUCTION

The Sterile Insect Technique (SIT) relies on the sustained and area-wide release of large numbers of sterile insects to allow them to compete for matings with wild individuals of a specific target pest population and reduce its reproductive potential. Prior to their release, sterile insects are shipped, emerged from the puparia, fed and matured, and then loaded into delivery vehicles for aerial or ground releases. The conditions under which these activities are conducted are as relevant to the overall success of SIT activities as is the production of a high quality sterile insect.

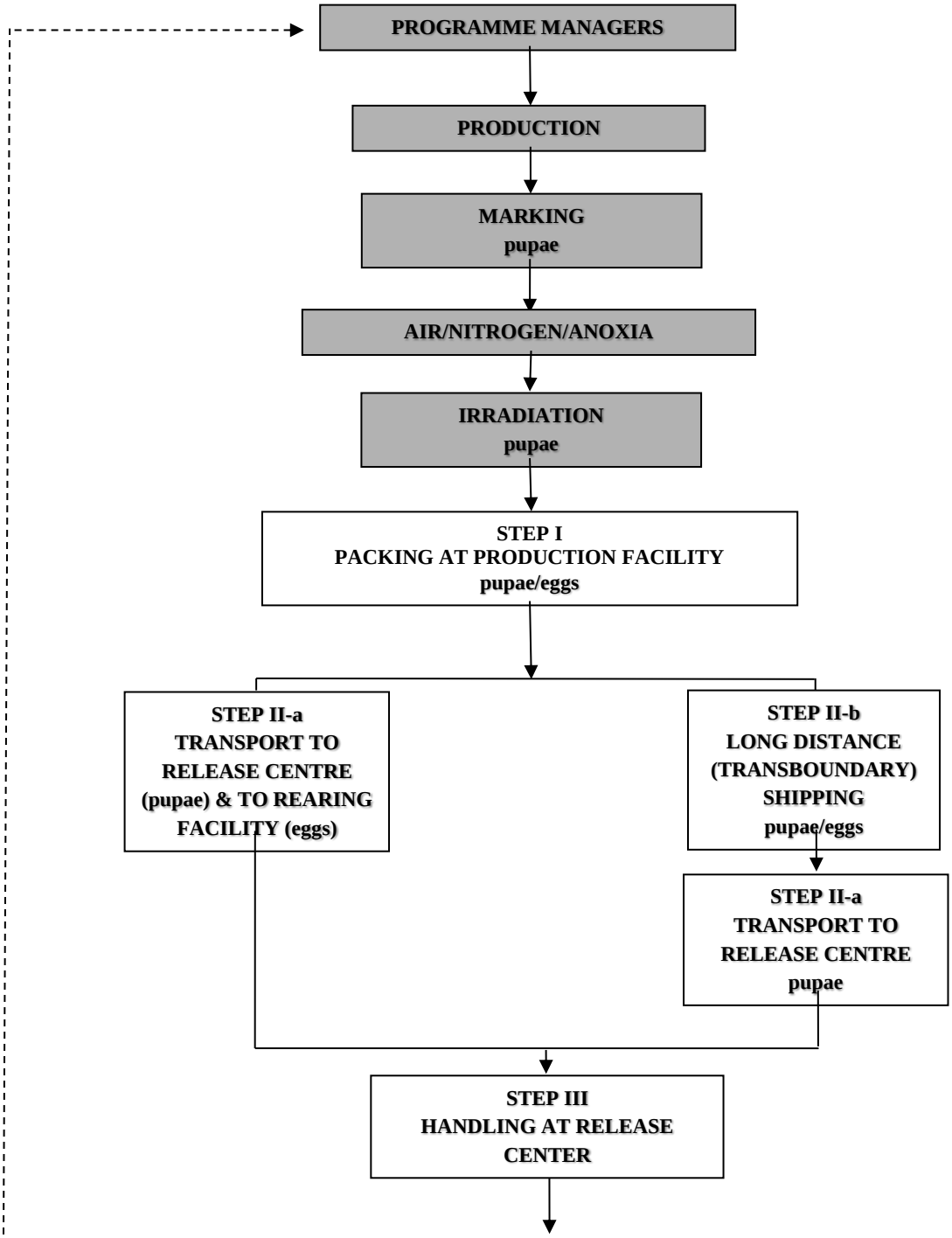
Successful area-wide (AW) Integrated Pest Management (IPM) programmes that incorporate the SIT have over several decades produced valuable information that has been useful to develop operational guidelines. Interactions and information exchange among operational programmes has been useful in advancing SIT procedures and technology. Researchers have also contributed by developing more effective SIT technology which has made SIT application more viable. This guideline has been prepared by people working in action programs that apply the SIT, as well as by researchers working on this very specific topic (see List of Contributors, **Appendix 1**).

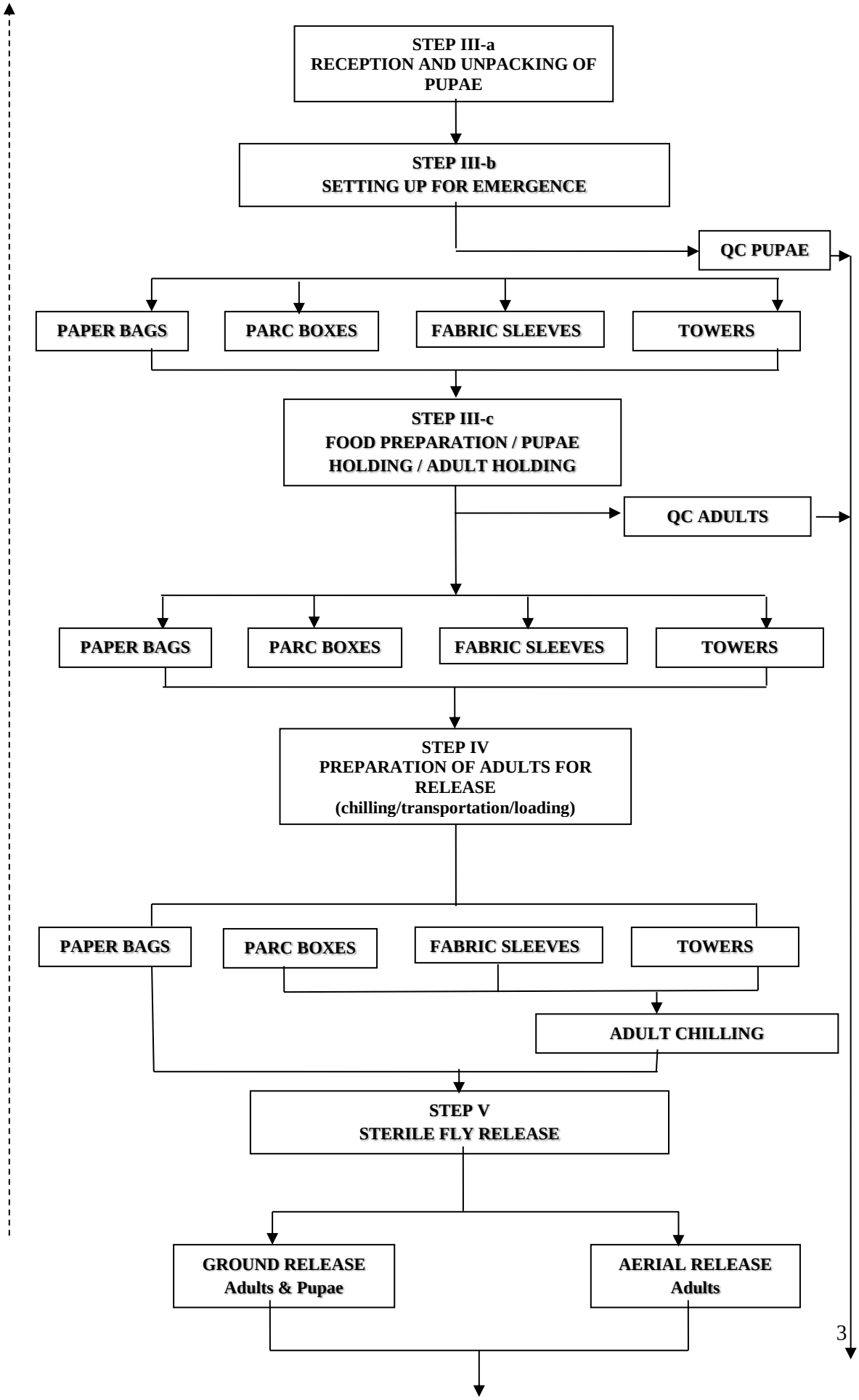
The SIT component of an operational programme can be clearly divided into two main areas of activity: 1) The mass rearing and sterilization through irradiation prior to insect transport and release, and 2) The post-production processes, involving the packing, shipping, handling, emergence, feeding, holding and release of sterile flies.

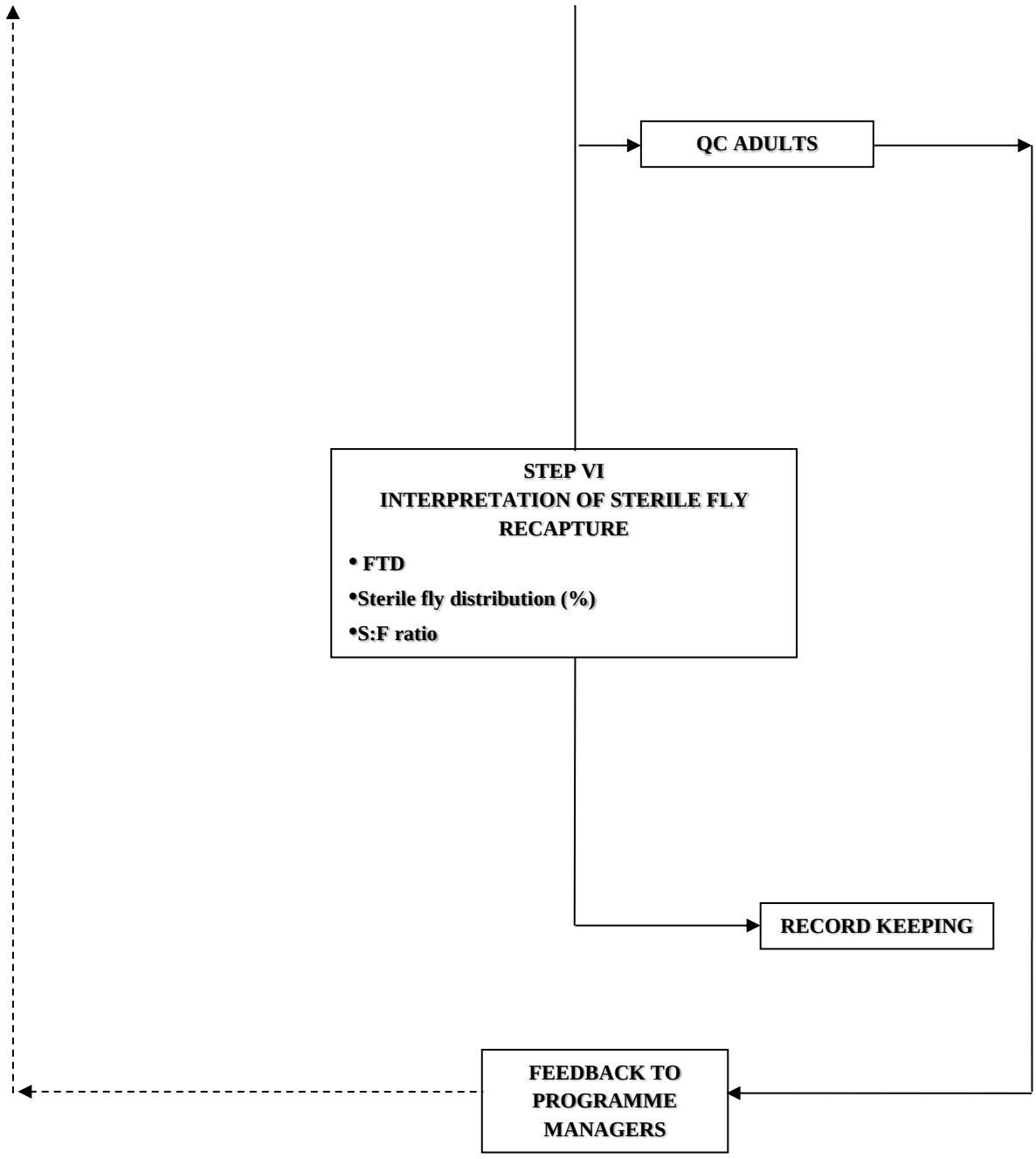
The post-production process is different from the mass rearing procedures. Generally, insects are handled in smaller batches and the focus is on the adult stage. Adults have entirely different demands for space and movement compared with life stages of the insect in the production facility and are generally held for shorter periods (several days) compared with weeks at the production facility. For this reason specific quality control tests are applied to the processes and to the product aimed at assuring high quality of sterile released insects. The product (sterile flies) quality control tests can be found in "Product Quality Control for Sterile Mass-Reared and Released Tephritid Fruit Flies, Version 6.0. Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture. IAEA, Vienna, Austria (2014)". http://www-naweb.iaea.org/nafa/ipc/public/sterile-mass-reared-v6.pdf

The procedures in this guideline are presented following a logical flow of the post-production processes, from packing after pupal irradiation to field release of sterile flies, as follows:

# FLOW CHART OF STERILE FLY RELEASE PROCESS




STEP III-a
RECEPTION AND UNPACKING OF PUPAE
STEP III-b
SETTING UP FOR EMERGENCE
QC PUPAE
PAPER BAGS
PARC BOXES
FABRIC SLEEVES
TOWERS
STEP III-c
FOOD PREPARATION / PUPAE HOLDING / ADULT HOLDING
QC ADULTS
PAPER BAGS
PARC BOXES
FABRIC SLEEVES
TOWERS
STEP IV
PREPARATION OF ADULTS FOR RELEASE
(chilling/transportation/loading)
PAPER BAGS
PARC BOXES
FABRIC SLEEVES
TOWERS
ADULT CHILLING
STEP V
STERILE FLY RELEASE
GROUND RELEASE
Adults & Pupae
AERIAL RELEASE
Adults


QC ADULTS
STEP VI
INTERPRETATION OF STERILE FLY RECAPTURE
• FTD
•Sterile fly distribution (%)
•S:F ratio
RECORD KEEPING
FEEDBACK TO PROGRAMME MANAGERS

# 2. PACKING AT MASS REARING FACILITY

*STEP I OF PROCESS IN FLOW CHART*

After irradiation has been carried out, sterile pupae should be adequately packed for transportation to the fly emergence and release (fly emergence) centre. Packing procedures for short and long distance transportation, including transboundary shipment, may vary as described below (Zavala et al. 1985, FAO/IAEA 2000, FAO/IAEA/USDA 2014 and **Appendix 4** this publication). Size and weight of packages are designed to minimize breakage.

### 2.1 Plastic Bottles

Sealed bottles should only be used for short-distance transport of irradiated pupae to a local fly release (fly emergence) centre (**Figure 2.1**), as it was used in Mexico. Air-conditioned or refrigerated vehicles are used for the transport; no additional packing or insulating material is required around the bottles. Plastic containers should be placed on the deck of the vehicle with proper brace stabilizer materials, to avoid excess movement.

In the case of vehicles without air conditioning, plastic bottles could be placed inside an insulated box and cooling units (hydrogel) to keep appropriate temperature (16 – 20°C).

**Figure 2.1** Plastic containers used to sterilize and transport medfly pupae in Mexico. (©FAO/Programa Moscamed Mexico, Guatemala, USA)

### 2.2 Cardboard Boxes

Polyethylene bags containing sterile pupae are loaded into secure cardboard shipping boxes for longer distance transportation to release centres. As an example, the shipping box used to hold the 4-litre bags of pupae that fit into the canisters of Hussmann irradiators is constructed of double-walled corrugated cardboard of 74 x 34 x 34 cm with a top and bottom full overlap. Inside the box, a central compartment, 46 cm long, is lined with additional layers of corrugated cardboard. Nine bags of pupae are placed lengthwise within this central compartment in three layers of three bags each. Layers, as well as bags within a layer, are separated by spacers of double- and single-wall, respectively, corrugated cardboard. The space remaining at either end of the box (≈10 cm of the

length of the box) is used to hold cooling units. These can be cooling units (hydrogel) prepared at the packing facilities, or using two packs of "blue ice", wrapped in newspaper (**Figure 2.2a**).

According to the capacity of the cardboard box, temperature must be kept at 16 – 20°C. In Australia 2-litre bags of pupae are placed in a cardboard carton, with ten of these cartons in a Styrofoam box (**Figure 2.2b**). In Argentina, a cardboard box of 42,5 x 33 x 27 cm and a Styrofoam box inside with 7 plastic bags of 2.8 l. pupae per bag is used (FAO/IAEA/USDA. 2014).

Once full, a box is sealed with carton staples (placing staples in locations where they will not hit the bags of pupae) and two bands of fibre-reinforced plastic adhesive tape (**Figure 2.3**).

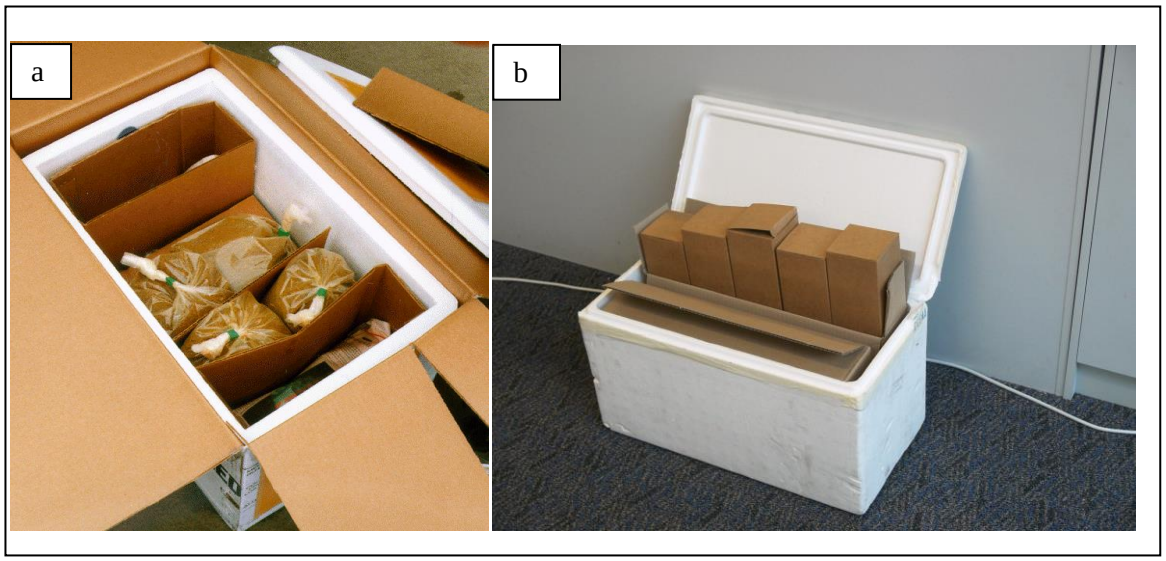



**Figure 2.2** a) Inside view of a box used to ship sterile Medfly pupae from Guatemala Moscamed rearing facility, b) Inside view of a box used to ship Queensland fruit fly in Australia. (©FAO/Programa Moscamed Mexico, Guatemala, USA/Queensland Fruit Fly Program Australia)


**Figure 2.3** Sealed boxes used for shipping sterile medfly pupae from Guatemala Moscamed rearing facility. (©FAO/Programa Moscamed Mexico, Guatemala, USA)

## 2.3 Plastic Baskets

The pupae are packed on plastic bags with very low levels of oxygen (hypoxia), to stop its metabolism and development during transportation. The bag dimensions are 15.3 x 7.3 x 46 cm and can contain around 300,000 pupae (equivalent to 5.0 liters of pupae or 2.5 kilograms depending on the size and weight of the pupae). Those bags are transported in air-conditioned or refrigerated vehicle from El Pino Production Facility in Guatemala to the medfly packing and release center located 200 km from the facility, on plastic baskets each one with dimensions of 39.5 x 60 x 20 cm. Four bags are placed in each basket.

## 2.4 Labelling

All boxes are properly labelled with the words: "Fragile" and/or "Biological Material". The words "Live Sterile Insects" and indication of the storage conditions ("This Side Up", "Handle with Care", "Keep Cool" or "Do not leave in the sun") should also be present on the boxes **(Figure 2.4).**

These words should be adopted as a standard for those programmes using SIT. Note the words "Keep Refrigerated Do Not Freeze" is misleading and should therefore not be used, since as mentioned in Section 3.1, the boxes should not be held at temperature below 20°C, except for certain specific conditions where 16 to 18 °C may be used.

To facilitate tracking of consignments, these should have complete information on the location of the addressee and a shipment number. Additionally boxes for each shipment have to be numbered consecutively in large, clear writing on the outside of the box, e.g. "Shipment 18, Box 3 of 24" (FAO/IAEA/USDA 2014).


**Figure 2.4** Three labels placed on boxes containing sterile medfly pupae shipped from Argentina (Mendoza rearing facility) to Spain (region of Valencia). (©FAO/ISCAMEN)

Every box should include specific forms with detailed shipment information including: Litres of pupae, collection number, basic quality control parameters (e.g. pupae weight, pupae/litre). The forms should include the corresponding supervisor signature for the different control points (i.e. irradiation, transportation, reception, quality control). Pupae containers (bags/bottles) must include radiation indicators inside the container. Containers should be sealed before irradiation, in order to ensure integrity. This procedure as a whole will assure traceability of the pupae container.

### 2.5. References

**FAO/IAEA/USDA. 2014.** Product quality control for sterile mass-reared and released tephritid fruit flies. Version 6.0. IAEA, Vienna, Austria.

**FAO/IAEA. 2000.** Gafchromic® Dosimetry System for SIT, Standard Operating Procedure. Joint FAO/IAEA, Division of Nuclear Techniques in Food and Agriculture. Vienna, Austria, 42 pp.

**FAO/IAEA/USDA. 2014.** Product quality control for sterile mass-reared and released tephritid fruit flies. Version 6.0. IAEA, Vienna, Austria.

**Zavala, J. L., M. M. Fierro, A. J. Schwarz, D. H. Orozco, and M. Guerra. 1985.**Dosimetry practice for the irradiation of the Mediterranean fruit fly *Ceratitis capitata* (Wied.). 23-30. *In* IAEA [ed.], High dose dosimetry, Proceedings of the International Symposium, STI/PUB/671.IAEA. Vienna, Austria.

# 3. TRANSPORTATION TO EMERGENCE AND RELEASE CENTRE (PUPAE) AND REARING FACILITY (EGGS)

*STEP II-a OF PROCESS IN FLOW CHART*

### 3.1. Pupae

During transport, boxes containing pupae should not be handled roughly or be subjected to excessive stacking and compacting to prevent accumulation of unwanted levels of metabolic heat. Post irradiation pupae are sensitive to excessive vibration: James (1993) reported that five hours transport in ambient temperatures with vibration resulted in up to 100% mortality in consignments. Excessive vibration during transport may also dislodge some dye from pupal cases, and dye is critical to the identification of sterile flies caught in traps.

Prior to shipping and during transit, sealed boxes should be placed in secure and clean facilities to avoid risk of carrying pests resulting in contaminated shipments (hitch-hikers).

Ideally, boxes of pupae should be held at or slightly below 20° C during transportation. In some instances, for example, pupae shipped from El Pino Guatemala to Florida USA, to compensate for high outside temperature and relative humidity, temperature during transportation is handled at 16 to 18°C. In Mexico, due to the short distance between the mass rearing and the packing facilities, cardboard-boxes are transported in refrigerated vehicles, at a temperature of 18-20$^{o}$C without "blue ice" (SAGARPA-SENASICA, 2013). Pupae should be treated as a perishable product, keeping it all the time under a "cold chain" approach. In all cases, the pupae containers inside the boxes must not be held below 0° C or spend more than a few minutes at temperatures above 30° C. Prolonged exposure to direct sunlight, could raise internal temperatures above 30° C. Data loggers should be placed inside the containers in order to record minimum and maximum temperatures during transport. For short distance transportation, air-conditioned or refrigerated vans should be used if ambient conditions are likely to result in overheating of pupae. The personnel in charge of shipping, movement and unshipping have to be trained and permanent supervision of the pupae is required.

The supervisor should complete a datasheet with the specifications and conditions of the sterile pupae being shipped. The minimum information that the datasheet should contain is shown in **Appendix 3**. The datasheet should be signed by the supervisor and a copy should always accompany the consignment. The supervisor should also file a copy of each of the documents (see Section 4.2.5) which accompany the consignment regardless of the destination (i.e. national or international).

#### 3.1.1 Process control

Upon arrival at final destination the consignment has been cleared by the national phytosanitary and customs authorities. The receiver must carefully check the datasheet that accompanies the consignment and verify: 1) that the datasheet has been signed by the shipper, and 2) that the content of the package matches the information reported on the datasheet. It is compulsory to verify the condition of the irradiation indicators attached to each pupae container. The indicators must clearly show that they have been exposed to the specified absorbed irradiation dose as explained in the Manual for Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies (FAO/IAEA/USDA 2014).

The receiver must then sign a statement that the product has been received according to specifications. Any discrepancy on the consignment content should immediately be reported to the shipper and a decision on keeping or discarding the consignment should be made immediately. Any visual sign on the indicators of inadequate pupae irradiation is sufficient to safely dispose of the whole consignment.

### 3.2 Egg Shipment for Medfly Genetic Sexing Strain (GSS-*tsl)*

Efficiencies in mass rearing can be obtained by using procedures to ship eggs from a main production facility (mother stock) to satellite rearing facilities that do not need to invest in maintaining large adult colonies and filter rearing systems (Fisher and Caceres 2000, Caceres et al. 2000). This enables a central production facility to supply eggs to satellite centres that produce only males for irradiation and release (Cáceres et al. 2007a and b, Mamán and Cáceres 2007).

#### 3.2.1 Handling, packing and transportation procedures

Medfly eggs (embryos) from genetic sexing strains using the *tsl* mutation are sensitive either to cold storage or high temperature treatment (to kill females) during the first 24 hour of embryo development. To avoid damage during egg transport, eggs collected up to 12 hours after oviposition, should be dipped in a chlorine solution (200 ppm) for 10 minutes or just rinsing the eggs with water and then bubbled in a water bath at 24$^{o}$C for 24 hours. Eggs for male only production should be incubated for an additional period of 12 hours, at 34 $^{o}$C, to kill female embryos. Embryos, either for colony or male only production, should then be mixed with either pre cooled water or agar solution (0.1- 0.2%) at 5$^{o}$C and stored in the appropriate container for transportation.

It has been demonstrated that eggs collected up to 12 hours after oviposition and pre incubated at 23-25ºC (pre-treatment), for 12 hours and then stored between 5-15$^{\circ}$C (transportation) for up to 72 hours, provide a suitable window for shipment.

Under these conditions, no significant reductions in egg viability and egg to adult survival were observed.

##### 3.2.1.1 Containers

The size and shape of the packaging container are typically a function of the quantity of eggs and the transportation time:

a) Plastic bags: For short transportation time between 24 - 48 hours, 0.5 to 1 liter of egg solution (1 vol eggs: 1 vol transportation medium) are sealed within polyethylene “Ziploc” bags that are ca 1.5 mil thick (mil is one thousandth of an inch = 0.0254 mm). Bags are placed in insulated shipping boxes that contain frozen hydrogel to maintain the temperature between 5 to 15$^{o}$C during transportation. Internal shelving should be placed inside the insulated shipping container to reduce possible damage to bags during the transportation. Size and weight of these packages are designed to minimize breakage. Transportation time should be as short as possible and should not exceed 48 hours.

b) Thermos: Either sealed insulated metal or plastic bottles should only be used for long-distance transport of eggs. Eggs are mixed with (0.1-0.2%) agar solution in 1:1 ratio (vol/vol) to avoid of the sedimentation and damage of eggs during the transport. The thermos is filled with 0.5 liter of eggs and agar solution. The flask should be maintained at room temperature during transportation. Shipment time should be as short as possible and should not exceed 72 hours.

c) Shipping boxes: Thermos flask or plastic bags inside insulated boxes are loaded into cardboard boxes. Size and weight of packages are designed to minimize breakage.

#### 3.2.1.2 Labeling

Shipping boxes should use the "universal" labeling, indicating presence of living material within the box as well as providing the information about proper maintenance and handling of the boxes. Boxes should be labeled as "Fragile" and "Keep cool do not refrigerate". The shipment should be provided with the information on the origin of eggs, their age and whether they were heat-treated or not (see Section 2.3).

### 3.2.2 Eggs processing after transportation

Thermos flasks or plastic bags should be carefully opened after delivery to the end-user and the temperature of the contents should be gradually increased to room temperature. Subsequently, depending on its microbial load, eggs could be re-rinsed in a chlorine solution at 200 PPM for 10 minutes and dipped several times with tap water of appropriate temperature, then mixed with water (1 egg : 20 water vol/vol ratio) and transferred and seeded onto diet in larval trays. In some cases, depending on embryos development, eggs are bubbled for around 6 to 12 hours at 34$^{o}$C to allow the embryos to finish their development.

### 3.2.3 Process control

After arrival at the production facility the temperature of the egg solution should be determined after opening the thermos or bags. In addition, information should be retrieved from the data logger placed together with the egg solution before and after the thermal treatment and inside transportation container to record temperature during pre and post shipment steps. A sample of 300 eggs should be taken from each batch of egg to determine egg viability to be compared with the control kept at the egg production facility.

## 3.3. References


**Cáceres, C., E. Ramírez, V. Wornoayporn, S. M. Islam, and S. Ahmad. 2007a.** A protocol for storage and long-distance shipment of Mediterranean fruit fly (Diptera: Tephritidae) eggs. I. Effect of temperature, embryo age and storage time on survival and quality. Florida Entomologist 90: 103-109.

**Cáceres C., D. Mcinnis, T. Shelly, E. Jang, A. Robinson, and J. Hendrichs. 2007b.** Quality management systems for fruit fly (Diptera: Tephritidae) Sterile Insect Technique. Florida Entomologist 90: 1-9.

**FAO/IAEA/USDA. 2014**. Product quality control for sterile mass-reared and released tephritid fruit flies. Version 6.0. International Atomic Energy Agency. Vienna, Austria. 159 pp.

**Mamán, E., and C. Cáceres. 2007**. A protocol for storage and long-distance shipment of Mediterranean fruit fly (Diptera: Tephritidae) eggs. II. Assessment of the optimal temperature and substrate for male-only production. Florida Entomologist 90: 110-114.

**SAGARPA-SENASICA. 2013.** Manual de procedimientos de empaque y colecta del Centro de Empaque de Mosca del Mediterráneo Estéril (CEMM) del Programa Moscamed, México. 41 pp.

# 4. LONG DISTANCE (TRANSBOUNDARY) SHIPMENT

*STEP II-b OF PROCESS IN FLOW CHART*

Transboundary shipment of sterile insects has taken place on a regular basis since the SIT was first developed. The total number of sterile insects shipped was estimated in 2003 at over 960 billion in more than 12,000 shipments to 22 recipient countries from 50 sterile insect production facilities in 25 countries. During this period of almost 50 years, only one problem associated with shipping live sterile insects has been recorded. This is a recent case with non-irradiated screwworms that were shipped to different locations for release. Human error was the cause of this incident that could have been prevented if standard operation procedures had been observed (FAO/IAEA/USDA 2014, Moscamed 2008). This single case shows that any system is subjected to failure and illustrates the importance of strict observance of standard operation procedures (SOPs) to mitigate the risk of hazards occurring. In 53 years, over 579 billion sterile pupae involving tephritid fruit fly pests (History of Transboundary Shipments of Sterile Tephritid Fruit Flies, see **Appendix 3**), no shipment of sterile insects has ever been rejected by national or international plant protection or regulatory authorities (Enkerlin and Quinlan 2004).

The risks from transboundary movement of sterile insects have been determined to be negligible (See **Appendix 4**) if procedures outlined in this guideline are followed. Some countries do not regulate shipment of sterile insects, others only require labelling and documentation, and still others are regulating sterile insects under their biological control measures. This guideline, in support of ISPM3 (FAO 2005), will assist factories or any other organization shipping sterile insects to follow standard operation procedures thus assuring safe shipment while facilitating trade.

For long-distance shipment, pupae are typically carried by commercial airlines in a portion of the cargo hold where temperature and air pressure are held at "cabin" levels. For long distance shipments airline routing should be carefully selected to minimize transhipment points and overall shipment time. Although pupae have been held under hypoxia for 40 hours for some programmes, quality begins to drop rapidly when hypoxia extends beyond ≈24 hours. Use of plastic bottles rather than bags and boxes increases the negative effects of extended hypoxia on insect quality (**Figures 4.1 and 4.2**).

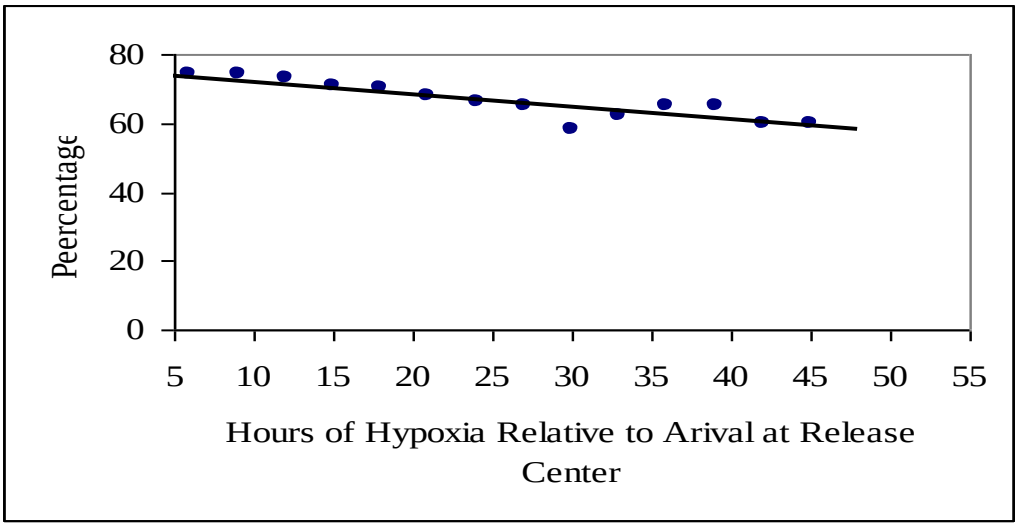



**Figure 4.1** Detrimental effects on flight ability from prolonged hours in hypoxia.

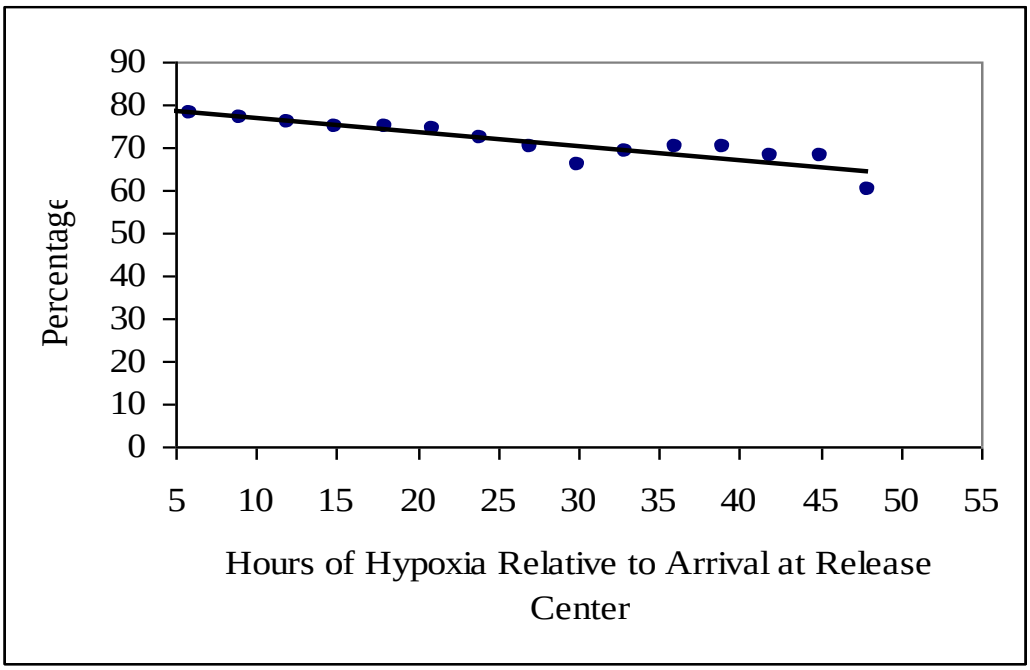



**Figure 4.2** Detrimental effects on emergence from prolonged hours in hypoxia.

**4.1 Operational Procedures (see also Sections 2 and 3 of this Guideline)**

**4.2 Normative Procedures**

This section provides guidelines for transboundary shipment and importation (either as a consignment in transit or for entry to the country of destination) of sterile insects for use in SIT control programmes of plant insect pests (**see also Appendix 5**). It covers shipment of sterile, mass reared insects, including those developed through traditional selection and mutation breeding.

The National Plant Protection Organization (NPPO) of each country should designate the proper authority for assuring safe shipment of sterile insects (either through or to their territory). It is up to the NPPO to coordinate with the producer/shipper regarding their responsibilities for achieving safe shipment, because producers of sterile insects may be private businesses as well as government, parastatal, joint venture or internationally owned facilities.

**4.2.1 Responsibilities of the producer/shipper of the sterile insects**

The producer/shipper may be the NPPO, a regional authority, a research centre, or a private organization. The producer/shipper should:

Make sure that sterile insects conform to international accepted quality control standards and operation procedures (FAO/IAEA/USDA 2014, FAO 2005), developed by the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, in cooperation with national governments, that offer years of experience in operating sterile insect production facilities and release programmes.

Take all necessary steps to ensure that exported sterile insects conform to relevant regulations of importing countries, especially concerning labelling and notification. Ensure that documentation includes instructions to handlers and officials at the point of entry on how the package should be treated. This will avoid damage to the contents and on action to be taken if the packaging is

breached. Documentation should also indicate whether it may be opened for customs inspection. Arrangements with the shipping company should be done so that packages containing sterile pupae are placed in a way that they can be removed first from cargo to limit the time between arrival and receipt at the release centre.

Maintain contact with the FAO/IAEA Joint Programme to facilitate awareness of new developments in operation procedures available in guidelines and manuals. Keep the Joint Division informed of any difficulties in compliance with the procedures or gaps in understanding of the procedures. The producer/shipper should give advance notice with full details of routing to the receiver to minimize delays and to alert officials at the point(s) of entry.

### 4.2.2 Responsibilities of the authorities prior to export

The NPPO in the exporting country should:

a) Certify that the shipment contains sterile insects that have been produced, sterilized and packed according to Manual for Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies (FAO/IAEA/USDA 2014) or other standard operation procedures developed by the Joint FAO/IAEA Programme in cooperation with national and / or local governments.

b) Verify that the shipment complies with the necessary documentation for safe transport.

c) Federal Phytosanitary Certificate for the shipment must also be extended.

### 4.2.3 Responsibilities of the authorities upon import (final or transit)

The authorities of the importing country should:

a) Make information available regarding the proper markings on packages to officials from any agency that may be a point of first contact with a diverted package of sterile insects so that it will be properly handled and notification will be made to the producer/shipper of the action taken.

b) Seek to verify that the packages have not been breached, and/or there is living material spilled in or on the packages.

c) Seek to verify the sterility of quarantine pests detected in regular surveillance, when the species detected is transiting or entering the country for use in SIT activities.

d) Take phytosanitary action if an exotic contaminant species of quarantine concern is detected in or on the packaging of a consignment of sterile insects.

e) If applicable, a pest risk analysis may be conducted to evaluate the additional risk and options for additional measures that may be considered.

### 4.2.4 Responsibilities of the importer

The importer may be the NPPO, a regional authority, a research centre, or a private organization. For the purposes of this standard, the primary responsibility of the importer regarding transboundary shipment is to notify the producer/shipper and appropriate authorities in the case of a missing or delayed arrival of a consignment of sterile insects to facilitate tracking the shipment and proper handling when located.

### 4.2.5 Shipping documents

Packages should be accompanied by the necessary documentation to guarantee timely and safe delivery. Shippers should be vigilant of the following:

a) Documentation should conform: (i) to relevant regulations of exporting and importing countries, especially concerning import permit, national transit permit, phytosanitary certificate, irradiation certificate, labelling and notification, and (ii) to transit regulations should the shipment transit through a third country (i.e., a country that is neither the country of origin nor the country of destination of the consignment) (**Figure 4.3**).

b) Documents should include clear instructions to handlers and officials at the point of embarkment, transhipment and entry on how the package should be treated to avoid damage to the contents and on action to be taken if the package is breached.

c) The documentation should indicate that package content is perishable and therefore rapid transit of the material should be allowed.

d) The receiver should have the necessary documentation to provide rapid feedback when the package is delayed.

e) The receiver might request data on the quality of the sterile insects being reared.

f) The receiver should request, for each consignment, a datasheet with a minimum of information as shown in **Appendix 2**.

g) Documents should also include clear instructions to officials at transhipment or entry points on how a lost package that is found is to be discarded.

A recommended practice is to include a copy of the radiation certificate with each shipment placed inside box number 1 of the shipment.


**Figure 4.3** "Transit" documents for shipment of sterile medfly pupae from Guatemala to Israel through the Netherlands. (©FAO/Programa Moscamed Mexico, Guatemala, USA)

### 4.2.6 Traceability

A system to trace the sterile insect shipments throughout the whole process is of primary importance. Procedures to facilitate tracking of consignments described in section 3 should be followed.

### 4.2.7 Action in case of non-compliance

Examples where phytosanitary action by importing or transit NPPO's may be justified regarding non-compliance with import regulations include:

a) Detection of a listed quarantine pest associated with sterile insect consignments for which it is regulated.

b) Evidence of failure to meet prescribed requirements (including bilateral agreements or arrangements, or import permit conditions) such as treatment and laboratory tests.

c) Interception of a consignment which does not otherwise comply with import regulations, such as detected presence of undeclared commodities, soil or some other prohibited article or evidence of failure of specified treatments.

d) Invalid or missing required documentation.

e) Prohibited consignments or articles.

f) Failure to meet 'in-transit' measures.

Type of action will vary with circumstances and should be the minimum necessary to counter identified risk. Administrative errors such as incomplete required documentation may be resolved through liaison with production facility. Other infringements may require action such as:

*Detention* - This may be used if further information is required, taking into account need to avoid consignment damage as far as possible.

*Destruction* - Consignment may be destroyed in cases where NPPO considers consignment cannot be otherwise handled. If destruction is required it must be done at least under supervision of end user.

### 4.2.8 Emergency action

Emergency action may be required by importing or transit NPPO in a new or unexpected phytosanitary situation, such as detection of quarantine pests or potential quarantine pests:

a) In consignments for which phytosanitary measures are not specified.

b) In regulated consignments or other regulated articles in which their presence is not anticipated and for which no measures have been specified.

c) As contaminants of conveyances, storage places or other places involved with imported commodities.

d) Emergency actions should result in destruction of consignment in cases where the NPPO considers consignment cannot be otherwise handled. If destruction is required it must be done at least under supervision of end user.

### 4.2.9 Records

Records should be kept by the NPPO of the exporting, transit and importing countries of all actions, results and decisions including:

a) Records of inspection, sampling and testing.

b) Non-compliance and emergency action (in accordance with ISPM No. 13: *Guidelines for the notification of non-compliance and emergency action*) (FAO 2001).

### 4.2.10 Communication

Producers and end users should ensure that there are communication procedures to contact:

a) Producer/end user and appropriate industry representatives.

b) NPPOs of exporting/transit/importing countries.

c) Have a list of contact numbers during and after hours.

### 4.3 References Cited

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# 5. HANDLING, EMERGENCE AND HOLDING AT RELEASE CENTRE

*STEP III OF PROCESS IN FLOW CHART*

### 5.1. Reception and Unpacking of Pupae

*STEP III-a OF PROCESS IN FLOW CHART*

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 2014).

Independently of the type of container used for the shipment, the supervisor of the irradiation facility has to make sure that all the bags in the cartoon boxes or bottles have received the established sterilization dose. No container should be shipped without verification that the insects have been irradiated. This is done by observing the colour change of the irradiator indicator. Normally, the indicator changes from red to black colour as indicative that the product has been irradiated.

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.

Radiation indicators are removed after all boxes/bags have been emptied; radiation indicators are counted and once properly verified should be destroyed to avoid being used in non-irradiated bags. If they are stored beyond their shelf life, some indicators may turn from black to red causing unnecessary concerns on proper irradiation procedures.

In Mexico, once pupae are taken out of the bags and packed in towers, cardboard boxes are returned to the mass rearing facility for reuse (SAGARPA-SENASICA, 2013).

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

a) Check the shipment documentation (see Section 4.2.5).
b) 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 2014).
c) Make sure that the holding room is set at the proper temperature (24 +/-1°C).
d) Verify that the boxes are kept at temperatures ranging from 16 – 20 °C (see Section 2.4).
e) Open the container and sample for quality control (see Section 13).
f) Safely dispose radiation indicators.

## 5.2 Procedures for Chilled Adults

### 5.2.1 Setting up for fly emergence

*STEP III-b OF PROCESS IN FLOW CHART*

In any chosen container for packing the pupae, there should be a minimum resting area of 0.5 $cm^2$ for each emerged fly. At the present time there are three basic systems used in which to emerge sterile flies for a chilled release: The Plastic Adult Rearing Container (PARC), fabric sleeves cages and 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 adding up to 55,000 pupae per box. Pupae are volumetrically dispensed into bags (PARCs), small plates (sleeves) 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). Adult diet is placed outside over the screen of the PARC box cover in order to force the adult flies to search for the food. When ready, PARC boxes are stacked together in sets of 6 boxes. The boxes are held together with a plastic rope. Then, each stack is manually moved to the emergence room.

In Mexico, PARC boxes are also used for emergence of parasitoids, placing 40,000 pupae distributed in 4 Kraft paper bags. 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 5.1**).

**Figure 5.1** PARC boxes used to emerge and hold sterile flies. (©FAO/Programa Moscamed Mexico, Guatemala, USA)

The fabric sleeves cage consist of a metal frame holding 20 horizontal sleeves with both ends connected to a bottomless bottle closed with a wide cap. Pupae are volumetrically dispensed in small plates inserted in both ends of the sleeve. A rod containing dry sugar and protein lays inside

the sleeve. Water is sprayed on the outside of the fabric every day after emergence. Collection of adults is done by shaking the cages in the vertical position with open lids after chilling (**Figure 5.2**).

**Figure 5.2** Fabric Sleeve cage, used to emerge and hold sterile flies. (©FAO/Programa Moscamed Valencia Spain)

At present time, there are 3 different tower types: Worley, Mexican and Guatemalan towers. In the Worley towers, pupae are placed into a hopper that dispenses measured amounts into each individual screen shelf. These shelves are then stacked into towers (**Figure 5.3**).

The Worley tower were designed for *A. ludens* to handle 75 aluminium shelves per tower (FAO 2007), with a capacity of 1 million pupae per tower; and lately adapted for *C. capitata*.

**Figure 5.3** Worley Tower's used to emerge and hold sterile flies. (©FAO/USDA Mission Texas)

The Mexican tower type was designed for *Anastrepha ssp*. This tower uses a higher aluminium rack with screens on the four sides, holding 18 shelves per tower. The packing center in Mexico (Centro de Empaque de Mosca del Mediterráneo (CEMM)), handles *A. ludens*, *A. obliqua*, and *C. capitata* in the redesigned Mexican type towers, (V&Z) Gutierrez el al. 2010) composed of 16 shelves, (**Figure 5.4**) each one of 81.7 x 70 x 10.3 cm, 1 container for pupae (55,000 *C. capitata*, 25,000 *A. ludens*, 20,000 *A. obliqua*), 2 trays for food (40 g for *C. capitata* and 20 g for *A.* spp), one adult resting device and one absorbent device called pillow for water retention and supply. Pupae are placed into a hopper and dispensed by means of a pupae dispenser machine (for accurate weight), (**Figure5.5**) into the pupae container, which is then placed in the individual shelf with all the other parts, to conform the tower (**Figure 5.6**).

In the Guatemalan Towers, the pupae are placed on individual shelves using plastic containers (12,500 pupae per container). Each shelf has 2 containers (25,000 pupae per shelf). A tower can hold 24 shelves (600,000 pupae per tower). The diet is placed on top over the sieve of the shelf. Then, each tower is manually moved to the emergence room. The packing centre in Guatemala uses PARC boxes (55,000 pupae per PARC box) as well as the towers for *C. capitata* **(Figure 5.7)**.

Fi**gure 5.4** Mexico towers used to emerge and hold sterile flies. (©FAO/Programa Moscamed Mexico, Guatemala, USA)

**Figure 5.5** Pupae dispenser. (©FAO/Servicios Aereos Mubarqui)

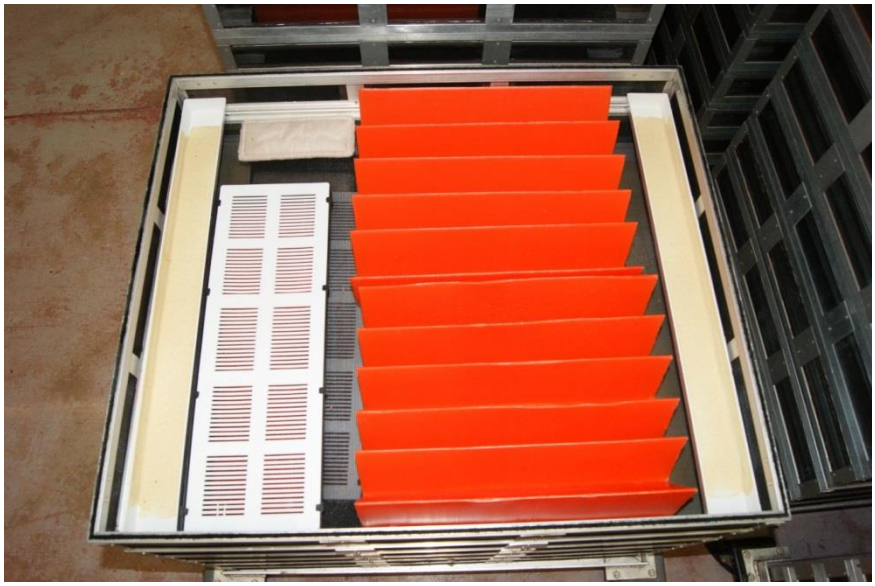

**Figure 5.6.** Mexican type tower shelves and components. (©FAO/ Servicios Aereos Mubarqui)

**Figure 5.7** Guatemalan towers used to emerge and hold sterile flies. (©FAO/Programa Moscamed Mexico, Guatemala, USA)

For amounts of pupae used in programmes utilizing these three emergence systems see **Table 5.1**.

**Table 5.1.**Amounts of pupae used in PARC boxes and towers in current operational programmes.

| **Fruit fly species** | **PARC Boxes** | | **Fabric sleeves cage** | | **Tower (Shelf) Worley** | | **Tower (Shelf) Mexican** | | **Tower (Shelf) Guatemalan** | |
|---|---|---|---|---|---|---|---|---|---|---|
| Medfly (C. capitata) | 6 | Bags | 20 | sleeves | 50 – 80 | Trays | 16 | Trays | 24 | Trays |
| | 45, 000 | Pupae/Box | 22,500 | Pupae /sleeve | 24, 000 | Pupae/tray | 55,000 | Pupae/ Trays | 25, 000 | Pupae/tray |
| | 660 | ml/ Box | 330 | ml/sleeve | 350-400 | ml/ tray | 480 | ml/ trays | 350-400 | ml/ tray |
| | 0.27 | million/set | 0.4-1.2 | million/cage | 1.2 – 1.92 | Million Pupae/tower | 0.88 | Million Pupae/tower | 0.6 | Million Pupae/tower |
| Mexfly ( *A. ludens* ) | NA | NA | NA | NA | 50 – 80 | Trays | 16 | Trays | NA | NA |
| | NA | NA | NA | NA | 13, 000 | Pupae/tray | 25, 000 | Pupae/ trays | NA | NA |
| | NA | NA | NA | NA | 400 – 440 | ml/ tray | 430–445 | g/ trays | NA | NA |
| | NA | NA | NA | NA | 0. 65 – 1.04 | Million Pupae/tower | 0.4 | Million Pupae/tower | NA | NA |
| West Indies fruit fly ( *A. obliqua* ) | NA | NA | NA | NA | NA | NA | 18 | Trays | NA | NA |
| | NA | NA | NA | NA | NA | NA | 20, 000 | Pupae/ trays | NA | NA |
| | NA | NA | NA | NA | NA | NA | 280–290 | g/ trays | NA | NA |
| | NA | NA | NA | NA | NA | NA | 0.32 | Million Pupae/tower | NA | NA |
| Diachasmimorpha longicaudata | 4 | Bags | NA | NA | NA | NA | NA | NA | NA | NA |
| | 2,500 | Pupae/bag | NA | NA | NA | NA | NA | NA | NA | NA |
| | 10,000 | Pupae/box | NA | NA | NA | NA | NA | NA | NA | NA |
| | 450-500 | ml/Box | NA | NA | NA | NA | NA | NA | NA | NA |

### 5.2.2 Food preparation and feeding

*STEP III-c OF PROCESS IN FLOW CHART*

In any food that is provided should be considered the nutritional requirements of male flies to sexual maturity and to its survival and longevity.

Food with agar base

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. gallons) 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. Mechanical mixers will ensure consistency of the diet medium. 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:

**Table 5.2**. Diet preparation.

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

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

The liquid is carefully poured or pumped with a mechanical dispenser into fibreglass trays (41 cm width x 77 cm length x 5-7.5 cm deep) (16"x30"x3"). The agar slab will be approximately 1.9 cm (3/4") thick. One agar square is placed on top of the screen of PARCs and tower shelves. If the agar squares are too thick they will be squeezed through the screens of the PARC/tower shelves. 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 PARC's and twenty equally sized pieces for each tower shelf.

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 shelves, a piece of diet material is placed on each of the shelves after they are loaded with pupae and then the shelfves are stacked on carts. An empty shelf 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 the water in the diet materials on the

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

Paste or dry food:

For the *Anastrepha* species (*A. ludens* and *A. obliqua*) and *C. capitata*, a different type of food called "Mubarqui" is now in use which is based on natural protein, lipids, carbohydrates, antioxidants and fat. Ingredients are: amaranth, glasé sugar, peanut and egg. It is in a solid fine powder with a clear brown alabastro color, according to Pantone color guide. This food is in use in both PARC's boxes and Mexican tower shelves. 1 kg of the Mubarqui food is mixed in 240 ml of water, then 40 g (*C. capitata*) and 20 g (*A.*spp) are spread in the food device dispenser. (**Figure 5.8**).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 (SAGARPA-SENASICA 2013). Food used for parasitoids is honeybee spread on the screen of the PARC box or Mexican tower type.

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

a) after peeling and toasting, the peanut is crushed to get granulated powder
b) the peanut is incorporated to the amaranth grain and mixed for 15 minutes
c) previously stirred egg is then slowly incorporated to the mixture and mixed for 20 minutes
d) after 15 minutes of resting, the mixture is placed on a tray to be cooked at 220$^{o}$C for 20 minutes
e) the mixture is finally ground to obtain fine powder

**Figure 5.8** "Mubarqui" solid powder food. (©FAO/Servicios Aereos Mubarqui)

### 5.2.3 Emergence and holding

The design of a sterile fly emergence facility is in function of the amount of biological material that will be received, packed, emerged and released in the field.

The design of the emergence rooms should ensure compliance of the following parameters:

- Temperature between 21 and 23°C
- Humidity less than the 70%

- Total darkness

The monitoring of temperature and humidity has to be done at least every 4 hours with hygrometers and thermometers.

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 time required for holding varies between species (minimum of 5 days for medfly, from 5 to 8 days for mexfly and 5 to 7 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.

Aromatherapy

The day before release, ginger oil (Spain and Guatemala) or orange oil (Mexico) is sprayed on the sterile adult flies to stimulate the sexual activity of the males (Shelly et al. 2004 and Teal et al. 2000) (for procedures to apply these semiochemicals see **section 5.2.4**). For those species with long reproductive periods, the use of hormonal treatments as juvenile hormones, need to be considered **(see section 5.4.3).** 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).

**Table 5.3** Environmental conditions and periods required for holding of sterile adults in PARCs, fabric sleeves and towers.

| Factor | PARCs | | Fabric sleeves | Towers | | |
|---|---|---|---|---|---|---|
| | ***C. capitata*** | ***D. longicaudata*** | ***C. capitata*** | **A. obliqua** | ***C. capitata*** | **A. l*uden*s (Tapachula 7 strain)** |
| Adult holding period (days) | 5 | 8 | 5 | 7 | 5 | 8 |
| Temperature range: ($^{o}$C) | 21-23 | 24-26 | 21-23 | 22-24 | 22-24 | 22-24 |
| ($^{o}$F) | 70-73 | 75-80 | 70-73 | 70-73 | 70-73 | 70-73 |
| Humidity range (%) | 60-70 | 60-70 | 60-70 | 60-70 | 60-70 | 60-70 |

## 5. 3 Procedures for Adults Packed in Paper Bags

*STEP III-b OF PROCESS IN FLOW CHART*

### 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/$m^2$with a double sealed on the bottom of the bag. Additional features can be added to the bag, such as containers for the pupae and structures (crumpled paper) for resting and increase the volume of the bags 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 2014).

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 100 cc (ca. 6000 pupae per bag). The paper bags used are 50 cm height x 26 cm width. Inside the bag additional paper is placed which provides support increasing surface to the bag and serves as resting area for the adult flies. The total surface of the paper bag is 2600 $cm^2$ thus the amount of pupae per $cm^2$ is 2.3 and roughly 1.5 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):

a) Paper bags are placed on the floor or on the table if pupae loading is done manually or on a conveyor if pupae loading is done mechanically, giving a minimum distance of 20 cm between each bag.
b) Pupae loading in the paper bags are done by means of a volumetrically measured cup.
c) 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.
d) Place the food mat inside the bag.
e) Once the pupae are placed inside the bag, and to avoid flies escaping, bags are closed by folding and stapling the opening with machines, manually or automatically, 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:

a) 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.
b) Place the bags in shelves or other structures; avoid direct contact with the floor.
c) 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.
d) Samples of pupae held separately are evaluated to determine the moment of the desired emergence or maturation level.
e) Once the required level is reached, the bags are shipped for aerial/ground release.
f) 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* |
|---|

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.

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 28). 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 5.8**).


**Figure 5.8** 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. (©FAO/PROCEM Argentina)

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:

a) 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.

b) 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 x 10 cm width).

c) 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 tephritid 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. 2005). Additionally the ratio of sugar to protein may affect adults; however, there are no clear guidelines 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. In Mexico, *C. capitata* is supplied with natural protein from amaranths, peanut and eggs (10%) and sugar (90%) in the Mubarqui food. (SAGARPA-SENASICA. 2013).

### 5.4.2 Semiochemicals 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 packing facility in Sarasota and at Los Alamitos in Los Angeles, site of the CDFA-USDA medfly packing 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 Worley tower, 24 hours prior to release (Shelly et al. 2004). ). In Valencia packing facility (Spain) and Retalhuleu, Guatemala, a cotton wick impregnated with GRO is placed during 6 hours near the fan in the room that holds the cages 24 hours prior to release. The amount of GRO will vary according to the size of the room (0,5 ml per cubic meter). GRO will penetrate into the cages through the fabric sleeves. In Mexico, 24 hours prior to release, glass vials with 0.27 ml of orange oil, per $m^3$ and a cotton wick are placed in the emergence room, a total of 230 ml per room (SAGARPA-SENASICA, 2013).

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. The evaluation of methodologies for practical use of these products in large-scale operational programmes has been done and the technology for the use of juvenile hormones into the holding protocols of sterile insect release has been reported (Gomez-Simuta, 2013).

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# 6. PREPARATION OF ADULTS FOR RELEASE

*STEP IV OF PROCESS IN FLOW CHART*

Sterile adult flies that are released using paper bags do not need to be chilled before release. In the case of the chilled adult release system, sterile adults are chilled in pre-cooled emergence rooms as described below. Basically the chilled adult release system allows for a more efficient handling of sterile flies which results in healthier sterile flies being released. This is reflected in a more uniform distribution of flies in the field and a better recapture rate. It also solves the problem of accumulation of great amounts of paper trash, a serious concern of the paper bag release method. Release methods, while operationally convenient, may not be always optimal in terms of sterile male performance. Therefore, the effects of different process need to be assessed. There is an indication for some species of fruit flies that chilling adult flies may have a detrimental effect on quality or quantity. Thus effects on sterile male performance of a cold knockdown procedure need to be investigated (IAEA 2004).

### 6.1 Chilling of Adult Flies in PARC Boxes

Procedures are as follows:

a) Determine that the flies have reached the time for release by checking the emergence grids (a device that holds 100 pupae in individual cells) and comparing it to the expected percent of emergence.
b) The required amounts for a day's release of stacked PARCs are moved from the emergence areas to a cold room for immobilization by exposure to temperatures in pre-cooled cold rooms. The rooms are kept at a temperature ranging from 0 to -3 $^{o}$C prior to fly exposure. Upon introduction of flies to the cold rooms, temperature must be kept at 2 to 5 $^{o}$C for 30 to 60 minutes depending on the volume.
c) The aerial release box is also pre-chilled at this time in the same room.
d) Once flies are determined to be immobile (a visual inspection of the flies is done to verify immobility); the straps are removed. Food is removed and discarded.
e) The PARCs are slammed on a table top to dislodge flies adhering to all surfaces within the containers; the lid is removed and bags inside PARCs are shaken to remove any additional flies and then the bags are disposed of.
f) Flies are then dumped into the collection hoppers that are in turn used to load the release box.

### 6.2 Chilling of Adult flies in Sleeve Cages

Procedures are as follows:

a) The day before the aerial release, determine the number of cages that will be transferred to the cold room according to the number of foreseen flights and emergence rate in the grids.
b) The required amount of cages is moved to the cold room the day before release. Exposition to GRO will be done in this room at 24 ºC on the day prior to release.
c) 4 hours before the collection of chilled adults, the temperature of the room is programmed to decrease following a smooth slope until reaching 4ºC.
d) Once flies are determined to be immobile, the lids are removed. The plates with empty puparia and the rod of food are also removed. The food rods will be reused 5 to 6 times.
e) Flies are collected and dumped in the release boxes by putting the cage in a vertical position and shaking.

### 6.3 Chilling of Adult Flies in Worley Towers

Procedures are as follows:

a) In the tower system, the ventilation fan is removed and the towers are moved into the cold rooms.
b) A "knock down" fan (high volume movement fan) is placed on top of each tower to facilitate the movement of air through the towers.
c) After of 10 to 30 min., at a temperature of 2 to 5 °C, flies are immobile (a visual inspection of the top tray will show this) and the fans are shut down and removed from each tower as the knock-down proceeds.
d) The towers are positioned under the vacuum and processed from the top down. Steps are: Food is removed and discarded; puparia are vacuumed from the edges of the tower shelf; flies are removed by tapping each shelf on the cross bars of the collection hopper (care should be taken that the shelves are horizontal when tapped on cross bars).
e) Flies are then dumped into the collection hoppers that are in turn used to load the release box.

### 6.4 Chilling of Adult Flies in Mexico Towers

a) Mexico towers are taken from the emergence room to the pre-cooled cold room, where they will be chilled for 45 ± 15 minutes with a temperature and humidity rate of 2 to 5$^{o}$C and 50 to 70 %.
b) Once the flies are immobile (knockdown), the fly collection process begins, consisting in placing a shelf on the collection table. Accessories for food, water, pupae containers and resting devices are taken out of the shelf.
c) Flies are then emptied from the shelf to the collection table and dumped into PARC boxes located under the table.
d) Flies are weighted and then loaded into the 3 release boxes needed for each flight and then transported to the airport in a refrigerated vehicle with temperatures between 10 and 14 $^{o}$C. (SAGARPA-SENASICA, 2013).

### 6.5 Chilling of Adult Flies in Guatemala Towers

a) The time needed for pupae to emerge as adults and reach the sexual maturity is 5 days.
b) In order to manipulate the adult insects, which have very high mobility, they are chilled by exposing them to temperatures of 2 to 5°C.
c) After 30 to 60 minutes of being at these temperatures (depending on the volume) the flies are knockdown, and they are totally immobilized.
d) Once the flies are knocked, they are removed from the boxes and the towers, and collected in boxes.
e) The weight is determined per box in order to put the amount needed in the release boxes, where the knocked insects from the collection boxes are dropped.

### 6.6 Loading and Transportation of Release Boxes with Chilled Adults for Aerial Release

Procedures are as follows:

a) The release box must be inspected to ensure the slide on the bottom is in place prior to loading.
b) Flies are collected (3 to 5 gr. samples) prior to release from each shipment for quality control tests as well as a means of determining the individual fly weights.

c) The number of flies per release box is calculated by dividing the total fly weight by the individual fly weight.
d) Care should be taken when loading release boxes with sterile flies to ensure against compaction of flies. In addition to causing damage to flies, compaction results in flies being released in balls instead of a steady stream affecting the uniformity of fly distribution. It also prevents the proper operation of the release equipment (see **Table 7.1**). Compaction can be reduced by eliminating excess humidity and reducing as much as possible vibrations inside the aircraft (Tween 2006).
e) The release box is then transported (if local situations require, air conditioned vehicles need to be used for transport) to awaiting aircraft where it is loaded on the pre-chilled release machine **(Figure 6.1 and Figure 6.2)**.
f) The slide is then removed from the release box enabling flies to drop onto the screw augers.

**Figure 6.1** Loading of release box into a truck for transportation to the airport. (©FAO/Programa Moscamed Mexico, Guatemala, USA)


**Figure 6.2** Loading of a release box into a fixed-wing aircraft. (©FAO/Programa Moscamed Mexico, Guatemala, USA)

### 6.7 Loading and Transportation of Paper Bags for Aerial Release

It is recommended that the truck that will take the bags to the airport is used exclusively for sterile fly transport and is never used in transport of insecticides or toxic substances. The truck should have shelves and a temperature control unit. To provide suitable conditions, the temperature must not exceed 20°C. The bags are loaded in shelves or other structures. It is strongly discouraged to pile up the bags, since it can result in severe damage to adults. For space saving, every other bag is placed upside down.

To prevent damage to the insects because of high temperatures, the bags are taken from the truck only when the aircraft is ready to be loaded. The bags are placed over trays and immediately are loaded onto the aircraft. The number of bags to be loaded depends on the capacity of the aircraft. Most common fixed-wing aircraft used are Cessna, Pipers or similar, which can carry 300 to 800 bags per flight equivalent to 1.5 to 5 million emerged sterile flies per flight (see **Table 7.2** and **Figure 6.3**). Nevertheless, precautions must be taken to avoid the cabin becoming crammed with bags, crushing bags, with the subsequent damage to the insects, **Figure 6.4** (Castellanos 1997, Programa Regional Moscamed 2002, Reyes et al. 1986, SAGARPA 1999, SAG 1994, Tirado and Gomez-Escobar 2005).

**Figure 6.3.**Loading of paper bags into a fixed-wing aircraft. (©FAO/PROCEM Argentina)


**Figure 6.4** Paper bags inside a fixed-wing aircraft. (©FAO/PROCEM Argentina)

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# 7. AERIAL STERILE FLY RELEASE

*STEP V OF PROCESS IN FLOW CHART*

Aerial release is more cost-effective than ground release for large-scale programmes and a more uniform sterile fly distribution is achieved compared to ground releases, which tend to clump sterile flies in localized sites or along release routes.

Once the release area is selected, it is divided in polygons, where flight lines are depicted. The basic tools in this step are digitalized maps, follow-up GIS software and GPS (see Section 10).

For aerial release a flight plan should be formulated at least 24 hours in advance. Plans will depend on the following:

- General strategy of the programme (suppression, eradication, prevention, containment)
- Progress made on the weekly coverage of the release zone
- Amount of sterile flies available for release on that day
- Established release densities
- Results achieved in sterile fly distribution and density in the previous weeks
- Availability of transport units and number of sterile fly recharging points in the area

At the present time there are two (2) basic systems for aerial release. These are the bag release and chilled fly release systems.

### 7.1 Aerial paper bag release

The bag release is a relatively simple process where flies are emerged within sealed paper bags and released as the bags are ripped open once they come in contact with the hooks or knives located at the end of the chute upon exiting the aircraft (**Figure 7.1**).

**Figure 7.1** Typical chute used in aircraft for paper bag aerial release. (©FAO/Programa Moscamed Mexico, Guatemala, USA)

The primary advantages of the bag release system are:

- That a minimal amount of accessory equipment is required for operation and a wide variety of facilities can be used to operate out of.
- Since the flies are never exposed to cold temperatures for immobilization prior to release, damage and reduced fly quality resulting from exposure to the cold is non-existent.

There are also some deficiencies in the bag release method. These include:

- Litter from bags throughout the release area is not environment-friendly in dry climates where they do not biodegrade rapidly.
- Space in aircraft is limited for bags and flies in bags are often damaged even with careful handling.
- Bags sometimes do not open, or only partially, allowing predators to enter bags before flies have found the exit.
- Flies are not watered prior to release and sometimes also not properly fed.
- Most importantly, sterile fly coverage within the target area is not as uniform compared with chilled adult release due to intermittent intervals (2 to 8 seconds) of release from the aircraft.
- In addition sterile flies may be subjected to higher predation rates because some flies stick to the bags until they reach the ground.

### 7.1.1 Spacing and altitude of paper bag release

Usually, flight lanes range from 100 to 600 meters apart, according to the species dispersion capability and desired sterile fly coverage. Closer lanes are required in areas with high host density and species considered to be weak fliers, whereas more open lanes are possible in areas where hosts are scattered and for fruit fly species considered to be strong fliers. In the case of medfly for temperate and semiarid environments, the most commonly used distance between lanes is 200 meters. Flight lanes should be straight or following altitudinal curves and lanes should always be kept parallel (Reyes et al. 1986, Diario Oficial de la Federación 1999).

Under conditions of calm winds no difference have been found between releasing sterile flies contained in paper bags from 200, 400 and 600 meters above ground level. However, lower release altitudes are preferred especially in areas subjected to strong dominant wind currents to prevent excess sterile fly or bag drift and in areas where predation due to birds is high and frequent (Reyes et al. 1986, Diario Oficial de la Federación 1999, SAG 1984). Releasing in the early morning is therefore preferable, when winds and temperature are moderate.

### 7.1.2 Calibration of paper bag release rates

According to the aircraft speed, required sterile fly density per unit area (hectare, square kilometres, acres or square miles) and size of the release area, a frequency for releasing the bags must be established. The labourer inside the aircraft must tear the bags and release them through a chute according with the established frequency. To estimate the frequency of paper bag release (in seconds/bag the following procedure is used:

1. To determine the size of the release area to be covered by one full paper bag load

1.1 Full paper bag load = 300 bags
1.2 Number of emerged sterile adult flies per bag = 6,400 (8000 pupae x 80% emergence)
1.3 Number of sterile flying adults per bag = 5, 120 (6,400 adults x 80% fliers)

1.3 Total number of effective (flying) sterile adult flies per load = 1,536,000 (5,120 sterile flies x 300 bags)
1.4 Required sterile fly density = 2000 sterile flies per hectare
1.5 Total release area:

$$\frac{\textit{Total number of sterile flies } 1{,}536{,}000}{\textit{Sterile fly density per hectare } 2{,}000} = \boxed{\textit{768 ha } (7.7\ km^2)}$$

2. To determine length of flight lanes of release area

2.1 Total square area (from 1.5) = 7.7 $km^2$ (~ 2.77 km x 2.77 km)
2.2 Length of one flight lane = 2.77 km (2,770 m)

3. To determine number of lanes in release area

3.1 Distance between lanes = 200 m
3.2 Length of square area (from 2.1) = 2,770 m
3.3 Number of lanes:

$$\frac{\textit{Length of square area (m)}}{\textit{Distance between lanes (m)}} \quad \frac{\textit{2,770 m}}{\textit{200 m}} = \boxed{\textit{13.8 lanes}}$$

4. To determine frequency (in seconds) of paper bag release
4.1 Speed of aircraft = 45 m/s
4.2 Length of flight lane (from 2.2) = 2.77 km (2,770 m)
4.3 Total number of lanes (from 3.3) = 13.8
4.4 Frequency:

$$\frac{\textit{(Length of flight lane)(No. of flight lanes) (2,770 } \sout{\textit{m}}\textit{)(13.8)}}{\textit{(Aircraft speed (m/s)) (No. of bags) (45 m/s)(300 bags)}} = \boxed{\textit{2.8 s/bag}}$$

With a full load of 300 bags (1.5 million sterile flies), each bag needs to be released every 2.8 seconds in order to have a density of 2,000 sterile flies per hectare. Considering that the speed of the aircraft is constant and that the maximum load in this case is 300 bags with a total of 1,536,000 effective (flying) sterile flies, to increase the sterile fly density, the frequency of bag release should be increased.

GPS and appropriate software can be used to verify that the aircraft is following the flight lines, as well as the correct swath distance (See Section 10).

According with the longevity of the insect (measured as specified in the quality control manual, FAO/IAEA/USDA 2014) the release interval should be adjusted. In medfly it should be carried out at least twice per week (See **Figure 9.1** in Section 9).

In order to evaluate the effect of the process on the quality of the sterile flies, samples are taken periodically before and after the release process in the aircraft (FAO/IAEA/USDA 2014).

### 7.2 Chilled Adult Release

The chilled fly release is primarily utilized and designed for large scale programmes. It is a more complex system designed to handle large volumes of flies.

The primary advantage of the chilled adult release method is that large numbers of flies can be carried on each flight and uniformly dispensed into the environment. Other benefits include no litter from bags; proper feeding and watering of flies prior to release; reduced predation and reduced labour.

There are also disadvantages to this method that include: chilled release equipment is often specialized and limited thus can be expensive to purchase and maintain; facilities are specially designed to accommodate the processes involved in emergence and fly release, thus expensive. Nevertheless, this method continues to be the most cost-effective.

There is a degree of damage to the flies from exposure to the cold temperatures needed to immobilize the flies and this is directly proportional to the length of exposure. For this reason if the target release area is located at a great distance from the emergence facility it may reduce the quality to a point that another setup location would have to be considered. Other factors affecting quality include condensation, compaction and damage from moving mechanical parts (IAEA 2004).

In Mexico, a large-scale study was conducted with medflies comparing these release methods: chilled adults, paper bags and small cardboard boxes (Villaseñor 1985). The release was made from a fix-wing aircraft using sterile flies marked with different colours for each system. The main parameters used to evaluate the methods were: a) field distribution assessed by % of traps with flies, b) density of recaptured flies assessed by FTD, with Jackson traps baited with trimedlure (male specific lure) and, c) cost of each release method. Results showed that the best sterile fly distribution and density was achieved with chilled adults followed by boxes and bags. On the other hand the most economic system was bags followed by boxes and chilled adults (Villaseñor 1985). Initially the main constraint of the chilled adult release system was the constant breakdown of the equipment, difficulty in acquiring spare parts and lack of specialized maintenance service. The new generation of chilled adult release machines use simpler mechanisms and are much more reliable, thus this constraint has been partially overcome as will be explained in the following Section.

### 7.2.1 Evolution of chilled adult release machines

Machines specifically designed to release chilled, sterile fruit flies have been in existence for more than 30 years.

There are four (4) basic components for these machines that are standard. These are:

- A means of cooling of flies during release (sterile flies are kept at a temperature of 3 to 4 $^{o}$C during release)
- A means of metering the flies
- A control system for the machines
- A release mechanisms

The first model was designed and fabricated by USDA in 1974 (**Figure 7.2**). It was first used for releasing sterile medflies in southern California in 1975-1976. This machine used a stack of collapsible bottomed trays to hold the chilled, immobile flies. The stack of trays was positioned over a funnel that channeled the flies toward a moving belt. The belt conveyed the flies to the release chute positioned at a forty-five degree angle from the fuselage of the aircraft. In operation, a photocell was used to detect the presence of flies on the moving belt. When none were detected, a motorized screw drive mechanism was actuated to release the bottom door on the next individual tray of flies thus dropping them onto the belt, breaking the photocell light beam and stopping the screw drive. The fly release rate was controlled by adjusting the speed of the conveyor belt. The machine maintained the flies at temperatures of 2-4° C for the duration of the flight.

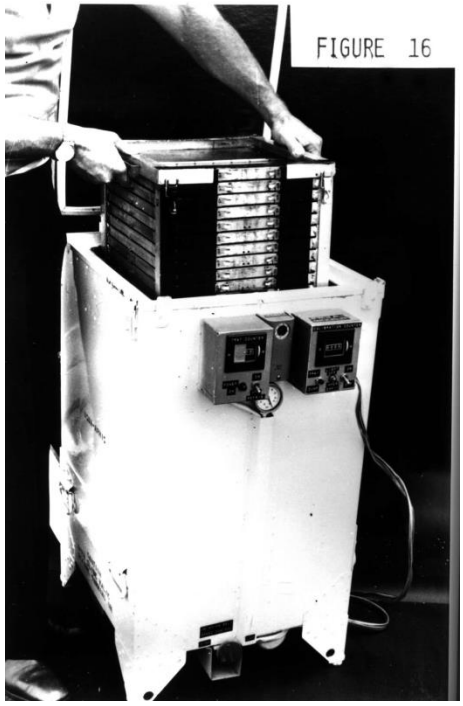


**Figure 7.2** Release machine with capacity of 5 million sterile flies per load used for medfly release in Southern California in 1975. (©FAO/CDFA)

In the first release machine, problems were found that included difficulty in loading flies, limited load capacity, clumping of flies due to excess water condensation, and frequent breakdown of mechanical components. Work immediately began on the design of a less complicated, larger capacity design and, when medfly outbreaks occurred in both northern and southern California in 1980, the improved model was put into service. In the new model, the stackable trays were replaced with a single box. The flies were supported within the box by collapsible wedge-shaped baffles. As in the earlier model, the photocell and conveyor belt were retained for metering and conveying flies to the release chute.

The first version of the new machine held two boxes of flies, each box having nearly three times the capacity of the 1974 model (**Figure 7.3**). Also, the refrigeration system consisted of standard automotive components and was much more trouble-free. The double box version was found to have more capacity than required for the release rates used at the time and was too large to fit into most single engine aircraft so later models were built with only one box. This model was used in all of the USDA fruit fly programs between 1980 and 1991.

**Figure 7.3** Release machine with a capacity of 10 million sterile flies per load. (©FAO/Programa Moscamed Mexico, Guatemala, USA)

The third generation of release machines replaced the mechanical baffles with fixed supports. Also, the conveyor belt was replaced with screw augers (**Figure 7.4**). The simplified design was found to be far more reliable. The release rate was controlled by adjusting the rotation rate of the three screw augers located beneath the box of chilled flies. Up to four speed settings could be programmed into each release and the pilot could change release rates with the push of a button.

**Figure 7.4** Double box release machine (DBRM). Shickel Corporation. (©FAO/Shickel Corporation)

This DBRM has an endless screw auger system to release chilled flies. The DBRM was design by USDA, APHIS and made by Shikel Corporation, Virginia USA. It is used in Guatemala Moscamed Program, and is operated manually by pilots.

The latest design developed by a Mexican company is the Mubarqui Smart Release Machine (MSRM) (Leal et al. 2013).The basic components of the MSNR are:

a) Cooling system and container: This container is made of stainless steel and insulated to keep biological material in suitable conditions for handling and transport by ground and air to the area or polygon where they will be released. It is equipped with devices as thermostat and vibrators to avoid air bags.
b) Central Control Unit: Is the device that receives instructions from the computer to turn them into actions such as start and end of the release mechanism, opening and closing of doors, modulating micro vibration to maintain precision rates. Release rate is programmed and controlled as needed by different vibrating feeder intensities with no damage to the biological material.
c) Navigation system: It guides the pilot to the release polygon and within the polygon. It also contains the controls for the automated release mechanisms.
d) Release mechanisms: It is installed inside the aircraft, designed and developed with vibratory feeders, automatic gates and linear actuators that regulates the conveyor belt system to the chute located outside the aircraft. The chute is shaped to avoid Venturi suction effect increasing the precision of the release rate. The mechanism is also equipped with video cameras.
e) Operation: The MSRM, works using a software developed by the Mubarqui company in Mexico, which is installed in a tablet with Android Operating System using the advantages of GPS systems. This device communicates via BLUETOOTH with the Central Control Unit. It performs all release related actions including automatic calibration, increasing or decreasing the release rate in polygons, outbreaks or in pest exclusion areas.
f) On the tablet screen, the pilot has all the information needed to navigate from the airport to the release area. Once the aircraft flies over the release polygon, it will adjust speed, maximum and minimum release altitude and swath of the lines. It also logs the flight path with all its specifications such as altitude and speed. It alerts for position, course and heading.
g) Release Method: The tablet receives via internet the release program of the day and the potential alternate polygons, as a "Release Flight Order". Immediately the pilot in command sets the information as effective flight order. The navigation program instructions will be shown sequentially and guide the aircraft to the release polygons. As soon as each polygon is reached, calibration of the release rate is automatically initiated according to the release flight order. In case weather conditions prevent the aircraft from flying on the polygon established as the first option, the pilot can change and setup as effective the alternate polygon.

At the end of the flight, the pilot synchronizes the tablet of the MSRM with internet and downloads flying files to MACX SYSTEM interactive website. The files will then be available for review and analysis by technical staff from field operations and informatics.


**Figure 7.5** MSRM release machine. (©FAO/Servicios Aereos Mubarqui)

### 7.2.2 Aircrafts and chilled fly release machines

Aircraft used for the different programmes often vary. Both single and twin engine aircraft, gas and turbine are utilized (**Table 7.1**). All release systems use 32 amp/24 volt electrical systems. Aircraft that operate with 12 volts will need to be converted to 24 volts.

**Table 7.1** Aircraft and machines for chilled adult release.

| Fruit Fly Species | Type of Machine[1] | Type of Aircraft | Capacity | Programme |
|---|---|---|---|---|
| *C. capitata* | Paper bags | CESSNA 172 | -- | Argentina |
| *C. capitata* | Paper bags | CESSNA 172 | -- | Chile |
| *C. capitata* | USDA | LET 410 UVE | 60 Million | Guatemala |
| *C. capitata* | USDA | CESSNA 207 | 2.5-3.5 Million | USA |
| *C. capitata* | Chilled release machine | BEECHRAFT KING AIR 90 | 10 Million | Portugal |
| *C. capitata* | Chilled release machine | NORMA ISLANDER | 5 Million | Israel |
| *C. capitata* | Chilled release machine | CESSNA 207 | 5 Million | South Africa |
| *C. capitata* | Chilled release machine | CESSNA 206 | 10 Million | Valencia, Spain |
| *C. capitata* | MSRM | CESSNA 401 & 402 | 60 Million | Mexico |
| *A.ludens* | MSRM | CESSNA 206 | 7 Million | Mexico |
| *A.obliqua* | MSRM | CESSNA 206 | 7 Million | Mexico |
| *C. capitata* | MSRM | CESSNA 172 | 5 Million | Neretva, Croatia |
| Tsetse | MSRM | GIROCOPTER | 15,000 | Senegal |

[1]For suppliers of chilled adult release machines see Appendix 5.

**Table 7.2** Common aircraft and release systems used.

| Fruit fly species | Type of release system | Aircraft | Programme location |
|---|---|---|---|
| *C. Capitata* | Paper bags | Cessna 172 | Argentina |
| *C. capitata* | Paper bags<br>Chilled adult | Cessna 172 | Chile |
| *C. capitata* | Paper bags | Pipper PA-28 | Chile |
| *C. capitata* | Chilled adult | Let 410 UVE | Guatemala |
| *C. capitata* | Chilled adult | Beechcraft king Air 90Ki | Guatemala |
| *C. capitata* | Chilled adult | Cessna 207 | South Africa |
| *C. capitata* | Chilled adult | Norman Islander | Israel |
| *C. capitata* | Chilled adult | Cessna 206 | Spain |
| *C. capitata*<br>*A. ludens*<br>*A. obliqua* | Chilled adult | Cessna 207 | Mexico |
| *C. capitata*<br>*A. ludens*<br>*A. obliqua* | Chilled adult | Cessna 208 | Mexico |
| *A. ludens* | Chilled adult | Maule | Mexico |
| *A. ludens* | Chilled adult | Cessna 205 | Mexico |
| *A. obliqua* | Chilled adult | Cherokee | Mexico |

### 7.2.3 Quality control of chilled adult release process

It is important to have a digital quality control system for aerial release process. This system should integrate all the information on the quantity and quality of released flies, when, where and how they were released, but must also indicate the field results in GIS maps.

There is a quality control assurance system called MACX used in Mexico for assessing the release process. MACX system is presented in specific versions for each project or campaign, for example PROMOMED for Mediterranean fruit flies in Chiapas, Mexico, Neretva Project in Croatia, PROMOFRUT for *Anastrepha spp.*fruit flies in San Luis Potosi, Nuevo Leon, Zacatecas, Sinaloa, Mexico and Tsetse fly release project in Senegal, Africa.The MACX system is designed to help in the follow-up of the chilled adult releases. It allows transmission to the base station during release in real time of data on the quality of the release process. The data is recognized, analysed, translated and re-transmitted to a web-site where it is available to supervisors and programme managers.MACX system is a tool to track shipments of pupae from the mass rearing facility to the different packing centers, quality control processes, sterile insect releases, trapping and integrated control activities. Emits, performs and is the linkage between program staff and the release company to streamline and eliminate errors in the process and service of sterile adult aerial release. Fruit fly progmmes in Mexico include the MACX system in the sterile fly release service contracts, as the right tool for quality assurance.

This system has been developed to work together with the Mubarqui smart release machines. The Mubarqui chilled adult release machine is equipped with sensor devices to recognize the internal conditions inside the release machine and its biological material. Main sensors are: a) sensor that measures the loaded volume and how it is being released during the flight, and b) humidity and temperature sensors. Since temperature and humidity are critical factors to maintain the quality of the release insects during the aerial release process, with the sensors these factors are checked in

real time. From the ground, the required humidity and temperature conditions are adjusted if needed and kept at the recommended levels (Leal Mubarqui 2005).

The aircraft equipped with the MACX system include a transmitter linked to the sensor devices of the MSRM and a GPS. The GPS recognizes second by second the position of the aircraft with high accuracy. The system also allows the base station to know the speed, the flight course in magnetic degrees, departure and landing time, as well as the flight duration in just a fraction of time. Altitude, line flight spacing and density is defined according to the parameters set by the program technical staff and these data is sent to the MACX system. By issuing a release order, information of the flight is automatically recognized by the Central Control Unit system and gives the pilot navigation instructions as well as the release rate or density previously planned.

The web-site to view the data of the MACX system in real time is: www.macxd.org.mx . This page not only has the real time report but also keeps historical data from the different release areas.

All equipment should be subjected to quality assurance protocols before use.

### 7.2.4 Spacing and altitude of chilled release

There are differences between programmes in the lane spacing and altitude of releases. For example, in the USA, in most chilled fruit fly releases the lane spacing used is normally 268 meters (880 feet). In preventive release programmes covering flat terrain, 536 meters (1,760 feet) is used between lanes. For *Anastrepha spp.* in Mexico, there is a tendency to use a 100 meter (320 feet) distance between lanes to ensure total coverage of the area. For medfly programmes 500 meters are recommended. Barclay et al. 2016, developed a model as a tool for programme managers assessment of optimal distance between release lanes for sterile Mediterranean fruit fly. The model is based on a diffusion equation to derive optimal spacing of flight lines and time interval between flights to achieve a reasonably uniform spatial distribution of released insects and to minimize costs.

There are many factors that need to be considered to assess above ground and/or altitude above sea level of releases. Some of these are; the flight ability and dispersal behaviour of the sterile insects being released; environmental conditions such as wind, temperature, cloud cover, fog, smog, and time of day of releases; geographical conditions including terrain, urban or rural, vegetation; other conditions affecting release altitude include governmental regulations on aviation and flight restrictions (no fly areas).

For example, in most fruit fly release programmes in the USA, Mexico and Guatemala, the altitude used for release is 300- 600 m (1000 – 2000 feet) above ground level (AGL). Chilled flies should preferably not be released lower than 150 m to avoid some chilled flies reaching the ground before they warm out and become active. However, other programmes in warmer climates use lower altitudes such as the *Anastrepha spp.* release programme in Mexico, which in flat terrain releases sterile flies at an altitude of 100 m above the ground and where flies are already active when reaching the foliage of the vegetation. On the other hand, releases carried out at altitudes above 600 m (2000 feet) AGL will result in excessive drift of chilled sterile flies.

### 7.2.5 Calibration of chilled adult release rates

Operational programmes use different methodologies to calculate chilled adult release rates. The following is one way of determining these rates.

To determine the number of chilled sterile flies released per second the following formula is used:

Adults flies released per second = M*A*V*Z

Where:

M= Number of adults per hectare
A= Width of lane spacing
V= Speed of the aircraft in km/h
Z= 0.0000278 Constant for determining adults per second

Example: To release 5,000 flies per hectare, the machine should release at a rate of 5,364 flies per second, if the speed of the aircraft is 144km/h with 268 m (880 feet) width of lane spacing.

*Flies per second = M*A*V*Z; 5,000 * 268 * 144 * 0.0000278*

*5,000 * 268 = 1,340,000 * 144 = 192,960,000 * 0.0000278*

*= 5,364 flies per second*

The auger or band speed should be adjusted up or down based on actual distance for release of sterile flies (see procedure below). Since airspeed and load size are usually constant, a very accurate release rate should be obtained by fine-tuning the release machine speed over several flights. The release machine should be subjected to a regular maintenance protocol. A backup release machine should always be available to assure continuity of the sterile release programme.

The actual distance for release is estimated as follows:

1. To determine total linear kilometres the number of square kilometres and lane spacing must be determined for the release area:

> *1($km^2$) divided by 268 m (lane spacing) = 0.373 linear km/$km^2$ times total area in square kilometres = total linear kilometres.*

2. To determine release rate:

> *Total flies per release area divided by total linear kilometres (step 1) in the release area = flies per linear kilometre released.*

3. To determine number of flies per kilogram:

> *Hand count and average 1 to 5 gram samples to determine the number of flies per gram and multiply this by 1000 to get the number of flies per kilogram.*

4. To determine number of flies per load:

> *Number of flies per load = number of flies per kilogram (step 3) times the number of kilograms per load*

5. Set actual distance for release of load:

The release machine auger (band) speed should be set to deliver the entire load over a set distance determined by the formula:

*Total flies per load (step 4) divided by the flies per linear kilometre (step 2) equals the expected distance covered in kilometres, per load.*

### 7.2.6 Release system used in Spain

The goal of the release is to reach a defined over flooding ratio and the wild populations in the field don't follow a homogeneous distribution. For this aim a real-time differential rate release system allows a more rational distribution of the amount of sterile reared insects available. Insects that are not necessary in areas where the desired over-flooding ratio is exceeded, are relocated to increase the number of insects in other areas with poor ratios. In addition, it allows releases in blocks with non-regular shapes as well as saving sterile flies in the ferries that connect the release blocks.

The release rates are fixed taking into account three information sources:

- A georeferenced map (raster file) providing desired release rates over the area. This raster has been previously calculated in the office taking into consideration variables such as pest and host distribution plotted on a map.
- A vectorial file providing the planned flight path.
- The GPS information during the flight.

Taking into account the map showing desired release rates and the flight path, the sterile fly load is distributed depending on the route length and number of hectares with susceptible fruit hosts under the flight path. The software transforms the flight path line into a series of signals or beacons and allocates a theoretical release rate to each of them. These calculations are done before the take-off. During the flight, depending on the actual speed and position, decisions are made in order to optimize the planned release file.

The release system that is hosted in the aircraft consists of four elements:

- A simple GPS antenna with a USB connection that takes on the position of the plane during the flight.
- Software installed in the laptop receives the position from the GPS through the GPS communication module. Before the take-off, the software has analysed the raster of the desired release rates and the flight path that will be followed by the aircraft. The path is divided into beacons spaced 100 meters apart. Each beacon is assigned a release rate corresponding to its position according to the raster map. During the flight, the software will communicate to the electronic device the release rate according to the position obtained by the GPS antenna in real time **(Figure 7.6)**.
- An electronic system communicates the software with the release mechanical device.
- A release mechanical device (double screw) is controlled by a servo engine according to the received signal from the electronic system.

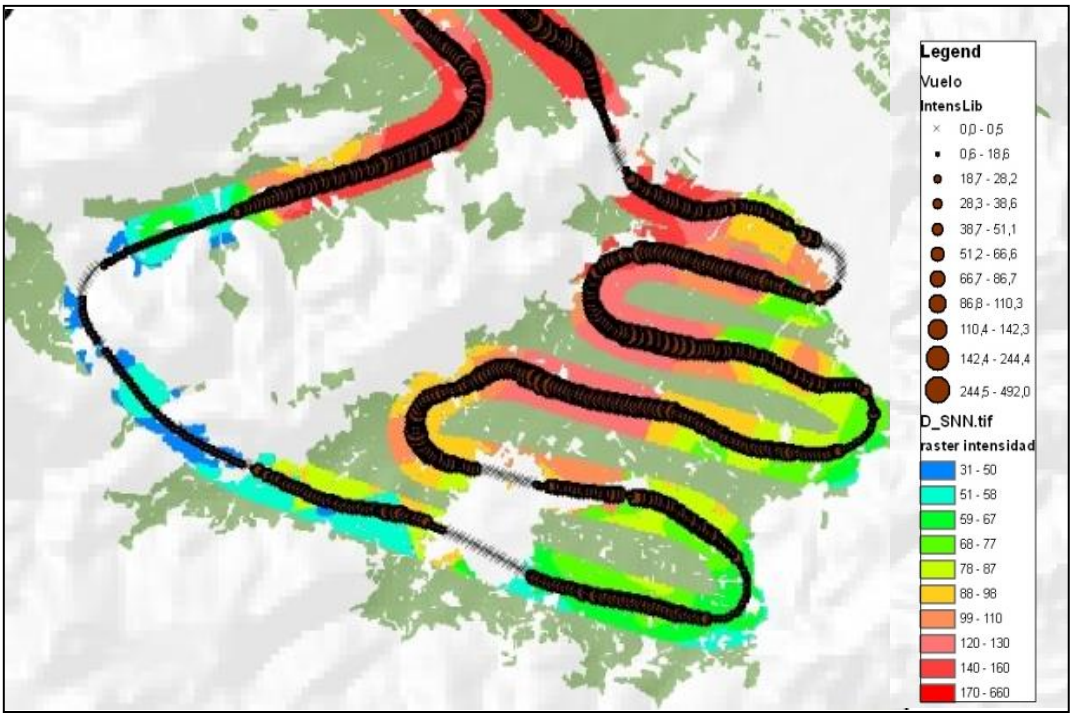


**Figure 7.6** Map of one portion of one release flight showing the actual rate of released flies as obtained from the log file recorded during the flight (displayed as series of beacons; the size of the dot represents the release rate, void dots mean no release) compared to the theoretical release rate as obtained from the flight path analysis module before take-off (displayed as coloured line; red colour means high rates of sterile flies to be released). (©FAO/Programa Moscamed Mexico, Guatemala, USA)

### 7.2.7 Pre-and post-release control of fly quality

The degree of damage to the flies caused by the release machine can be assessed by quality control tests.Samples of the sterile flies are collected at three points in the release process to determine flight ability and longevity:

- Control: The first sample is collected before release from the top of the release container full of flies and serves as the test control.
- Pre-release: The second sample is collected before release from the bottom beneath the release chute and serves as a measure of damage done by compaction of flies in the release container and damage done by the auger mechanism or endless band.
- Post-release: The third sample is collected in a netting bag from the release machine and is a measure of damage caused during transportation and release flight time. A minimum of three samples of 100 sterile flies each should be collected at each of the three points.

For flight ability tests the flight tubes used should contain 100 flies each, be properly label as “control”, “pre-release” and “post–release” and then placed in an area with a controlled environment. The percent flight ability and longevity for each sample is determined by counting the number of flies remaining in each tube at the end of a 24 hour period. This number is subtracted from 100 to determine percent fliers and longevity (FAO/IAEA/USDA 2014, SAGARPA-SENASICA 2013).

A difference of more than five percentage points between each step is an indication that excessive damage is being done to the flies. If this is found to occur, the aircraft and release machine should be immediately taken out of service and the source of the problem corrected.

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# 8. GROUND STERILE FLY RELEASE

Ground releases are commonly used where aerial releases are neither cost-effective nor efficient (discontinuous distribution and relatively small areas), or where additional releases are required to provide a higher density of flies for a particular reason, i.e. hotspots as indicated by monitoring traps, or where a high risk area is known to exist and needs to be treated with more flies than can normally be supplied aerially.

Ground releases can be divided into two general methods, adult and pupal. Adult release is the most widely used method and the pupal release method could be applied only under certain specific conditions.

### 8.1 Adult Ground Release

This method is generally based on pupae being delivered into the release centre and the pupae being held in containers (e.g. paper bags, plastic bins, cardboard boxes, etc.), allowed to emerge, held for a period of time to allow a full emergence and development to maturity, and then released by a ground mechanism. This method minimizes predation compared to the pupal release, however, conditions in the holding containers need to be well managed to ensure released adults have good survival and competitiveness. Adults are usually released 2 to 5 days (varies with species) after emergence and are approaching sexual maturity (to greater degrees with different species). This procedure hopefully facilitates minimum adult losses prior to sexual activity. The main variations in this technique are the release containers and the adult holding densities.

Generally, adults in containers should be transported from the release centres to the release sites in cool conditions (<20 $^{o}$C) to minimise stress within the container. The frequency of release may be affected by circumstances such as supplies of pupae, staggered emergence, and unfavourable weather conditions.

#### 8.1.1 Containers used for ground release

The most widely used containers for ground release are plastic cylindrical bins, PARC boxes and paper bags.

##### 8.1.1.1 Cylindrical bins

Ideally only 15,000 pupae (Sproule *et al.* 1992, Horwood and Keenan 1994, Perepelicia *et al.* 1994) should be placed in release bins (45 litre plastic) with a maximum of 25,000 (this is for Queensland fly with an average weight of 10 mg – thus needs to be adjusted for other species). Crumpled paper should be placed in the bottom of the bin to provide additional resting space to allow expanding of wings and to absorb excreta. The inside of the bin should be sand blasted to allow adults to grasp the walls. Round bins are not as space efficient as square or rectangular containers and require larger vehicles for transport to release the equivalent number of flies (**Figure 8.1**). Ventilation within bins (through screen-covered openings) is more important than with PARC because the bins are deeper (James 1992, Horwood and Keenan 1994). Respiration gases such as carbon dioxide and ammonia from excreta may pool in the bottom of any container, particularly bins, and adversely affect adults emerging in the bottom of the container (see Sections below).

**Figure 8.1** Bins in the back of a trailer ready for release. The trailer is usually covered to afford shelter to the adults during transport. Longer distance transport should be by air conditioned vehicle. Road transport should be kept to a minimum as this form of transport causes stress to the adult over extended travel. The small air vent can be seen and may be a limitation to this method. (©FAO/ Queensland Fruit Fly Program Australia)

#### 8.1.1.2 PARC boxes

A variant of the adult release method is the release using PARC boxes. These boxes have a 50 litre volume and have a larger floor area than the bins. Pupae are not as deep on the floor and are less likely to overheat. Additional crumpled paper or other dividers can be added to increase resting space for adults. Both these methods may have ventilation problems and volatile waste products (carbon dioxide, ammonia, humidity) may build up in containers and decrease survivability (Horwood and Keenan 1994, James 1992). Holes may be cut in the PARC boxes and covered with gauze or fly screen to assist volatiles to escape the containers. Generally these containers may result in larger numbers of flies (>15,000) deposited in a smaller number of locations, compared with techniques which use smaller release containers such as paper bags.

Ventilation is very important for these methods to draw waste products out of containers. In the holding room, containers are frequently stacked in pyramids to maximise space. These pyramids interfere with air flow and managers needs to ensure that air flow and waste removal is optimised (**Figure 8.2**).

**Figure 8.2** PARC boxes with and without ventilation in the sides. The internal divider for additional standing space can also be seen. Pink pupae can be seen in the bottom of boxes and emergence has not started. Boxes are stacked five high and room ventilation must ensure that waste gases do not accumulate in the boxes. (©FAO/ Queensland Fruit Fly Program Australia)

With both these methods, managers must assess overcrowding stress. Additionally, an excess depth of pupae (particularly in containers with a small base) may contribute to pupal overheating and be detrimental to emergence (Dominiak *et al.* 1998).

Where methods re-use containers or parts of them (plastic bins, PARC boxes, tubs, drinkers, etc), managers need to be aware that cleaning is an important component to minimise the chance of fungal or other pathogens adversely impacting the programme. Care needs to be taken in the choice of cleaning agents as some residues may be detrimental to adults. Sometimes minor changes in cleaning agents (due to supply problems) may significantly adversely impact on adults.

#### 8.1.1.3 Paper bags

Pupae are placed in paper bags (e.g. Kraft No. 20) and adults emerge in and are distributed in the same bags. One linear meter of paper is placed inside the bag to provide adult flies with a resting surface of approximately 2,400 $cm^2$. This method places smaller numbers of pupae in bags and is suited for more releases to be made from more points. This should result in a better distribution of flies over the landscape, compared with plastic bins, PARC boxes or large cages. Commonly bags are about 20 cm length x 10 cm width x 35 to 45 cm height and contain about 4,000 to 8,000 pupae with an expected 80 to 85% emergence.

#### 8.1.1.4 Other types of release containers

There are other containers that could be used for ground release such as the mesh cages and nylon mesh bags (**Figures 8.3, 8.4** and **8.5**). These containers have been tested experimentally in Australia; however, have not been used in large scale operational programmes (Dominiak *et al.* 1998, 2000a, 200b, 2003, Meats *et al.* 2003). They were developed to overcome ventilation problems of solid walled containers such as plastic bins and PARC boxes. These may carry higher adult numbers than bins/boxes as there is no accumulation of waste volatiles. The mesh sides provide easy surface for adult flies to stand. Cages with dimensions of 1.8 m length x 0.7 m width

x 1.2 m height may be seeded with 200,000 pupae, with an expected 86% emergence (Dominiak *et al.* 1998). Larger cages often suffer the same distribution problems as bins in view of the limited number of release points.

**Figure 8.3** Two large mesh cages on a trailer. The sides are held with Velcro (i.e. material which has two sides, sticky hook side and a fur side – it can be pulled apart and pushed together to make a seal) and can be easily pulled open to release adult flies. Cages are transported in utility vehicles or trailers because of their size. This method usually releases large numbers of flies in a small number of confined release points. (©FAO/ Queensland Fruit Fly Program Australia)

Distribution can be improved by the use of smaller cages (50 cm length x 50 cm width x 50 cm height) which contain 16,000 pupae (Meats *et al.* 2003). Field managers need to determine which cage size is suitable for their circumstances. Pupal depth should not exceed 9 mm as the accumulated heat results in decreasing emergence and increases deformed adults (Dominiak *et al.* 1998). Some species also have a lower emergence resulting in different adult populations in cages. This factor may determine the number of pupae placed in cages.

**Figure 8.4** Small mesh cages are easily opened using Velcro lids. These do not have the ventilation problems associated with bins or boxes. Smaller release containers such as these cages allow smaller releases at many more release points than the large cages. (©FAO/ Queensland Fruit Fly Program Australia)

Another similar method is the nylon mesh bag. These bags (~90 cm length x 90 cm width) may contain as many as 80,000 pupae and result in 80% emergence (Dominiak *et al.* 2000a). Bags have Velcro joins in the side panels to facilitate adult release and subsequent washing. These bags are hung on wire racks for emergence. The nylon mesh allows air to circulate through the bag and waste products do not accumulate.

**Figure 8.5** Nylon bags hung on a rack. Pink pupae can be seen in the bottom and adults can also be seen on the bag sides. Water is provided by the wettex or cloth at the top. The bag has Velcro joins in the side panels for easy opening and cleaning. (©FAO/ Queensland Fruit Fly Program Australia)

### 8.1.2 Description of adult ground releases procedures

Once the adult fruit flies have emerged in bags, they are loaded in the releasing vehicles, which must have a shelter to protect the bags from direct sun, rain, wind, etc. Precautions must be considered to avoid excess of movement of the bags during transport. Also, it is not recommended to pile up or compress the bags to avoid unnecessary damage to adults caused by the excessive handling. These release vehicles should be conditioned with at least two levels of racks where paper bags are placed to avoid piling and compressing.

Prior to release, it is critical to know the location of the hosts, in order to efficiently release flies in the field. For this purpose, a host census or data base, as well as the location of detection sites must be determined in advance.

To help the flies to escape from the paper bags, the bags are torn from top to bottom. Handling needs to be with care to avoid damaging the flies.

Traditionally, paper bags and other release containers (e.g. PARC box, plastic bins, etc.) are taken by air-conditioned vehicle to pre-designated release points. These locations should preferably be more than 100 m from any monitoring site. The vehicle is stopped and the container is taken from the vehicle to the site and the adults released under or into the tree canopy. These activities usually take several minutes to complete. This process requires a series of stops and may be considered time inefficient. This may be a minor concern where labour costs are low. The number of release points per hectare needs to be determined, depending on the desired coverage, and the estimated flight distance of insects. Standard or pre-determined release points have been commonly used in

the past, however, there is an increasing trend to roving releases where small numbers are released from a moving vehicle from many points. Fixed point (James 1992, Dominiak *et al.* 1998) and roving releases result in slightly different distributions in the field, and use varying levels of resources – managers need to assess which method is appropriate for their circumstances. Fixed point releases may be located by GPS coordinates and researchers and managers can better understand flight distances, dispersion and distribution factors (See Section 10).

Paper bags may be placed in host trees usually on a weekly basis and old bags are removed in the following distribution cycles (**Figure 8.6**). Some countries have concerns about environmental pollution issues and this may need permission from local authorities. These large numbers of small releases allows better distribution of sterile flies, however, are labour intensive and may be less acceptable in countries where labour costs are high.

**Figure 8.6** This paper bag has been torn open to show the flies inside. Normally the adult flies leave the bag through opening. These empty bags are removed from the tree during the next bag distribution cycle. (©FAO/ PROCEM Argentina)

In the PARC box or plastic bin releases where larger numbers of pupae are involved, unmerged pupae should be returned to the vehicle for re-use and possibly a subsequent second release however this does not occur with bag releases. Unmerged pupae should not be poured on the ground as dye may become lost from the pupal case before emergence and hence compromise the integrity of identification services. Unmerged pupae should be returned to base for destruction.

An alternative is to release flies from a slow moving vehicle in a roving release (**Figure 8.7**) (Salvato *et al.* 2003). This is more time efficient however requires some other considerations. This method minimises the stop/start nature of the fixed point release method and is commonly used in paper bag release, however, other small containers may be used.

Adult flies may be distributed mechanically from a machine, similar to aerial release, however, this adds significantly to the cost of the programme.

**Figure 8.7** Paper bags are stacked on trays in the back of a small truck. Bags may be inverted to save space. Bags need to be torn open to allow flies to escape the bag at release sites. (©FAO/ PROCEM Argentina)

Bags or other small containers may be stacked on the back of a tray-back vehicle and the release person tears the bags or opens the containers and introduces adults into the air stream. Releases are made at regular times or distances but the vehicle does not stop. This option may have occupational health and safety aspects which are strongly regulated in some countries. There also needs to be a systematic approach to ensure spent containers are kept separate from unused containers. Fruit flies tend not to fly in winds >4km/hr and therefore releases from an open cage while the vehicle is moving is unlikely to be successful (Dominiak *et al.*2002a).

An additional option is to chill the adult flies (3 to 6 $^{o}$C depending on the species) prior to release. This ensures that only adults are placed in the release containers. This avoids the need to return the puparia to the release centre. Generally these containers are held at below flight threshold temperatures (~17 $^{o}$C) up to the point of release. After release from these containers, adult flies quickly warm up and fly to trees.

Both these approaches have some general limitations. High temperatures (>30 $^{o}$C) should be avoided as many fly species prefer not to expend energy and not fly at these temperatures. It is generally not recommended to release during rain. Releases when ambient temperatures are below flight threshold are also discouraged as released flies have a low probability of reaching the protection of trees.

### 8.1.3 Situations under which to conduct ground releases

There are various possible situations to conduct adult ground releases (some of these can also apply to other containers used for ground release):

a) **Routine ground releases in predetermined spots:** According with the particular conditions of the area (host distribution, urban v/s rural, accessibility given by roads, topography, distances, required permission to enter properties, etc.), the distribution of the material is pre-determined, identifying every spot where a bag is to be placed. A specific list containing the places is prepared and must be taken in the vehicle during the process of

releasing. In order to meet the desired density, the number of bags for every spot must be specified. This releasing method makes it difficult to cover the area homogenously, and because of that, it is not recommended for general use in extended areas. To conduct this releasing method, the bags are distributed as homogenously as possible. Two general methods are commonly used, namely from vehicles in movement and stopping at every releasing spot:

- Releases carried out stopping the vehicle: At every pre-established releasing spot, the vehicle stops and the bags are placed within the canopy of host trees having both, foliage and fruit. Avoid placing bags within a radius of 100 meters from a trap. As an example, a small vehicle can carry 150 to 300 bags, to cover an area of 400 to 500 has, releasing a density of ca. 2,500 to 3,500 adults per ha (8000 sterile pupae per bag x 85% emergence).

- Releases from moving vehicles: For releases carried out from moving vehicles, the bags are torn and released at regular intervals of 50 to 100 meters. The vehicle usually moves at a speed of 40 km/h. As an example, a large vehicle with capacity of 1,200 bags to cover an area of 3,000 ha per day.

**b) Complementary preventive ground releases in high risk areas:** Some areas require more flies as a preventive measure, because of the risk associated based on historical data. The number of additional bags should be such that the regular fly density in the area is increased.

**c) Complementary ground releases in hot spots or detection areas:** Increased fly releases are sometimes required in a hotspot or following a detection that meets the emergency response trigger, which is 2 or more adult flies, a gravid female or an immature stage detected for the case of Medfly. For implementation of eradication actions the area where the fly find occurred can be defined as:

- 200 meters radius around the detection point (12.5 ha). 10 bags are placed within that radius. Based on experience, ca. 40,000 flying males are expected in the area of 12.5 ha (8,000 sterile pupae per bag x 85% emergence x 60% fliers) (ca. 5,500 sterile flies per hectare).

- 1 $km^2$ (100 ha) around the detection point, where 100 bags are placed. Based on experience, ca. 400,000 sterile flying males are expected in the area of 100 ha (ca. 4,000 sterile flies per hectare).

**d) Complementary ground releases in places difficult to access:** Complementary releases may be required to cover places not easily reached by airplanes (deep valleys, mountainous zones, foggy or hazy zones or other climatic adversities) or zones with aircraft exclusions (airports, military zones). According to the pest situation in the area, the release procedures can be matched either with regular release or high risk zone release.

**e) Back-up ground releases:** Ground releases may be required as a back-up to aerial release when flights are cancelled due to adverse climatic conditions. Regular ground release is used to cover the area.

### 8.2 Pupal Ground Release

Pupal release has been conducted as a routine operation with success only in the case of Australia. Other experiences using this release method have generally not been satisfactory mainly because of

substantial sterile fly losses during emergence and wing stretching due to predation by birds, ants and other predators. Thus a critical pre-condition for use of this release method must be low predation rates.

### 8.2.1 General concepts

Pupal ground release is based on pupae being distributed directly into the field, and the emergence and maturation occurring with minimum human interference. In general, these methods are likely to gain best results if predation (by birds, ants, lizards and other creatures) is minimal. It is also important to produce pupal body weight as higher pupal weights are usually associated with higher survival and competitiveness attributes (Dominiak *et al*. 2002). The main advantage of this method is its low release cost and the virtual absence of any infrastructure requirement. However there are many areas where pupal release would be unsuited and managers need to assess their circumstances. It appears best suited to small release programmes where predation is not a major concern.

One advantage is that there are also indications that adults become acclimatised to the local weather as the pupae are exposed to variable temperatures for the two days between release and adult emergence (Meats 1973, 1984). Indications are that this is particularly valuable when releases are done in autumn and spring, when adults held at constant temperatures are unlikely to fly at lower temperatures (lower than 17°C) (Dominiak *et al.* 2000a). Apart from the adaption to local climate, pupal releases do not suffer any overcrowding stress and adults leave the site when they are ready. Therefore emergence may be extended and is not limited to particular time constraints required by most adult releases. Adults emerge and disperse daily into the environment compared with the sudden large delivery of flies in one day using adult ground releases.

This regular flow of adults leaving the site results in a steady delivery of adults into the environment without any requirement for human operators to revisit the area. Both overcrowding and irregular delivery of flies into the field are potential short comings of adult ground release programmes.

### 8.2.2 Covered pupal releases

Unsheltered pupal release, involving the distribution of unprotected pupae onto the ground is not successful, even with low predation rates, due to climatic influences, particularly heat. Even if these pupae emerge into adults, there is a high chance that the dye on the pupal case may be removed by rain or dew formation. This method has little chance of being successful and is not generally supported.

Ground release of covered pupae therefore attempts to replicate nature where adults emerge from pupae placed underground. In these methods, pupae are poured directly onto the ground and covered in material called a “bed”, and beds may be up to 1 m across and contain 800,000 pupae with 80% emergence possible (Dominiak and Webster 1998). The material holds the pupal case firmly and minimises energy loss during emergence, compared with circumstances where pupal cases can move during emergence in adult releases. Several materials have been evaluated.

#### 8.2.2.1 Sawdust

Dry sawdust has been tried, but hard woods appear to contain toxic compounds which decrease emergence. Dye maybe added to the sawdust to supplement the normal dyeing process associated with dye on the pupal case **Figure 8.8** (MacFarlane and Betlinski 1987).

**Figure 8.8** A bed on the ground using sawdust. Researchers are evaluating emergence and adult survival. Coverings which do not crust maximise adult emergence from the bed. Dry abrasive coverings may damage the fly. (©FAO/Queensland Fruit Fly Program Australia)

#### 8.2.2.2 Sand

Several types of sand have also been evaluated. In general, sand which forms a crust after drying out does not affect emergence of the adults from the pupal case, but adults have difficulty breaking through the crust. Double washed river sand is recommended (Dominiak *et al* 2000b).

#### 8.2.2.3 Vermiculite

Emergence through dry materials often results in superficial damage to the insects cuticle and predisposes the insect to moisture loss and early death. Therefore it is considered that moist vermiculite is better than dry vermiculite, using a mixture of 4 litres of water per 4 litres of vermiculite (Dominiak *et al.* 2003b). A layer of approximately 5 to 10 cm of vermiculite seems to be ideal; however, this needs to be evaluated for different fruit fly species and for different grades of vermiculite. Moist vermiculite appears to be the ideal covering, providing a medium to hold the pupal cases during emergence and to prevent the loss of body weight (Dominiak *et al.* 2002). Moist vermiculite also does not remove dye from pupal cases, however, free water does and this should be avoided.

### 8.3 Pupal Release Methods

There are several methods to house pupae and bedding materials. The basic "bed" technique is to pour the material (vermiculite, sand, sawdust or other) on the ground to a depth of 25 mm, pour evenly the pupae over the bed, and cover the pupae with up to 10 mm of the material. This method has several disadvantages. If placed in full sun, pupae may overheat and die. In some areas, meat eating ants may predate on emerging adults. Ants appear unlikely to harvest covered pupae but some species of ants may take exposed pupal cases. Birds (such as crows or seagulls) may learn that scratching through the bed may offer and easy meal – this will vary in different areas and different bird species (Dominiak *et al.* 2000a). Rain may minimise the dye marking of emerged adults and therefore this method may be more suited to dry regions. The advantages are that up to

800,000 pupae may be deployed at one site with virtually no resources (Dominiak and Webster 1998).

**Figure 8.9** Tray release being prepared. Pupae are first poured onto the base and are being covered by vermiculite. The two house bricks will hold the top tray above the base (see background) and allow the flies to escape from between the two trays.

The ideal container appears to be a white styrene foam box (commonly used to supply vegetables to markets – 30 cm x 58 cm x 29 cm). These are low cost and commonly available. They provide insulation against extremes of temperature. These containers need to allow holes or portals (~3 cm x 10 cm) for the adults to leave the container. These containers can comfortably hold 240,000 pupae, although 80,000 was more commonly used, covered with 6 litre of moist vermiculite (Dominiak *et al.* 2003b). Ideally these portals should have some covering to prevent rain from entering the container and drowning the pupae or adults. Pupal frass should be returned each week when the container is recharged with pupae. During cooler periods, the emergence can be encouraged by placing containers at least 1 m off the ground; this prevents the effects of the cold ground on the pupae. The styrene foam also affords some protection from extremes of temperature.

Flies emerge from containers and obtain nutrition from the two drinkers. Food in the drinkers may be water and sugar, or also include protein, depending on the research results for different species. Bricks create weight to prevent wind turning the container over. In wetter climates, there should be some mechanism to prevent rain entering the boxes (**Figure 8.10**).


**Figure 8.10** Foam box for release being used in late winter. (©FAO/Queensland Fruit Fly Program Australia)

The bucket release allows pupae to emerge and for the adults to leave via the holes. Food and water are suspended from the lid in small containers (**Figure 8.11**). Buckets can be hung in trees however branch pruning is necessary to avoid ants predating on pupae or adults. Buckets require a lid to keep out rain and minimise bird predation.

**Figure 8.11** Bucket release. (©FAO/Queensland Fruit Fly Program Australia)

For ground pupal release a low cost water based food source may be made available by a pet drinker (Dominiak *et al.* 2003b). These containers often have a three litre capacity and would provide food and water for a week.

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# 9. STERILE FLY RELEASE DENSITIES

*STEP V OF PROCESS IN FLOW CHART*

**9.1 Factors to Consider for Establishment Sterile Fly Density** (from Hendrichs et al. 2005)

### 9.1.1 Pest aggregation

Aside from the absolute population density, the degree of population aggregation or dispersion is important. Sterile insects are often released by aircraft, and are thus distributed fairly homogeneously over the target area, irrespective of whether the target pest is distributed evenly or clumped. Pest insects with a clumped distribution require higher release rates (Barclay 2005) as compared with a homogeneous pest distribution, to obtain the required sterile to wild male ratios (Vreysen 2005), and thus pest aggregation also affects strategy selection and its cost. Only if the released insects can find the same aggregation sites and aggregate in a similar manner as wild insects, so that adequate sterile to wild male over-flooding ratios are obtained in those sites, is there no need to increase release rates to compensate for such clumping.

### 9.1.2 Sterile male longevity

The density of the sterile male population in the field, which fluctuates in relation to the release frequency and the sterile male mortality rate, should not decrease below that needed to maintain the critical overflooding ratio (**Figure 9.1**, upper graph) (Barclay 2005; Kean et al. 2005). Therefore, the frequency of release and number of sterile males released has to be carefully assessed in relation to the average longevity or survival of the sterile males, to effectively avoid periods when insufficient sterile males are present in the field (**Figure 9.1**, lower graph).

As generations normally overlap in multivoltine species, releases for such pest species have to be continuous, with survival determining whether releases have to occur once a week (New World screwworm), twice a week (Mediterranean fruit fly, tsetse), or even daily basis (pink bollworm). The importance of assessing the survival of sterile male insects in the natural habitat must be emphasized here, as their actual survival in open field conditions is often drastically lower than in protected field-cage situations, where sterile males have easy access to food and are protected from predation (Hendrichs et al. 1993). In addition, mass-rearing conditions often inadvertently select for short-lived individuals (Cayol 2000). A shorter sterile male lifespan, although not directly representative of competitiveness, often requires higher release frequencies, and thus can significantly increase programme costs compared with longer-lived sterile insects (Hendrichs et al. 2005).

Different species have different average life expectancies in the field, varying from days to weeks. In Queensland fruit fly, the majority (about 80%) of recaptures are made within 3 to 4 weeks of releases (Dominiak and Webster 1998, Dominiak *et al.* 2003a, Meats 1998). In Medfly, Cunningham and Couey (1986) determined that Steiner traps baited with trimedlure caught almost 94% of the total sterile fly recapture 24 hours after release.

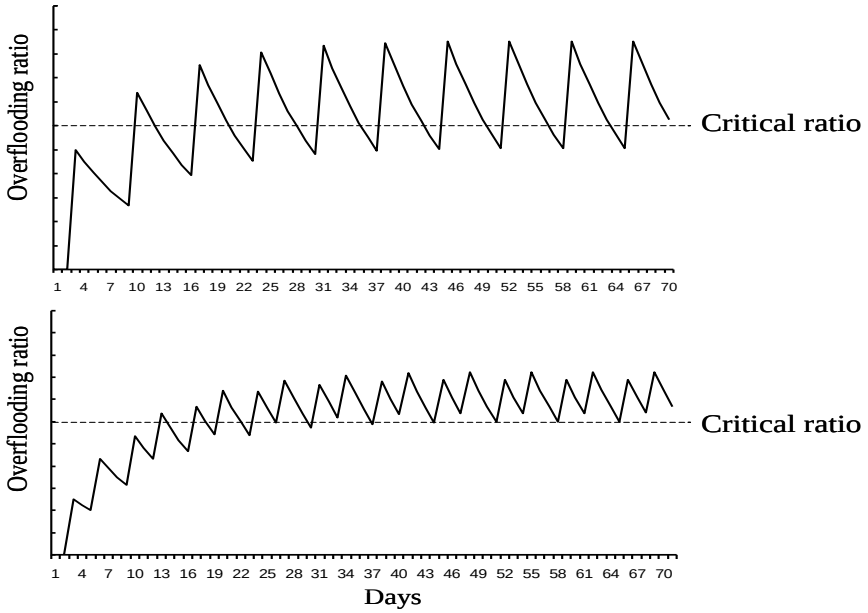


**Figure 9.1** 1 Effect of sterile insect longevity (assume daily mortality rate of sterile males is 0.1) on sterile to wild over-flooding ratio. Upper: Due to only weekly releases, sterile insect population routinely decreases significantly below the critical over-flooding ratio; Lower: twice-a-week releases overcome this problem (from Hendrichs et al 2005).

### 9.1.3 Topography and other conditions of target area

The topography of the target area, combined with the density of roads, has major implications for programme implementation and the selection of an intervention strategy. A flat terrain and a good road network will facilitate most field activities (including ground release in some cases), whereas mountainous areas, dense vegetation, and the absence of roads will complicate implementation. In most of the larger programmes, releases and some of the population reduction activities use aircraft (usually with fixed wing), and the topography and presence/absence of a road network are less critical. Monitoring, however, is mostly ground-based, and extreme terrain conditions make eradication campaigns (which have a more intensive monitoring component) much more complex and costly than programmes following a suppression strategy (which have less intensive monitoring activities). Conversely, the absence of a good road network is advantageous for the establishment of efficient quarantine procedures in support of an eradication strategy. Travellers frequently carry fruit (some of which is infested with fruit flies), and visitors bringing fruit as gifts are common in some cultures. While some fruit flies generally do not fly very far, they are commonly transported in infested fruit by travellers on road networks (Dominiak *et al.* 2000). Irregular reintroductions of infested fruit may act as a source of reinvasion after eradication has been achieved. The regulation or exclusion of this risk fruit via roadways is a key component of any sterile programme.

Likewise, topography influences the requirements of sterile insects or bait sprays, e.g. mountainous areas have a larger surface area per square kilometre as compared with two-dimensional conditions, demanding higher sterile insect release rates. Furthermore, helicopters, which are more expensive to operate than fixed-wing aircraft, are often needed in difficult terrain for safety reasons and to properly treat narrow valleys.

Some production areas are surrounded by desert conditions (Mavi and Dominiak 2001) in what may be described as production oasis surrounded by rural deserts. These conditions occur for example in Australia, Chile, Mexico, and there is no need to treat the surrounding areas as both wild and sterile fruit flies will not survive. In most tropical and subtropical situations, however, where conditions are similar to the surrounding areas, larger areas need to be treated. Modelling can be

used to evaluate if this desert and oasis principle is present (Yonow and Sutherest 1998, Yonow *et al.* 2004, Dominiak *et al* 2003a)

### 9.2 Assessing Release Densities

To establish sterile insect release densities for action programmes that work in fruit fly infested areas, it is important to determine, first, the level of the wild population (for methods to accurately determine the absolute population density, see Ito and Yamamura 2005). It can be also roughly estimated by using a trapping scheme as described in IAEA (2003).

The procedure is as follows:

This procedure assumes that the response of the sterile released flies and the wild flies to traps is equal.

a) Determine the fly/trap/day (FTD) value for the fertile (wild) population:

$$FTD_{wild} = \frac{\text{Total captured wild flies}}{\text{(Total No. Traps) (avg. days in field)}}$$

b) Determine FTD value for released sterile flies, as follows:

$$FTD_{sterile} = \frac{\text{Total re-captured sterile flies}}{\text{(Total No. Traps) (avg. days in field)}}$$

c) With the information from (a) and (b) calculate the sterile:wild ratio present in the field.

$$FTD_{sterile}/FTD_{wild} = \text{Ratio}$$

d) Determine an appropriate S:F ratio according to the action programme objective (**Table 9.1**).

**Table 9.1**. Minimum recommended initial release ratios depending on the action programme objective.

| Programme objective | Avg. Ratios* (for Medfly) |
|---|---|
| Suppression | 25-100 :1 |
| Eradication | 100-150: 1 |
| Containment | 50-150: 1 |
| Preventive Release** | 25-50:1 |

*Minimal S:W ratio. This ratio will continue to increase as $FTD_{fertile}$ is reduced due to suppression and SIT application.

**Suggested ratio to ensure a minimum amount of sterile flies required to outnumber potential entry. Based on the assumption that one wild fly is caught per trap per cycle, irrespective of whether a wild fly is caught or not.

e) If the calculated ratio S:F does not meet the objective of the action programme (see **Table 9.1**) additional non SIT suppression measures need to be implemented before sterile insects can be released (i.e. bait sprays) or additional sterile flies have to be released to increase the over-flooding ratio. Only when the target of $FTD_{fertile}$ of 0.1 has been achieved, should sterile releases be initiated. 0.1 is a rough FTD value, above which it is normally recommended not to use sterile insects except for hotspot situations, (IAEA 2003). ISPM 26. 2006.

*Example:*

*Assuming that 5 traps in 1km$^2$ (100 ha) exposed in the field for 7 days captured 3 wild flies, then:*

*a) $FTD_{fertile}$ = 3 flies/(5 traps x 7 days)= 0.085*

*b) The same calculation using $FTD_{sterile}$:*

*Assuming 1,000,000 sterile flies were released in the same 1 km$^2$ area and that 3,000 flies were recaptured.*

*$FTD_{sterile}$= 3,000 flies/(5 traps x 7 days)= 85.71*

*c) Current sterile:fertile ratio*

*$FTD_S/FTD_F$ = 85.71/0.085 = 1008 ($1008_S$:$1_F$)*

*d) Required number of sterile flies for a 50:1 ratio*
*1,000,000 released sterile flies*
*1008 current sterile:wild ratio*
*50 required sterile:wild ratio*

*(1,000,000*50)/1008) = 49,600 sterile flies in 100 ha (1 km$^2$)*

*e) Number of sterile flies per hectare*

*(49,600/100) = 496 sterile flies/ha*

*If the ratio S:W needs to be increased there are two options to achieve the desired ratio:*

*a) Additional suppression measures (i.e. bait sprays) can reduce $FTD_{wild}$ from 0.085 to $FTD_{wild}$ = 0.03, therefore the new S:W ratio is, 142:1 (0.085/0.03*50)*
*b) Increase the sterile fly numbers to achieve the required ratio of steriles (ie. 142); to calculate the new release numbers, substitute the new ratio in d) above.*

*(1,000,000*142)/1008) = 165,675 sterile flies in one km$^2$ or 1,657 in one hectare*

As the control process progresses the initial S:W ratio will increase. This ratio will continue to increase as long as the $FTD_{fertile}$ continuous to decrease due to suppression measures and release of sterile flies is kept constant (**Figure 9.2**).

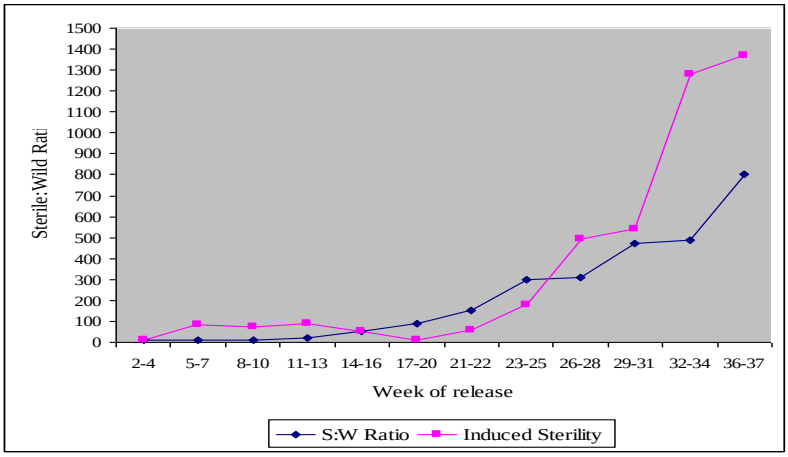


**Figure 9.2** Increased S:W ratio as result of SIT control.

Recapture of sterile flies is affected by the release mechanisms, release rates, seasonal changes in trapping efficiency and the environmental conditions of the area such as topography, vegetation and host density. **Figure 9.3** illustrates the effect of the release rate on the number of sterile flies/trap/day (FTD) in an oasis environment where a range of 500 to 1000 sterile flies per hectare where released in Oasis North and 1000 in Oasis East. **Figure 9.4**, illustrates the sterile FTD fluctuation due to changes in climate conditions of the same areas presented in **Figure 9.3**. Managers should be aware of these variations to decide on the most appropriate number of sterile insects to be released in order to maintain the required sterile:wild ratio.

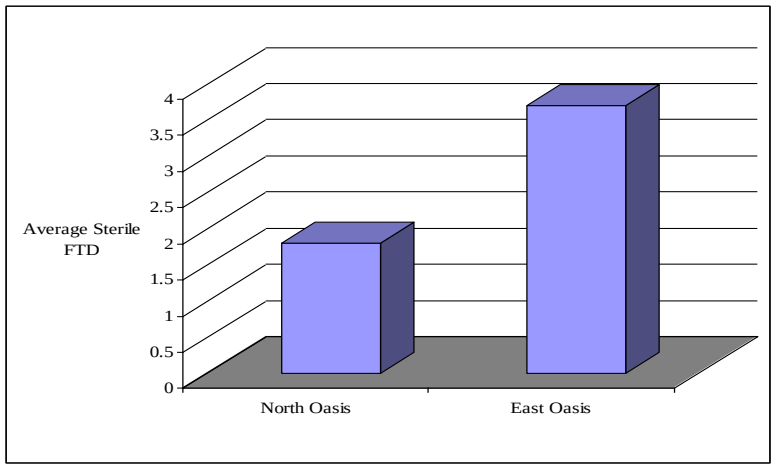


**Figure 9.3** Effects of release densities on the number of sterile flies per trap per day (FTD) using the bag release system in the North (500-1000 sterile flies/ha) and East Oasis (1000 sterile flies/ha) in the Province of Mendoza, Argentina, 2004 -2005.

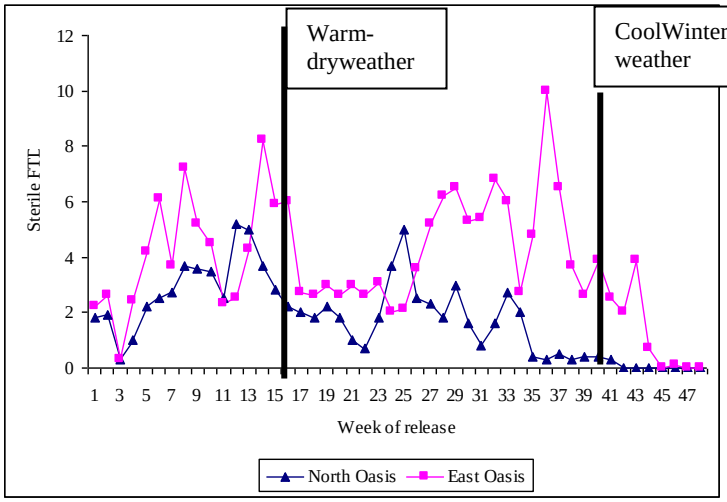


**Figure 9.4** Fluctuations of sterile flies per trap per day due to changes in the climatic conditions in the North and East Oasis in the Province of Mendoza, Argentina, 2004 -2005.

The Regional Medfly Program in Guatemala and Chiapas, Mexico, is applying an Excel spreadsheet calculator that allows determining sterile insect release densities and their respective sterile:wild ratio for aerial release blocks (Rendon, 2010). The computer model operates at the regional program aiding program managers, as follows:

For trapping purposes, each aerial release block uses Phase IV and Jackson Traps at a 9:1 ratio, on average the trap density used at the operational program is of 2 traps/$Km^2$. Phase IV traps (similar function of the more widely used Multilure traps) are baited with food based attractants/Biolure to detect wild flies (in average 60 % female and 40% male flies) and male specific Jackson Traps baited with Trimedlure to catch wild and sterile male flies (**Figure 9.5**). This trapping array allows to assess sterile: wild release ratios on a weekly basis. To calculate the S:W ratios, all male and female wild flies captured in Phase IV and Jackson Traps are recorded. For sterile male capture only sterile males captured in Phase IV Traps are considered. The reasoning behind excluding male captures from Jackson Traps is based on action program data, in which it has been determined that the number of sterile males captured in Jackson traps is ca. 7 times greater from those captured with food based attractants/Biolure Phase IV Traps. Continuous release of high-density sterile males flies over the same areas where the trapping network operates, including the male specific Jackson Trap, results in a high recapture rate of sterile males flies compared with wild male flies. To overcome this problem and avoid having biased information in favor of sterile male flies present in the field, Jackson Traps are not considered in the calculations of recaptured sterile males. In addition, with respect to wild insect capture, a comparison of wild fly captures in food based attractants/Biolure Traps and Jackson Traps, reveals that the capture of wild flies is similar in number for both traps but that food based attractants traps, also favor the capture of unmated and mated females (**Figure 9.6**).

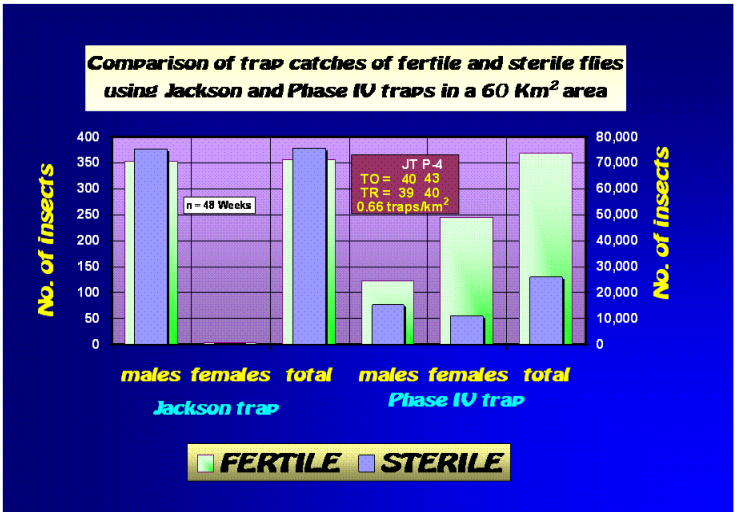


**Figure 9.5** Number of fertile and sterile flies captured using Jackson /Trimedlure and Phase IV/Biolure Traps.

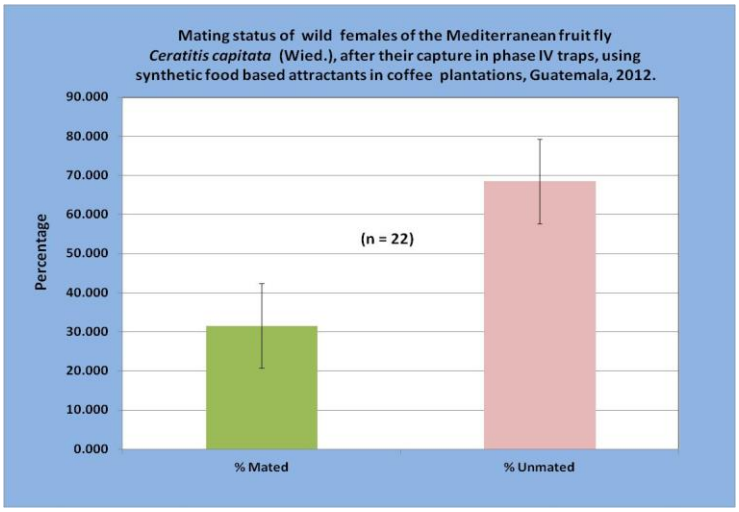


**Figure 9.6** Proportion of gravid females flies and non-gravid captured in Phase IV Traps.

Procedures to apply the Excel Spreadsheet Calculator:

a) The exclusion of sterile male capture from Jackson Traps for the sterile: wild ratio calculations, allows for a single recapture rate (from Phase IV combined with food based attractants) to enter the calculations. The calculation of the sterile: wild ratio is achieved by determining first the value of FTD (flies/trap/day) for both sterile and wild flies and then dividing the $FTD_{(sterile)}/FTD_{(wild)}$ = sterile: wild ratio.

b) FTD = Number of flies captured $_{(sterile\ or\ wild)}$/Total # of traps within the block * average number of days of trap exposure in the field (7 or 14 day trapping cycle).

c) To adjust the operational release densities it is recommended to take into account the information collected during 4 weeks of program data to observe the trend of pest population and reduce the variability of the data and increase the confidence in the decision making.

d) In the event of no wild flies being caught, a wild capture of 0.25, 0.50, and 1.00 flies per release block is assumed for free, low prevalence and suppression areas, respectively. A zero value for wild flies will prevent the Excel model from computing a recommended sterile fly density, thus, a rate of approach for specific areas is assumed in order to continuously maintain the release of sterile males over the target area. The assumed capture of 1 fly/month is equivalent to 0.25 flies/week in the free area; for low prevalence 2 flies/month and 4 flies/month in the suppression area.

e) The sterile:wild ratios used in these calculations were generated from experimental field data (**Figure 9.7**) that uses the optimum sterile fly density and S:W ratios for areas with continuous hosts such as coffee plantations (Rendon, 2008). These information was used to determine the S:W ratios required for prevention and eradication in free areas, prevention and eradication in low prevalence areas and suppression and containment in suppression areas (Table 9.2). The recommended ratios should be validated for its use in coastal areas, highland valleys and mountainous areas with mixed and scattered fruit hosts.

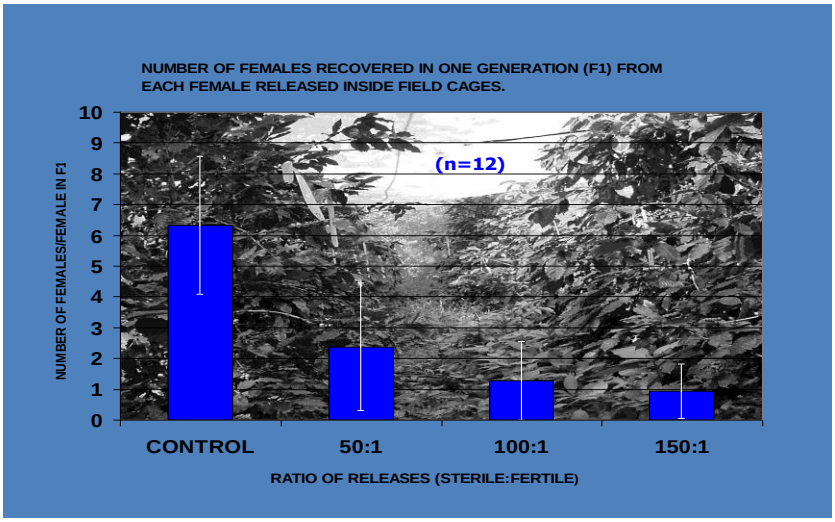


**Figure 9.7** Sterile to wild ratio required for population suppression and eradication.

**Table 9.2** Appropriate ratios for the target objective in each working area.

| Working areas | Objectives | Sterile:Wild ratio |
|---|---|---|
| Free | Preventative release | 25-50 to 1 |
| | Eradication | 150-200 to 1 |
| Low prevalence | Preventative release | 25-50 to 1 |
| | Eradication | 150-200 to 1 |
| Suppression | Suppression | 100-150 to 1 |
| | Containment | 25-50 to 1 |

**Table 9.3**, taken from the FAO/IAEA trapping guidelines (FAO/IAEA, 2003) describes the FTD values (wild population size) which define pest area status (infested, suppression, eradication and exclusion). Within this context, it has been determined, based on local action program data, that the use of sterile insects as part of the eradication effort is optimized when the FTD values have been reduced to an FTD figure lower than 0.05. Population reduction to this FTD value is achieved by other control methods such as the application of aerial and ground bait sprays, bait stations, other biocontrol methods and mechanical control (i.e. fruit stripping). Reduction of the wild population to less than 0.05 $FTD_{wild}$ warrants the use of lower release rates of sterile insects and leads the program activities to pest eradication if the appropriate sterile:wild ratios are reached (**Table 9.2**, **Figure 9.7**).

**Table 9.3** Matrix of the different trapping scenarios.

| Trapping Survey | Trapping Applications | | | |
|---|---|---|---|---|
| | **Infested Area FTD > 1** | **Suppression FTD: 1 - 0.1** | **Eradication FTD: 0.1 – 0.0** | **Prevention (Containement, Exclusion) FTD: 0.0 – 0.0** |
| Monitoring | x | x | x | |
| Delimiting | | x | x | |
| Detection | | | | x |

FTD = Fly/Trap/Day (values used only as reference)

Example of application of the Excel Spreadsheet Calculator for an area under population suppression:

In a block of 15,000 ha (150 $km^2$), located in suppression area, there are 300 traps (270 phase IV and Jackson 30) exposed in the field for 7 days. Assuming that in current week in

Phase IV Traps there were: 28 wild female flies, 18 wild male flies and 5,929 sterile male flies; in Jackson Traps there were: 3 wild male flies. Then:
Current release rate=75,000,000 sterile flies at a density of 5,000 flies/ha.
*$FTD_{sterile}$= 5,929 flies/(270 traps x 7 days)= 3.1529*
*$FTD_{wild}$ = 49 flies/(300 traps x 7 days)= 0.0233*
*Current sterile:wild ratio=$FTD_S$/FTDw=3.1529/0.0233 = 135*
*Required ratio for a suppression area is 150:1 (**Table 9.2**)*
*New release density=75,000,000*150/135=83,333,333/15,000ha=5,543 flies/ha*

The required adjusted release density for 5 consecutive weeks is shown in **Table 9.4** and **Figure 9.8**.

**Table 9.4** Densities required according to S:W ratios achieved in a suppression area.

| Week | Density release flies/ha | Total released flies/Km$^2$ | FTDw (♂+♀) | FTDs (♂) | Current S:W ratio | Required S:W ratio | New rate sterile flies/km$^2$ | New rate sterile flies/ha | FTDw[1] | Upper limit viable density (sterile flies/ha) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5,000 | 500,000 | 0.0233 | 3.1529 | 135 | 150 | 554,252 | 5,543 | 0.05 | 6,000 |
| 2 | 5,543 | 554,300 | 0.0148 | 2.9204 | 197 | 150 | 421,362 | 4,214 | 0.05 | 6,000 |
| 3 | 4,214 | 421,400 | 0.0150 | 2.0156 | 134 | 150 | 470,406 | 4,704 | 0.05 | 6,000 |
| 4 | 4,704 | 470,400 | 0.0125 | 1.8230 | 145 | 150 | 484,979 | 4,850 | 0.05 | 6,000 |
| 5 | 4,850 | 485,000 | 0.0171 | 2.4805 | 145 | 150 | 502,779 | 5,028 | 0.05 | 6,000 |

[1]FTDw = 0.05 is the pest population limit set by the Moscamed Program for SIT use when the objective is population suppression.

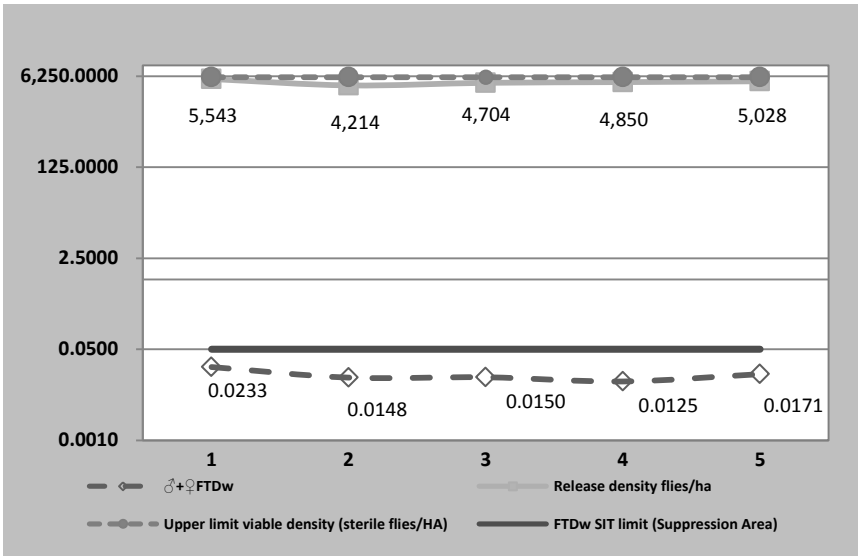


**Figure 9.8**Required release densities of sterile insects/ha to achieve a 150:1 sterile♂: wild ♂ ratio compared to Male Flies/Trap/Day (FTD) captured in a resident population in a suppression area.

Example of application of the Excel Spreadsheet Calculator for an area under population eradication:

Assuming a total of 21 wild flies (males and females) caught in the two trap types.

Current release rate=30,000,000 sterile flies at a density of 3,000 flies/ha.
*$FTD_{sterile}$= 4,069 flies/(270 traps x 7 days)= 2.1529*
*$FTD_{wild}$ = 21 flies/(300 traps x 7 days)= 0.0098*
*Current sterile:wild ratio=$FTD_s$/FTDw=2.1529/0.0098 = 220*
*Required ratio for an eradication area is 200:1 (**Table 9.5**)*
*New release density=30,000,000*200/220=27,272,727/15,000ha=2,731 flies/ha*

The required adjusted release density for 5 consecutive weeks is shown in **Table 9.5** and **Figure 9.9**.

**Table 9.5** Densities required according to S:W ratios achieved in an area under eradication.

| Week | Density release flies/ha | Total released flies/Km$^2$ | FTDw ♂+♀ | FTDs ♂ | Current S:W ratio | Required S:W ratio | New rate sterile flies/km2 | New rate sterile flies/ha | FTDw[1] | Upper limit viable density (sterile flies/ha) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3,000 | 300,000 | 0.0098 | 2.1529 | 220 | 200 | 273,120 | 2,731 | 0.01 | 3,000 |
| 2 | 2,731 | 273,100 | 0.0085 | 1.9204 | 225 | 200 | 242,895 | 2,429 | 0.01 | 3,000 |
| 3 | 2,429 | 242,900 | 0.0080 | 2.0156 | 253 | 200 | 192,334 | 1,923 | 0.01 | 3,000 |
| 4 | 1,923 | 192,300 | 0.0070 | 1.9980 | 286 | 200 | 134,552 | 1,346 | 0.01 | 3,000 |
| 5 | 1,346 | 134,600 | 0.0036 | 2.0189 | 562 | 200 | 47,869 | 479 | 0.01 | 3,000 |

[1]FTDw = 0.01 is the pest population limit set by the Moscamed Program for SIT use when the objective is population eradication in an area.

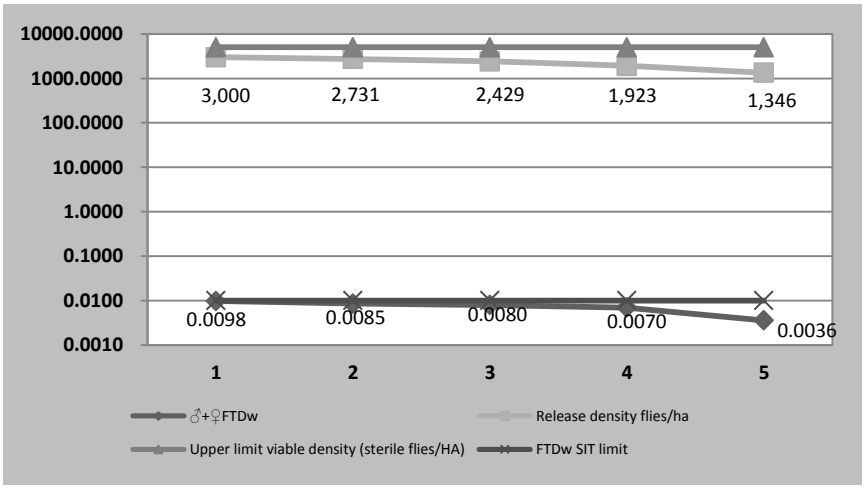


**Figure 9.9**.Required release densities of sterile insects/ha to achieve a 200:1 sterile♂: wild ♂ ratio compared to Male Flies/Trap/Day (FTD) captured in a resident population in a low prevalence area.

The Excel model allows sterile fly densities to be adjusted, resulting in an optimization of sterile fly use which is the basis for area-wide SIT programs. When the established S:W ratio and the FTDw

are below or above the set limits required to achieve the program goal (suppression, eradication, prevention (contention and exclusion)) using the initial sterile fly density, the model works-out an adjusted density (above or below the initial density) in order to reach the goal. In the example given above of an area under population eradication, the model adjusted the sterile fly density to a lower number on a weekly basis. This as a result of having the required S:W ratio to eradicate populations above the set ratio of 200 to 1, as well as an FTDw below the set level of 0.01. In the example, the S:W ratio was 220, 225, 253, 286 and 562 and the FTDw 0.0098, 0.0085, 0.0080, 0.0070 and 0.0036 in five consecutive weeks, as shown in **Table 9.5** and **Figure 9.9**. Adjusting the initial sterile fly density from 3,000 flies/ha to 1,346 flies/ha without jeopardizing the objective of eradication, represents a substantial cost saving for a program. The opposite may also occur, this is, a situation where the initial sterile fly density is insufficient to obtain the required S:W ratio of 200 to 1 and as a result the FTDw is above the pest limit of 0.01. In this case the model will adjust for a higher density compared with the initial one.

The existing SIT programmes, their objective and actual sterile insect release densities are shown in **Table 9.6**. Programmes that are initiating area-wide SIT operations should determine their required release densities considering the conditions under which activities will be conducted such as assessed wild FTDs, objectives of the programme (suppression, eradication, etc.) and established over-flooding ratios. In practice over-flooding ratios (sterile:wild) have varied from as low as 50:1 (Wong et al. 1986) to 200:1 and as high as 1000:1 (Fisher et al. 1985, McInnis et al. 1994).

**Table 9.6** Release densities for different fruit fly SIT programmes and their respective programme objectives.

| Country | Fruit fly species | Objective | Aerial Release Density (Male Flies*/Ha) | Main Host and Area Characteristics |
|---|---|---|---|---|
| Argentina | Medfly (*C. capitata)* | Eradication<br>Prevention | 500-3,000<br>250-1500 | Stone and soft fruit (peaches, plums, apples and others)/Oasis-Valleys with extreme high/low temperatures. |
| Australia | Qfly (*B. tryoni*) | Prevention<br>Eradication | 1,000<br>Not available | Soft fruit (tomatoes)/stone (peaches, plums)/Flat and dry area. |
| Brazil | Medfly | Suppression | 1,000-2,000 | Mango and grapes subtropical conditions in a valley |
| Chile | Medfly | Prevention<br>Eradication | 1,500-2,500<br>>3,000 | Guava, mangoes/isolated valleys surrounded by mountains and desert. |
| Guatemala | Medfly | Containment<br>Eradication | 5,000 | Continuous coffee, mixed host rural areas/coastal, valley and mountainous area. |
| Israel | Medfly | Eradication<br>Suppression | 1,000 | Citrus and urban backyard hosts |
| Japan (Okinawa) | Melon fly (*B. cucurbitae)* | Prevention | Not available | Garden crops and urban backyard hosts |
| Jordan | Medfly | Eradication | 1,000 | Citrus and urban backyard hosts |
| Mexico | Medfly | Eradication | 2000- 5,000 | Continuous coffee, mixed host rural areas/coastal, valley and mountainous area. |
| Mexico | Mexfly *(A. ludens)* | Suppression | 2,500 | Citrus, Guava, mangoes production areas/coast, oasis, mountainous area. |
| Mexico | West Indian fruit fly (*A. obliqua)* | Suppression | 2,500 | Mangoes, coast and mountainous areas. |
| Peru | Medfly | Eradication | 1,000-2,000 | Olives/oasis |
| Portugal (Madeira) | Medfly | Suppression | 3,000-5,000 | Mixed fruits and vegetables |

| | | | | |
|---|---|---|---|---|
| Spain | Medfly | Suppression | 1,000 - 2,000 | Citrus |
| South Africa | Medfly | Suppression | 1,200 | Grapes/isolated valleys – dry with irrigation |
| Thailand | Oriental fruit fly (*B. dorsalis)* | Suppression | 5,000 | Pilot areas of mango orchards with no isolation |
| | Guava fruit fly *(B. correcta)* | Suppression | 5,000 | |
| USA California | Medfly | Prevention<br>Eradication | 250<br>1,000 | Urban (Jungle) fruit and vegetables.Variable climate and topography. |
| USA Florida | Medfly | Prevention<br>Eradication | 500<br>1000-1400 | Citrus and urban host/Coastal area, tropical. |
| USA Hawaii | Melon fly | Suppression | Not available | Experimental- Tropical, melon, squash. |
| USA Texas | Mexfly | Suppression | 650 | Citrus and urban host/semi-arid with irrigation |

*Adjusted for percent emergence, however, not for flying males.

### 9.3 References


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# 10. GPS-GIS SUPPORT TO STERILE RELEASE PROGRAMMES

*STEP V OF PROCESS IN FLOW CHART*

The Geographic Information Systems (GIS) are computerized systems to acquire, store, analyze and display geographic information to support the decision making process. The Global Position System (GPS) was developed in the 70s by the Department of Defense (DoD) of the U.S.A to provide a location system in any part of the world. Both systems, together with the Information Technologies (IT) -specifically the databases (DB) - have been developed and now are accessible for anyone with interest. Also their cost has significantly decreased.

Prior to the development of the current Global Positioning System – Geographical Information System (GPS-GIS) in use today, flying and releasing was done by visual means both on the ground and in the air. Personnel were stationed at various positions on the ground with flags and/or balloons to guide aircraft along flight paths and to define the release areas. This was a very inaccurate and time consuming operation that required numerous personnel in sometimes harsh environmental conditions. Pilots were required to visually fly areas utilizing landmarks that were often hard to define or lacking altogether. Maps were few and the ones that were there were normally out of date.

With the current GPS-GIS capabilities, the actual position and location of where the aircraft is required to fly can be actually recorded and verified during the flights. Data such as position of aircraft, in either geographic (latitude/longitude) or projected (x,y) coordinates, altitude flown, speed of aircraft, lane numbers of release, deviation from the lane, flight time, time to cover a release area, speed of the release machine operation, whether the release machine is operating or off is actually recorded and provided after each flight.

### 10.1 Mapping of Release Areas

When a programme area is initially defined, the most recently availablemaps are normally obtained and used to determine how and where a release will be done. The points that define the boundaries of the area are put into the open source or commercially available GIS mapping system which in turn will use this data along with the lane spacing and direction of flight to map the flight lanes. If there are no maps or if the maps have changed drastically from when they were published, these systems can still be utilized.

The design of the release polygons (areas or blocks) can be done with the support of GIS. GIS layers such as elevation of the terrain (topography), host availability, wild insect populations are needed in order to design the release polygons adequately. The orientation of the flight lanes is defined by the topography, especially if the terrain is steep, in which case the lanes have to be perpendicular to the main slope.

After the release polygon and flight lanes are designed, estimations of the flight time to cover the areas can be conducted for budgeting and scheduling purposes.

The system can map the boundaries and lanes with data provided or the boundaries can be flown and recorded in the flight data recorder. Then the mapping systems are used to draw the actual release areas and flight lanes. All the data recorded can be downloaded after the flight, and some systems allow the live monitoring of the flights.

Currently, there are two options for GIS software that can be used for mapping a release area:
- Private proprietary software like ArcGIS from ESRI
- Free Open Source Software (FOSS) like Quantum GIS

Both private and FOSS software largely meet the requirements for design, support and follow up of the aerial releases.

Private software requires a license that is normally expensive, which is disadvantageous for small programmes. Although FOSS was not very user friendly several years ago, it has now become a widely used tool with almost all features that can be found in private software. IAEA is now finalizing a tutorial on the use of GIS FOOS that will be release by the end of 2013.

GIS software also allows designing curvilinear release paths that fit better in release blocks with a non-regular shape. Release paths must always keep the requirement of being parallel at a predefined distance (Figure 10.x). The snake lines can help to shorten the duration of the flight, reducing costs significantly.

If releases are done following long curvilinear path, the previous requirement is not necessary but instead of it, the system must be able to accept track files with a high number of beacons and coordinates. **Figure 10.1**

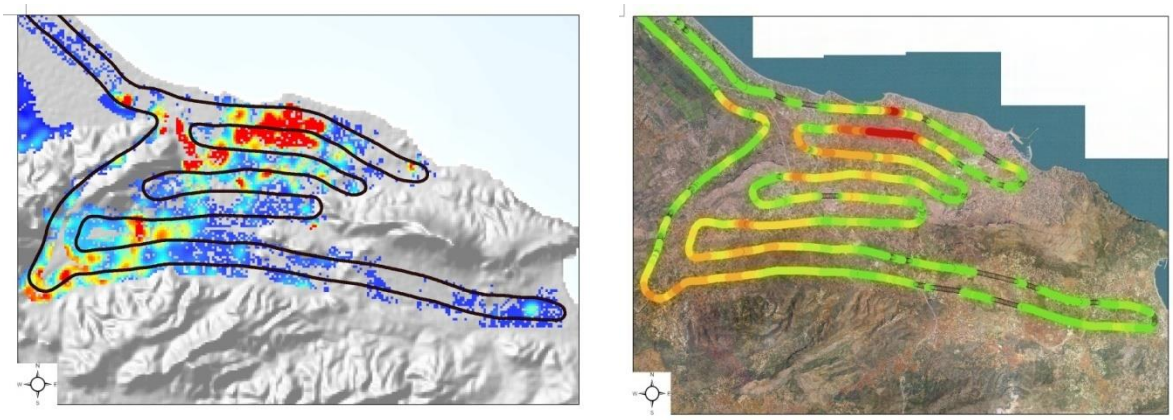

**Figure 10.1** Release block with non regular shape and curvilinear release path adapted to it. The colour scale on the left map show desired release intensity and on the right map shows the calculated release rate at every beacon on the release track. (©FAO/Programa Moscamed Mexico, Guatemala, USA)

### 10.2 Common Requirements for GPS-GIS in Aerial Releases

The system needs to be able to record and display the date and time of the entire flight from takeoff to landing and differentiate between standard flight and flight when the release system is on/off. The system should provide immediate deviation indications that are sufficiently accurate to keep the aircraft on the desired flight path and also other features:

- A compact moving map display with polygon feature that will alert the pilot when the aircraft is entering or exiting a specific geographic polygon. This map display can be used by the pilot of the aircraft to make the turns within the release polygon.
- Software designed for parallel offset in increments equal to the assigned swath width of the application aircraft.

- The system must be able to manage two or more release polygons in on flight.
- The system should be able to turn off the release machine in areas defined as "exclusion zones" (areas that cannot or not need to be released).
- The system should have the capability of release at different densities as needed (see section 7.2.1).
- A course deviation indicator (CDI) or a course deviation light bar must be installed on the aircraft and in a location that will allow the pilot to view the indicator with direct peripheral vision without looking down. The CDI must be capable of pilot selected adjustments for course deviation indication with the first indication at 1 meter or less.
- The system must display to the pilot the current lane number and cross-track error. The lane advance may be set manually or automatically. If automatic is selected, the pilot must be able to override the advance mode to repeat applications of single or multiple lanes.
- The system must be equipped with software for flight data logging that has a system memory capable of storing a minimum of 4 hours of continuous flight log data set at any desired interval, either time or distance intervals. The full logging record will include position, time, date, altitude, ground speed, cross-track error, release on/off, insect release machine auger or motor RPM, aircraft registration number, pilot name, and job name or number.
- The system should be provided with sensor to log other required information, such as humidity and temperature in the release machine, and the weather parameters.
- The flight data log software shall be compatible with GIS software.
- The system must be malicious software protected.
- The system must compensate for the lag in logging release on/off. The system will display release on/off at the boundary without a saw tooth effect. Must be capable to end log files, rename and start a new log in flight.
- When viewed on the monitor or a printed copy, the flight path will clearly differentiate between release on/off.
- The software must be capable of displaying the entire flight in slow motion and stop and restart the replay at any point during the flight (not essential). Must be able to zoom any portion of the flight for viewing in greater detail and print the entire flight or the zoomed-in portion.
- Must have a measure feature that will measure distance in meters or feet between lanes or any portion of the screen. Must be able to determine the exact latitude/longitude at any point on the monitor.
- Flight information software provided with the system must have the capability to interface with other mapping software. The interface process must be "user friendly", as programme personnel will be responsible to operate the system in order to access the information.
- The system must provide a detailed report of the incidents during the flight, mainly significant deviation of lanes. This report should include at least the number of line, altitude of deviation, the location where the deviation occurred.
- A "Users Manual" must be provided with the equipment and the data logger software.
- All recorded flight information at the end of each day will be provided to the programme personnel. Information should be provided on external USB Memories or if another means is used, a downloading device to enable programme personnel the ability to retrieve information must be provided. Some systems are provided with technology live transference of data, but costs are relative high.

### 10.3 Common Requirements for GPS-GIS for Ground Releases

For ground releases, all monitoring trap site coordinates should be recorded using GPS. Releases should not occur within 100 m of a monitoring site. Release staff should be provided with paper or electronic devices to ensure the 100 m buffer is maintained. If releases are made too close to traps,

high numbers of sterile males will be trapped. Large numbers of sterile flies in traps may artificially indicate a high recapture rate, suggesting the sterile fly population is higher than it really is. Large numbers of sterile flies in traps create an additional and unnecessary work load for identification services. Additionally, if a single wild fly enters the trap with hundreds of sterile flies, dye transference becomes increasingly likely, creating uncertainty and additional work for identification services. Sterile flies are expensive to produce and distribute and should not be wasted by releases near monitoring traps. The use of GPS-GIS technology helps to avoid these problems and ensures efficient use and monitoring of the SIT operations (IAEA 2006). Also, having the coordinates of ground releases sites and analyze them before going into the field with a GIS, can help to schedule in a better manner the field activities, i.e. release in the early morning and with appropriate weather conditions.

### 10.4 Post-processing

The data logged during the flights and the releases polygons can be overlaid with other information, such as elevation, sterile and fertile captures in the traps in order to evaluate the performance of the release activity. The following parameters can be better analyzed and represented with the support of GIS.

- Altitude over the terrain: The GPS installed in the aircraft reports the altitude over the sea level (AOSL); however pest management programmes are interested on measuring the altitude over the terrain (AOT) (**Figure 10.2**). This parameter can be estimated with the GIS if a Digital Elevation Model (DEM) of the terrain is available, with the equation:

  AOT = AOSL – DEM

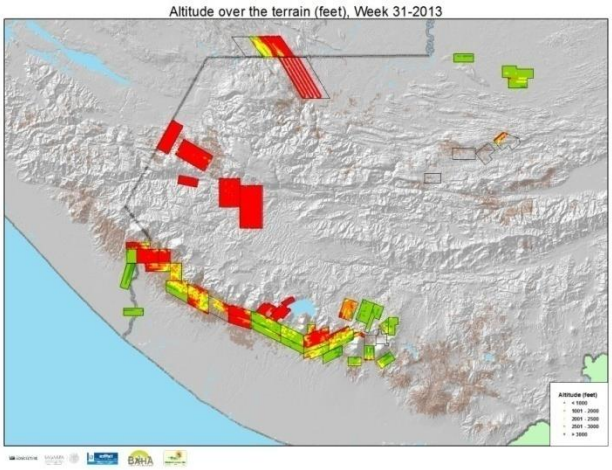


**Figure 10.2** Maps with the AOT for the Regional Medfly Program in Guatemala and Mexico. (©FAO/Programa Moscamed Mexico, Guatemala, USA)

- Sterile captures within the release polygons: Using the trapping network and considering the release polygons as Area of Interest (AOI), different analyses can be conducted to evaluate the performance of the release activity. The analyses can be done at polygon, trap level or using interpolated information from the traps. Number of sterile captures and percent of traps within the block with sterile captures can be indicators of this performance. If these two variables are low, decisions to improve the release activity should be made.

  Sterile Captures outside the release polygons: Captures outside the release polygons are also important to analyse. If too many flies are captured outside the blocks, it can be considered that sterile insects are not reaching the targeted area (**Figure 10.3**).

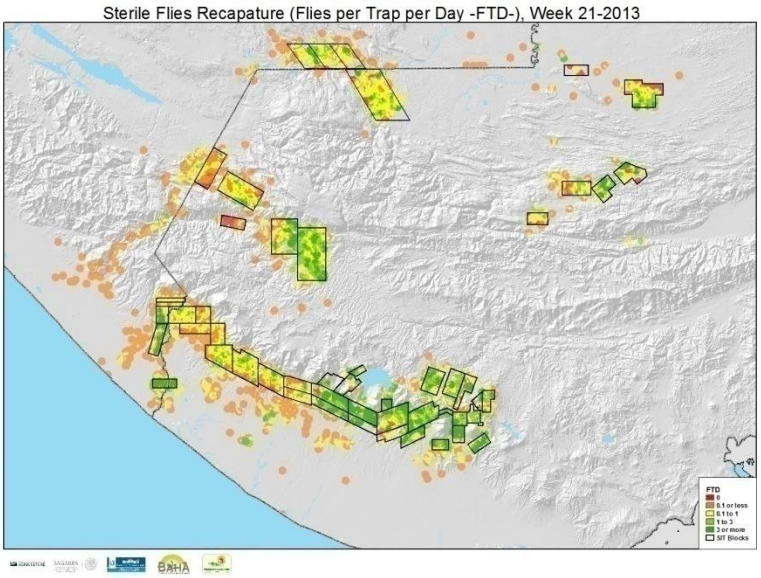


**Figure 10.3** Map of the sterile recapture from the Regional Medfly Program in Guatemala and Mexico, where the sterile captures are evaluated inside and outside of the release polygons, using interpolation techniques. (©FAO/Programa Moscamed Mexico, Guatemala, USA)

- Sterile to wild ratio (SWR) – the sterile to wild ratio can be also used to adjust the release densities (**Figure 10.4**).

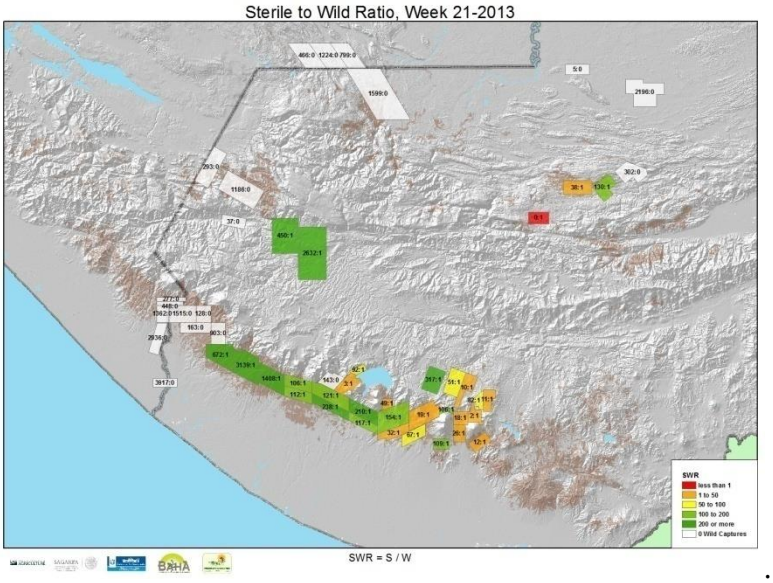


**Figure 10.4** Map of the sterile to wild ratio from the Regional Medfly Program in Guatemala and Mexico, which shows the sterile to wild ratio of each of the release blocks. (©FAO/Programa Moscamed Mexico, Guatemala, USA)

If the information of the captures is analyzed at trap level or with interpolated information, problematic areas within the polygons can be addressed, and specific decisions in those areas can be made.

For example, the sterile to wild ratio of the whole block can be explained due to the wild captures. Even in the blocks with relative good sterile fly recapture, the SWR is low due the high number of wild captures (**Figure 10.5**).

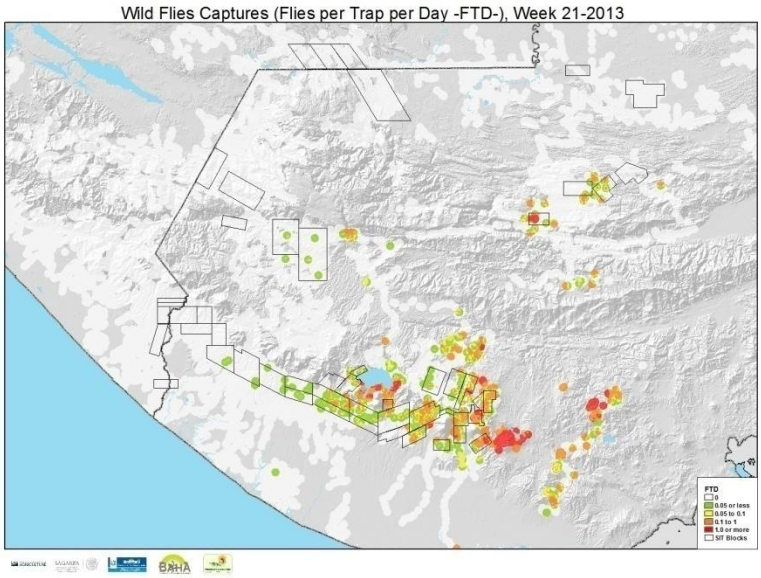


**Figure 10.5** Wild flies captured transformed to flies per trap per day (FTD) in the release blocks. (©FAO/Programa Moscamed Mexico, Guatemala, USA)

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# 11. INTERPRETATION OF STERILE FLY RECAPTURE

*STEP VI OF PROCESS IN FLOW CHART*

## 11.1 Background

Application of the SIT against fruit flies was first attempted at least 45 years ago (**Table 11.1**). These early programmes demonstrated the potential for significant population reductions up to and including eradication.

A chronology of all significant field trials and operational programmes up to 1992 was compiled by Klassen et al. (1994). This list includes multiple species of tephritid fruit flies.

**Table 11.1.** Early recorded tephritid fruit fly programmes or pilot test applying the SIT (from Robinson and Hooper 1989).

| Country | Fruit Fly | Area ($Km^2$) | Sterile Flies Released | Time frame | Sterile flies per ha per week | Population Reduction | Comments |
|---|---|---|---|---|---|---|---|
| USA – Hawaii | Medfly (*C. capitata*) | 31 $km^2$ | 187 mil. | Ca. 1 year (end July 1960) | 116 | 90% | Pilot test |
| Marianas / Rota | Melon fly (*B. cucurbitae*) | 85 $km^2$ | 257 mil. | 11 months (Sept. 1962-July 1963) | 720 | Eradication | First successful eradication of an insect species other than screwworm with SIT approach |
| Nicaragua | Medfly | 48 $km^2$ | 40 mil. | 9 months (Sept. 1968-May 1969) | 278 | 90.1 egg 91.1 larvae | 2 km wide buffer around release area sprayed |
| Costa Rica | Medfly | 2.5 $km^2$ 48 $km^2$ | 2 mil./wk 48 mil. | 1964 1968-1969 | 8,000 Not available | 90 % | Promising results; compared with two controls |
| USA – California | Medfly | 258 $km^2$ | 500 mil. | 1975 (7 months) | 646 | Eradication | Ground applications of bait sprays were applied with unsuccessful control |
| Tunisia – Porto/Farina | Medfly | 6 $km^2$ | 250 mil. | 1972 (9 months, March –Nov.) | 11,000 | 97 % | Equally effective as chemical control plot comparison |

The first organized attempt at documenting and evaluating data from tephritid eradication programmes using SIT, evolved during the 1981 San Jose/Santa Clara, California, USA, Medfly Project. Trap catch figures were entered manually on drawn grid maps as total flies per square mile. From 1984-1987, data for each trap was displayed electronically on a grid printout representing the release area. Flies retrieved per trap indicated the actual numbers counted by the identification section.

R. H. Cunningham indicated a need for a more timely reporting tool to capture sterile fly distribution. A model report was developed to display distribution of fly numbers within each square mile. As well, this report provided additional information that was absent from the previous reporting system. This report now included an account of the total number of flies retrieved in each trap rather than a single number for the square mile. This since has been referred to as the Cunningham Report (**Figure 11.1**).

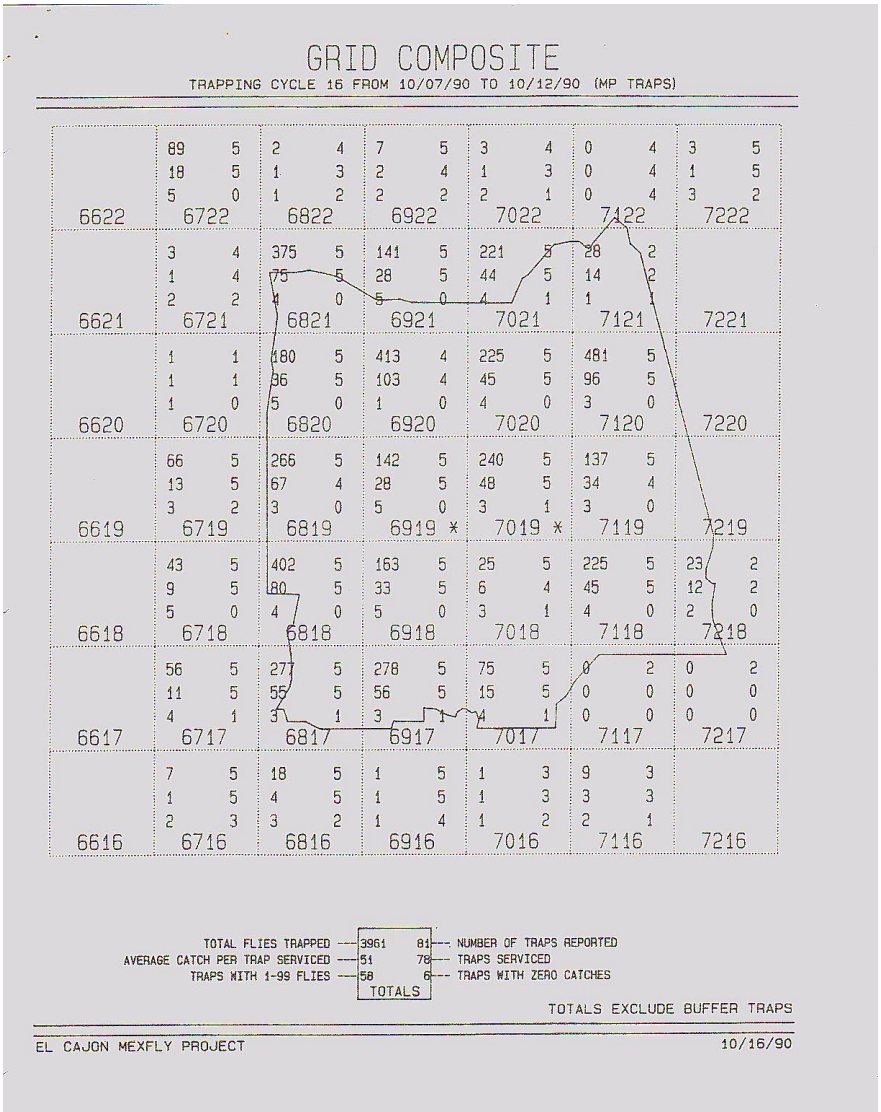


**Figure 11.1**.Cunningham report.

In the California programmes of the early 1990s the reports were again modified due to the increase in size of the treated area and sterile fly numbers being recovered. Sterile flies retrieved were grouped into categories and displayed electronically on a grid printed using colour codes. Basic categories are as follows: 1) skipped or lost traps; 2) zero flies trapped; 3) 1-99 flies trapped; 4) 100-999 flies caught; and 5) 1000+ flies caught. Procedures used since in eradication and preventative release programmes generally follow the reporting system used in the Cunningham Report. Minor modifications have been incorporated based on local needs without endangering the integrity of the data presentation. Recent advances in GIS and spatial analysis now allow for more detailed and useful evaluation methods for sterile fly recapture (see Section 10).

### 11.2 Recapture Indices and Evaluation Parameters

There are certain conditions that the sterile flies should meet to assure proper performance in the field. Some of the most important are: sterile fly age and nutritional reserves when released, longevity, host finding and mating competitiveness. Managers will have to ensure that these conditions are met in order to release competitive insects in the field. Interpretation of recapture, based on the following indices, will assist in measuring sterile fly performance:

a. Sterile fly distribution in the field (percentage of traps with capture)
b. Sterile fly/trap/day (FTD) as a measure of sterile fly relative abundance and survival
c. Sterile to wild ratio (S:W Ratio)

Achieving the established values for each index, together with adequate quality control parameters, will ensure proper performance of the sterile insects in the field. These indices are usually calculated in large areas of grouped traps, usually inside a release polygon and are a summary of the entire area. Although these measures are useful to give a general idea of the distribution of sterile flies in the field, they do not permit the identification of localized trends and potential issues within release polygons. Newer methods combining the data in GIS with spatial analysis methods make it possible to conduct analysis at the trap-level instead of averaging values over a larger area and can identify smaller scale issues that can affect the performance of SIT. Decisions can be taken to resolve identified issues by changing flight parameters (altitude, direction of lines, densities), evaluating trap placement, or conducting supplementary activities such as ground release. S:W ratio can also be evaluated at a finer scale using an index that compensates for traps with zero wild or sterile captures (e.g., (S-W)/(S+1)) to show localized areas with undesirable ratios. Identified problem areas can be monitored or analysed for several time periods to determine how consistent this pattern occurs.

In addition, application of area-wide SIT can be assessed by a series of evaluation parameters that can be summarized as follows:

a. Egg sterility measurements
b. Determining larval infestation levels in the preferred host in the area
c. Reduced presence of wild flies in traps

The SIT evaluation parameters should be selected based on the objectives of the action programme. For example:1) re-establishing export protocols once levels of immature and adults detected decrease below a set threshold, in cases of low prevalence areas, 2) declaration of fly free area with three generations of the pest without detection in cases of eradication programmes, etc. Other evaluation parameters could be used to document programme progress.

### 11.2.1 Description of recapture indices

a) Sterile insect distribution in the field

Sterile insects should be properly distributed in the area, and a minimum of 90% of traps with sterile fly capture over a release area would be an acceptable level of fly distribution. Attention will have to be paid to areas with consistent lack of sterile flies which will mean problems with trapping or in the efficiency of the sterile fly distribution. One solution for low recapture in particular areas would be to add additional sterile flies.

b) Sterile Fly/Trap/Day (FTD) as a measure of sterile insect relative abundance and survival

Adequate sterile flies presence in the field (measured by sterile fly recapture in FTD) (IAEA 2003) will allow for sterile:wild fly interaction. Action programmes should ensure that the minimum required ratios of sterile to wild flies are present in the area at all times (See Section XI). Knowledge of sterile insect survival (FAO/IAEA/USDA 2014) is relevant to define if additional releases are needed and when they are needed to ensure sterile fly availability in the field.

c) Sterile to wild ratio (S:W Ratio)

The S:W ratio should be defined and assessed according to the objective of the programme (see Section 8.2) (FAO/IAEA/USDA 2003). This critical over-flooding ratio should be maintained above the pre-established minimum at all times within the area of concern. Trapping should be used to corroborate sterile:wild ratios. Additional releases would be necessary if sterile fly numbers drop due to sterile fly mortality, migration of sterile or wild populations or other causes. Results of the S-W ratio can also be used to calculate recommended release densities using the actual density applied in a given block and the most recent trapping results of the sterile and wild flies. See section 9.

### 11.2.2 Description of evaluation parameters

a) Egg sterility measurements

This measurement is performed by collecting host fruit in the field. Field collectors should ensure that oviposition marks are present before removal of the fruit from host trees. Fruit should be taken to facilities for dissection. Eggs extracted from the fruit should be processed as described in the Sterility Tests Section (Procedures Section 2.5) of the Manual for Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies (FAO/IAEA/USDA 2014). This test would be difficult to implement under high availability of fruit and very low population levels inherent to eradication programmes.

b) Determining larval infestation levels in the area in the preferred host

Larval infestation levels are measured as numbers of larvae/kilogram of fruit host. In this case, host fruit with infestation symptoms should be collected from preferred hosts from the area subjected to sterile insect releases and brought back to the fruit processing laboratory. Fruit is allowed to finish ripening in order to allow final larval development and egression under laboratory conditions. Measurements of fruit weight should be taken and the number of larvae per kilogram of fruit estimated. This will provide a value of infestation that can be compared periodically to determine the progress in population reduction. This procedure is described in detail in the Fruit Sampling Section of Moscamed Programme Field Operations Manual (Reyes et al. 1986, Programa Regional Moscamed 2003, Programa Moscamed 1990).

c) Reduced presence of wild flies in traps

The predicated result of SIT is to reduce population numbers as releases continue over time. This result should be reflected in a reduction of the wild population as measured by trap captures and the corresponding $FTDf_{ertile}$ index. The results of a fruit fly control programme can be compared periodically using the $FTD_{fertile}$ index over time.

d) Negative trapping for at least three generations.

In the case of an eradication programme, after a number of generations of sterile insect release, it is expected that the wild population will be eliminated from the treated areas. An assessment of this condition would be to measure the absence of wild flies by maintaining the same level of trapping for at least three generations after the sterile fly release programme has been completed (IAEA 2003). The negative trapping over the course of three generations will confirm eradication (FAO 2006). The time should be adjusted based on the life span of the different developmental stages of the insect which is determined by the prevailing environmental conditions present in the area and by the trade protocols (Tassan et al. 1983 and Anon. 1997).

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# 12. DESIGN OF A FRUIT FLY PACKING, HOLDING, CHILLING AND RELEASE CENTRE

## 12.1 Background

The design of a packing, holding, chilling and release centre is of primary importance in the application of the SIT. In operational programmes that use the SIT, it is a common practice for managers to adapt existing buildings such as warehouses into this type of facilities resulting in less than optimal conditions for managing the sterile flies.

The Moscamed Program in Mexico, in the past 36 years has used 4 different types of construction for this purpose. The first was an existing building at about 2 kilometers from the airport, where the sterilized pupae were packed in large aluminum cages and held in conventional rooms equipped with window air conditioning packages until adult emergence and maturity. Adults were then transported to a chilling room to prepare the flies for aerial release. An electromechanical release machine with trays was used to release from small aircrafts. This system was then replaced by the bag system and because of the greater demand for space, and many failures of the release machines at that time, years later a new warehouse was built within the Moscamed Program´s mass rearing facility complex, with newer equipment to facilitate handling of the bags and prevent significant quality loss of sterile flies. In addition, an aircraft runway was built on the side of this packing center that operated for a few years until it was canceled due to lack of minimum safety measures. Because of the need for space for other activities within the mass rearing facility and because of the problems originating from long distance transportation of adult flies to the airport, the center was moved again to another existing warehouse at about 15 kilometers from the airport. The building of a new packing center in the Moscamed Program in Guatemala, under the guidance of the United States Department of Agriculture (USDA), triggered in 2007 the decision to build a new packing centre specifically designed for the needs of the program in Mexico.

The construction of a fruit fly packing centre should consider among others things:

a) Selection of site. The packing centre should be located near an official airport to avoid excessive transport time. In the child adult aerial release system, sterile flies are dumped in a refrigerated box where they accumulate causing compaction that can affect the quality of the sterile flies.
b) Placed within the area of influence of the programme, to reduce as much as possible the required aircraft ferry time to reach the release target areas.
c) Availability of basic services. It is important to have public electricity and clean water supply and located within an industrial area in order to have easy access to spare parts for the machinery in use.
d) Should be well communicated with easy access to the main network of roads.

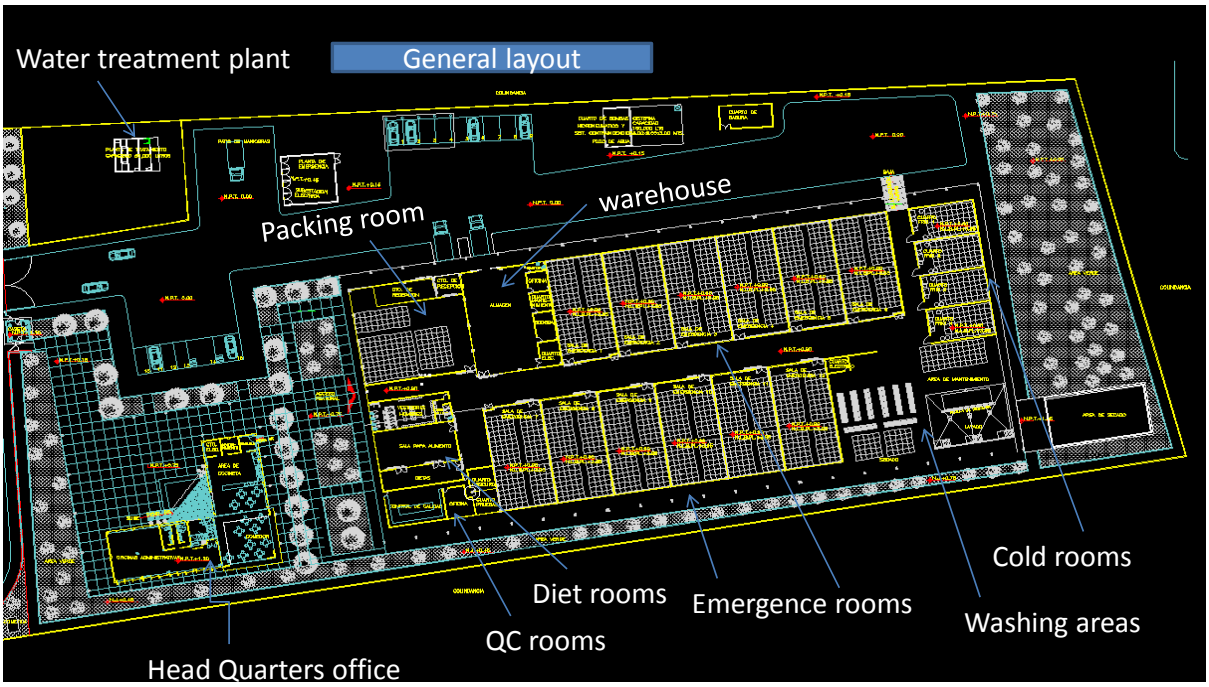


**Figure 12.1** General layout of the Mediterranean fruit fly packing, holding, chilling and release centre in Tapachula, Chiapas, Mexico. (©FAO/Programa Moscamed Mexico, Guatemala, USA)

e) Packing system. At the present time there are 4 basic packing systems: Bags, PARC boxes, sleeves and towers (see Section 5).
f) Size. Depending on the estimated sterile fly volumes and packing system, there will be different space requirements.
g) Equipment. To meet the needs of each of the environments in the different working rooms such as: Temperature, humidity and air quality (see below equipment requirements for the different working rooms).
h) Design of the building with a logical work flow, following the biological stages of the insects (in this case pupa and adults), specific environmental conditions and handling requirements as well as technical supporting areas.

Once the packing system is selected, the amount of flies per unit of packing space needs to be determined. This will depend on the amount of square centimeters of resting surface for the flies, available per unit.

For example, for the tower system used in Mexico (Mexican Tower Type): Each Tower has 16 shelves with the following dimensions: 82 cm length, 70 cm width and 10 cm height. Each shelf contains 2 food dispensers, 1 pillow for water supply, 1 pupae container and 2 plastic resting areas for the emerged adults.

It is normally recommended to have a maximum density of 2 flies per square centimeter; nevertheless in order to have better quality, the numbers for each shelf in use is of 55 thousand pupae equivalent to 1.47 flies per square centimeter. Before a change in sterile fly density is conducted, quality of the sterile flies should be assessed through quality control tests (emergence,

and fly ability) following the Manual for Product Quality Control for Sterile Mass-Reared and Released Tephritid Fruit Flies (FAO/IAEA/USDA 2014).

Total surface for the shelves is determined by the sum of the shelves and the resting areas as follows:

a) Shelf:
82 cm length
70 cm width
10 cm height

Length x height x 2: 82 X 10 X 2= 1,640 $cm^2$
Width x height x 2: 70 X 10 X 2= 1,400 $cm^2$
Base and cover of the shelf: 82 X 70 X 2= 11,480 $cm^2$
Total surface of the shelf 14,520 $cm^2$

b) Resting areas:
120 cm length
40 cm width
Length 120 x length 40 x resting areas 2 = 19,200 $cm^2$

c) Total surface shelf and resting areas= 33,720 $cm^2$

d) Flies per square centimeter

55,000 pupae / 90% emergence= 49,500 flies

49,500 flies / 33,720 $cm^2$ = 1.47 flies per $cm^2$

**12.2 Emergence and Holding Rooms**

Emergence and holding rooms have special needs such as space, temperature, humidity and air quality. There are two main criteria to establish the type of rooms: One is for large single rooms where pupae containers are placed for a number of days, separating the containers using space or special marks for each day. The other is to have one room for each day, providing the sterile flies with a quiet place with no disturbance to avoid waste of valuable energy that will be required in the field after release.

For the single large room, the size of the room is calculated taking into account the amount of flies and days needed from the packing to the release day. For single day rooms, the size and number of rooms is computed based on the amount of pupae to be packed per day.

From the Mexican experience, the best is to have single emergence and holding rooms for each day. Smaller spaces facilitates handling of environment conditions including temperature, humidity and air quality compared with larger areas, securing the quality of the sterile flies.

The size of the emergence and holding rooms is calculated as follows:

For a volume of 1 billion sterile pupae per week and using the Mexican Tower Type,
55,000 pupae per shelf (at 1.47 flies/$cm^2$) x 16 shelves per tower= 880,000 pupae per tower

A mass rearing facility of 1 billion flies per week will produce 142 million flies (pupae) per day requiring 161 towers per day (880,000/142). In this case the size of the emergence and holding room is: 161 towers x 1 $m^2$ per tower = 161 $m^2$ tower space per day. This total tower space is considering that 1 $m^2$ per tower will provide space between towers for temperature and humidity control and to allow oversight work. In addition, the rooms will require 1 $m^2$ between the wall and the towers to avoid temperature and humidity build-up and 2 linear meters from the towers to the room entrance for the in and out movement of equipment and supplies.

Under the concept of single emergence rooms, the amount of rooms will depend on the amount of days that the flies will be held before release, ideally one room for each day. If the flies are released at the $6^{th}$ day, then 12 emergence and holding rooms are needed. During the release date ($6^{th}$ day), the towers are transported to chilling room where they will remain until chilling and release. Releasing flies close to sexual maturity, will allow sterile males to immediately look for mates. In some instances this would require holding flies at list for an extra day, in which case another room will be needed.

Temperature control in the emergence or holding rooms is of primary importance, especially in those areas where the ambient temperature and humidity are high, like in tropical areas, but even in cold environments humidity and air exchange in the emergence rooms is very important to preserve the quality of the flies. Temperature, humidity and air quality are managed by 2 integrated air conditioning packages of 25 ton, 440V, 3F-4H, 60HZ, 10,000CFM and 1 drying dehumidifier of 120 pints/day, 127V, 1F-2H, 60HZ, and 250 CFM. Allowing more than 10 air interchanges per day to assure air quality.

**12.3 Chilling Rooms**

Sterile fly knock down for the different types of packing systems (see Section 6). The amount of sterile flies to be released per day and the capacity of the machines used to release the flies, will determine the number of chilling rooms needed per day. For a daily production of 142 million pupae (aprox. 1 billion per week), 4 chilling rooms are required with a capacity of holding 25 million flies. The 4 chilling rooms will be used at least twice per day. The size of each room is 75 $m^2$, thus, the total area required is 300 $m^2$. Three release machines with a capacity of 20 million flies each are placed inside fix-wing airplanes; 60 million chilled flies per flight, sometimes there is a need for release fewer amounts of flies per flight. A total of 9 machines and 3 airplanes to release this daily production volume are required. It is advisable to have an extra airplane with three release machines in case of a failure.

Same as in the emergence and holding rooms, the control of the environment inside the chilling rooms is critical. Temperature to immobilize the flies for easy handling in the release machines should be -2 to 0 $^oC$. Humidity content of the flies before release in the field is of primary importance and should not exceed 70 RH. Excess humidity will cause congestion of flies and obstruction of the airplanes release shut. Correct selection of the equipment is essential. The

equipment used in each chilling room, in this example is: 3, 48,000 BTU Condensers, 220V, 3F-4H, 60Hz; 6, low profile 24,000 BTU evaporators, 220V, 2F-3H, 60Hz, TSS -4°C 2,800CFM; and 1, 1,400 m3/h air processing dehumidifier and 1 drier, 440V, 3F-4H, 60 HZ.

### 12.4 Reception, Packing and Quality Control Rooms

The reception room is dedicated to prepare the pupae received from the production facility for the packing process. The packing room is divided into two rooms, one for the pupae dispensers (20 $m^2$ each) and one to accommodate the conveyors and all the components for the Mexican type towers as well as the towers required for one day of pupae production (320 $m^2$) (see Section 5).

At the packing room there is a specific equipment for the evenly distribution of the pupae in the containers that are placed in each shelf of the Mexican type tower. The specifications of the equipment are as follows: These equipment has a volumetric dispenser capable of handling from 10 to 3000 g, an accuracy of 1.5 g and 70 dispensations per minute, although, due to the amount of pupae to be dispensed in each pupae container, only a maximum of 25 dispensations are conducted per minute. This is done through 10 distribution heads, a linear vibrator, a central vibrator and power of 1KW, 220 V, 2F-3H, 60 HZ. In addition, two conveyors are required to transport the different parts of the towers.

Rooms to conduct quality control (QC) tests are required. A set of routine and periodic quality control tests are required to determine the effect of packing, holding, chilling and release, as well as to verify that the sterile insects received from the production facility fulfill minimal quality requirements as specified in the Manual for Product Quality Control for Sterile Mass-Reared and Released Tephritid Fruit Flies (FAO/IAEA/USDA 2014).

Quality control tests are conducted during the different steps of the process at the emergence and release centre. For this purpose, three rooms of different size to conduct QC tests are required; the first to perform flight ability test and the second for longevity or stress without food and water, with specific photoperiod requirements. Each room has 20 $m^2$; the third room of 80 $m^2$ is for the set-up of the different tests and office space for the personal involved in quality control.

These rooms should have the same environmental conditions compared with the rooms where pupae and emerged flies are kept. Therefore, the equipment requirements should be the same as in the emergence rooms.

### 12.5 Supporting Areas

Packing facilities will generate sewage waters from the sanitary and industrial process. Sanitary waters are originated in the offices and sanitary rooms, the industrial water is produced when the equipment is washed carrying solid residues such as fruit fly food residues, fruit fly body parts and puparia.

Waters are treated using extended aeration with about 10 to 14 hours in order to have enough material degradation. Extended aeration is used to treat industrial waters containing mainly soluble organic material, whereas bacteria are used to treat the more complex substances contained in this type of water. An important advantage is that this type of treatment produces low quantities of activated mud. The 3 important steps for the treatment of this type of industrial waters are: 1.

Cribbed to separate the solid materials from water; 2. Aeration to treat the water and 3. Precipitation of solids. Treated water can be used for recycling in the same industrial process or sanitary services, resulting in substantial water savings or for irrigation of green areas within the complex. The water should comply with the corresponding environmental legislation. The treatment area is of 48 $m^2$, with a volume treatment capacity of 200 $m^3$.

Areas to support the activities performed at the facility are required such as warehouse for the supplies and equipment (200 $m^2$), diet preparation room (176 $m^2$), washing and maintenance of the equipment (900 $m^2$), offices (70 $m^2$) dressing and sanitary rooms (80 $m^2$), area for drying materials (230 $m^2$), machinery room (220 $m^2$), waste containers area (24 $m^2$), cistern (80 $m^2$), pumping-engine (37 $m^2$) and water treatment areas (48 $m^2$). For a facility designed to handle 1 billion pupae per week, the minimum surface required for the supporting areas is 2,065 $m^2$. There are other independent areas, such as: Office space (head quarters) (507 $m^2$), green areas and internal access roads, (13,740 $m^2$).

In summary, the total surface area of the Packing and Release Centre of the Moscamed Program in Mexico with a capacity to process 1 billion sterile flies per week is 5,662 $m^2$ and the total area including the packing centre, head quarters, green areas and internal roads is 17,143 $m^2$ (Table 12.1).

**Table 12.1** Area in square meters required for the different working areas and total area for the entire packing and release centre of the Moscamed Program in Mexico.

| ROOMS | NUMBER | AREA ($M^2$) | TOTAL AREA ($M^2$) |
|---|---|---|---|
| Emergence and Holding | 12 | 231 | 2,772 |
| Chilling | 4 | 75 | 300 |
| Reception | 1 | 65 | 65 |
| Packing | 1 | 340 | 340 |
| Quality Control | 1 | 120 | 120 |
| Supporting areas | 11 | 2,065 | 2,065 |
| Head quarters | 1 | 507 | 507 |
| Green areas and internal roads | 1 | 13,740 | 13,740 |
| Total | -- | -- | 19,909 |

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# APPENDIX 2
# DATA SHEETS FOR SHIPMENT OF STERILE PUPAE

A copy of this datasheet should be present within each box of the consignment.

| **Name and address of the facility (origin):** | **Name and address of the recipient:** |
|---|---|
| ……<br>……<br>……<br>……<br>…… | ……<br>……<br>……<br>……<br>…… |

| **Consignment General Information** | | | |
|---|---|---|---|
| **Irradiation date:** | | **Irradiation dose (Gy):** | |
| **Packing date:** | | **Shipping date:** | |
| **Total No of boxes:** | | **Total weight (kg):** | |

| | **Box Number within the Consignment** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Elements*** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **Total** | **Observations** |
| Number of pupae containers inside the box[1] | | | | | | | | | | | *a* | |
| Weight (kg) | | | | | | | | | | | *b* | |
| Number of pupae containers with radiation sensitive indicator | | | | | | | | | | | *c* | |
| Number of indicators that were exposed to the recommended dose[2] | | | | | | | | | | | *d* | |
| Number of indicators countersigned at the origin, after irradiation | | | | | | | | | | | *e* | |

[1] Plastic bags, "sausages" or other

[2] "Visual determination"

Observations: _____

_____

Authorization: _____

(a) Ideally a=c=d=e

(b) This value should be equal to the total weight reported under “General Information”

(d) Should it differ from value in (a), the consignment should be disposed safely and not used

# APPENDIX 3
# HISTORY OF TRANSBOUNDARY SHIPMENTS OF STERILE TEPHRITID FRUIT FLIES

| Year | Tephritid species | Site of production | Amount[1] shipped (million pupae) | Recipient | Observations |
|---|---|---|---|---|---|
| 1963-1990 | Mexican fruit fly, *Anastrepha ludens* | Monterrey, Mexico | Unknown | Texas, USA | |
| 1970/71 | Mediterranean fruit fly, *Ceratitis capitata* | Seibersdorf, Austria | Unknown | Procida, Italy, and Greece | Relatively small amount since sterile flies were used for field trials |
| 1970 | Mediterranean fruit fly | Costa Rica | Unknown | Nicaragua | Relatively small amount since sterile flies were used for field trials |
| 1975-1977 | Mediterranean fruit fly | Madrid, Spain | 302 | Canary Islands | |
| 1978 | Mediterranean fruit fly | Seibersdorf, Austria | Unknown | Guatemala | Sterile pupae shipped from the IAEA laboratories (Seibersdorf) to a packing and emergence facility in Guatemala for field trials and staff training in SIT techniques |
| 1979-2015 | Mediterranean fruit fly | Chiapas, Mexico | 326,400 | Guatemala | Transboundary shipmentshave been carried out periodically for the past 36 years |
| 1989-1994 | Mediterranean fruit fly | Chiapas, Mexico | 6,670 | California, USA | To assist the CDFA in eradication of medfly outbreaks |
| 1990 | Mediterranean fruit fly | Chiapas, Mexico | 552 | Chile | Sterile flies donated by the Mexican government to Chile |
| 1989-1990 | Mediterranean fruit fly | Seibersdorf, Austria | Unknown | Israel | Pilot trials |
| 1994 | Mediterranean fruit fly | Seibersdorf, Austria | 60 | Tunisia | Pilot trials |
| 1996-2000 | Mexican fruit fly | Chiapas, Mexico | 2,511 | California, USA | To assist the CDFA in eradication of Mexican fruit fly outbreaks |
| 1994-2015 | Mediterranean fruit fly | El Pino, Guatemala | 146,900 | California, USA | To assist the CDFA in eradication of medfly outbreaks |
| 1997/98 | Mediterranean fruit fly | Madeira, Portugal | 206 | Israel | In support of pilot suppression programme |

| Year | Tephritid species | Site of production | Amount[1] shipped (million pupae) | Recipient | Observations |
|---|---|---|---|---|---|
| 1997-2000 | Mediterranean fruit fly | El Pino, Guatemala | 3,700 | Israel | In support of pilot suppression programme |
| 1998-2015 | Mediterranean fruit fly | El Pino, Guatemala | 89,400 | Florida, USA | To assist the State of Florida in eradication of medfly outbreaks |
| 1999-2000 | Mediterranean fruit fly | El Pino, Guatemala | 720 | South Africa | In support of pilot suppression programme |
| 2011-2013 | Mediterranean fruit fly | Valencia, Spain | 425 | Croatia | In support of suppression programme |
| 2010, 2012, and 2014 | Mediterranean fruit fly | Bio-Fly Israel | 1,489 | Croatia | In support of suppression programme |
| 2008 - 2015 | Mediterranean fruit fly | Bio-Fly Israel | 560 | Jordan | In support of suppression programme |
| **TOTAL** | | | 579,895 | | |

[1]Amounts are approximate figures of shipped sterile pupae.

# APPENDIX 4
# TRANSBOUNDARY SHIPMENT OF STERILE INSECTS

**Prepared by an FAO/IAEA Consultants Group**
**30 July to 3 August 2001, Vienna, Austria**

## PREAMBLE

A Consultants Group Meeting was held to discuss the potential risk[3] from transboundary[4] shipment of sterile insects for pest control programmes. This meeting took place in Vienna at the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, from 30 July through 3 August 2001. The group of consultants (see Annex 1) was called together in response to requests for guidance from national plant protection organizations (NPPOs) in light of the growing demand for alternatives to pesticide use as an exclusive control measure and the increasing interest from the private sector to invest in the Sterile Insect Technique (SIT).

The aim of the meeting was to characterize the potential risk posed by transboundary shipment of sterile insects shipped for SIT programmes and to reach conclusions regarding the level of risk. In the process of this analysis, the group identified some routinely applied procedures, including best practices for shipment that reduce the risk to a negligible level. However, there currently are no internationally recognized guidelines for regulating shipment of sterile insects.

Harmonized guidance regarding regulation of the shipment of sterile insects will facilitate trade while addressing concerns about shipment of what could be quarantine pests. This document was developed as a discussion paper for consideration by the Interim Commission on Phytosanitary Measures (ICPM), the governing body for the International Plant Protection Convention (IPPC).

One possible result of this discussion paper will be the development of an international standard providing guidance on measures pertaining to the transboundary shipment of sterile insects. Alternatively, this topic could be added to the International Standard on Phytosanitary Measures (ISPM) regarding biological control agents (IPPC, 1996) at the time of its revision. However, certain provisions in the ISPM on biological control agents are inappropriate when considering sterile insects (e.g. holding in quarantine for the next generation). In addition, the IPPC Glossary of Terms (IPPC, 2001) definition of biological control excludes the SIT.

In the interest of harmonization, similar discussions may be needed at the Office International des Epizooties (OIE) and the World Health Organization (WHO) regarding the use of sterile insects for control of human or animal diseases.

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[3] "Risk" in this context includes both the likelihood and the consequences of an adverse event occurring

[4] "Transboundary" in this context refers to entry (Customs and Agriculture clearance) of a shipment into the importing country as well as transit shipment through a third country. Transit may or may not involve transloading.

## EXECUTIVE SUMMARY


- The increased use of the Sterile Insect Technique (SIT) to suppress or eradicate insect pest populations is resulting in increased shipment of the sterile target insect pests from one country to another, often passing in transit through other countries. These transboundary shipments are not subjected to international standards for biological safety.
- As the SIT becomes more commercial, the need for guarantees that the sterile insects can be safely and legally shipped are essential to encourage financial investments in commercial sterile insect mass rearing facilities. Also, international regulations are required to reduce the need for independent development of national regulations that may hinder the insect control programmes.
- The objective of the Consultants Meeting was to prepare a discussion paper for consideration of the Interim Commission on Phytosanitary Measures (ICPM), the governing body for the International Plant Protection Convention (IPPC), as a first step towards developing an international standard or other guidance on the transboundary shipment of sterile insects. Additional discussions may be needed to address shipments of sterile insects for control of pests of veterinary and medical importance.
- The scope of the discussions was limited to radiation-sterilized insects for use in Sterile Insect Technique (SIT) control programmes against plant insect pests. Insect strains produced artificially by genetic engineering or other modern biotechnology methods were excluded.
- Four potential hazards were identified with regard to transboundary shipments of sterile insects:
    1. Outbreak of the target pest in a new area, where it does not already occur.
    2. Increase of fitness of the local pest population through the introduction of genetic material from the escaped insects into an area where the pest already exists.
    3. Unnecessary regulatory actions being initiated following false identification of captured sterile insects and conclusion that it is a quarantine threat.
    4. Introduction of exotic contaminant organisms in a shipment, other than the target species for the SIT programmes.
- Transboundary shipment of sterile insects has taken place on a continuous basis for nearly 50 years. The total number of sterile insects shipped was estimated at 962 billion in more than 12,000 shipments to 22 recipient countries from 50 sterile insect factories in 25 countries. During this long period and many precedents, no problems associated with the hazards listed above or any other have been identified, and thus the shipment of sterile insects have never been subjected to any regulatory action.
- The potential risks of the identified hazards were evaluated using a scenario analysis technique.
- The events considered for hazard 1, were: sterilization failure, shipment packages opened accidentally, escape, survival and reproduction of the sterile insects. For hazard 2, in addition to the above sequence of events, the escaped insects would have to reproduce with a local population and undesirable traits established in the population. For hazard 3, the critical points would be shipment packages opened accidentally, escape, survival and captured insects not recognized to be sterile. Hazard 4 is not unique to sterile insects and was thus not assigned a risk, as it is possible in shipments of goods of any type.
- For each hazard the calculated estimated risk was:
    1. $0.5 \times 10^{-18}$
    2. $0.5 \times 10^{-23}$
    3. $1 \times 10^{-11}$

4. Many-fold less likely than the risk of moving biological control agents

- It was concluded by the consultants that the present systems of transboundary shipment of sterile insects for SIT programmes is very safe. However, international regulations should be developed for approval by the Interim Commission on Phytosanitary Measures (ICPM) to facilitate commercial development of the SIT.

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## I. INTRODUCTION

There is a growing demand for cost effective control of insect pests of plants, as well as insects of veterinary and medical importance. At the same time insecticides are under greater scrutiny for potential toxicological and environmental impacts. An alternative insect pest control method is the Sterile Insect Technique (SIT). This involves mass production of the target insect species, sterilization using ionising radiation and repeated release into the target population. The release of sterile insects that target a population of the same species is a form of "birth control". The sterile insects mate with the wild population but fertilization results in no viable offspring. Repeated releases of sterile insects lead to a reduction in the pest population.

The SIT differs from classical biological control, which involves the introduction of exotic biological control agents, in the following key areas:

1. Sterile insects are not self-replicating and cannot become established in the environment.
2. Autocidal control is by definition intraspecific.
3. SIT used against an established pest never introduces an exotic species into the ecosystem where the SIT programme is being implemented.

The SIT has been used for nearly 50 years for eradication, suppression and control programmes of both plant and animal pests (e.g. Mediterranean fruit fly (medfly, *Ceratitis capitata*) and New World screwworm (NWS, *Cochliomyia hominivorax*). Because of the limited number of facilities for rearing and sterilization, sterile insects are often shipped for release in other locations. Transboundary shipments have gone from production facilities to release sites in countries throughout the world. Demand for SIT is rising and new commercial facilities may be constructed soon to meet this demand.

### *I-A Background on transboundary shipments*

Transboundary shipments of sterile insects have been made on a continuous basis for the past 46 years. The first shipment of sterile NWS was from its production site at the USDA/APHIS mass rearing facility in Florida, USA, to the Caribbean island of Curaçao in 1954. This effort resulted in the eradication of the NWS from the island that same year. This was the first eradication of an insect pest population using the SIT.

Most of the transboundary shipments of sterile insects have originated from production facilities in North and Central America for shipment to at least 22 countries in 4 continents including the Americas, Europe, Africa and Asia (see Annex 3). One example is the ongoing shipment of sterile medfly pupae from the production factory in Tapachula, Chiapas, Mexico, to the packing and emerging facility in the southwest of Guatemala. Since 1979, biweekly ground and air shipments have been carried out amounting to 280 billion sterile flies (ca. 4,830 tons) in 21 years. Another important case is the ground and air shipment, since 1992, of 104 billion sterile NWS (ca. 1,733 tons) from the screwworm factory in Tuxtla Gutierrez, Chiapas, Mexico, to all of Central America, Panama and the Caribbean.

In Europe, most transboundary shipments of sterile insects have been carried out in support of SIT pilot projects. The first case involved sterile Mediterranean fruit flies shipped from the FAO/IAEA

Agriculture and Biotechnology Laboratory in Seibersdorf, Austria, to the island of Procida, Italy, in 1970. There are some other examples of transboundary shipments of sterile insects produced in Europe such as the case of the 206 million sterile Mediterranean fruit flies shipped from the mass rearing facility in Madeira, Portugal to Israel during 1997/98.

Other cases involving Europe include transit shipments of sterile pupae from Guatemala, Central America, through Amsterdam, Frankfurt or Madrid, to Israel and South Africa and from Mexico, through Frankfurt, to Libya, (see Table in Annex 3).

In the past 46 years, at least 962 billion sterile insects (equivalent to about 18,000 tonnes) have been shipped domestically and internationally. None of these shipments has ever been prohibited from transit or entry for phytosanitary reasons by the 22 recipient countries or numerous transiting countries. The sterile insects are shipped by air cargo (commercial airlines or charter planes) or by ground in refrigerated trucks. They are packed in labelled, sealed containers to prevent contamination or escape. These safeguards are in place to protect the integrity of the sterile insects and not that of the public, property or the environment in the event of a massive escape. The same measures serve as safeguards against the hazards identified in this document, however, thereby greatly reducing any risk.

*I-B Existing Guidelines*

Internationally recognized guidelines on many steps in the mass rearing and sterilization of insects and quality control (materials used in production, the product and process) already exist (see References Section IX) but there are no internationally recognized guidelines for regulating shipment of sterile insects. Some countries do not regulate shipment of sterile insects, others only require labelling and documentation, and still others are regulating sterile insects under their biological control measures. In order to encourage a harmonized approach to national treatment of this method of plant pest control, some guidance on the risks involved will be very useful.

## II. SCOPE

This discussion paper characterizes the risks involved with the transboundary shipment and importation (either in-transit through third countries or directly to the importing country) of sterile insects for use as autocidal control agents in control programmes of plant insect pests. Mass production site hazards and risks related to the release of sterile insects did not fall within the terms of reference of this Consultants Group.

Shipment of sterile, mass reared insects was considered including those developed through traditional selection and mutation breeding, for example sexing strains. Sterile insects resulting from strains which may be created artificially by genetic engineering or other modern biotechnology methods were excluded.

This discussion paper is also limited to the shipment of sterile insects resulting from radiation-induced sterility and does not deal with sterile insects resulting from the application of other sterilization techniques (e.g. chemosterilants or transgenically-induced sterilization).

## III. HAZARD IDENTIFICATION

A key objective of the Consultants Group was to identify and characterize potential phytosanitary hazards associated with the transboundary shipment of sterile plant insect pests. The Consultants identified hazards and distinguished independent events leading to the occurrence of each hazard. This provided a format for estimating the likelihood and characterizing the consequences of each hazard in a scenario analysis[5]. Figure 1 shows the scenarios for each of the hazards.

Four potential hazards were identified as follows:

| HAZARD | PRIMARY EVENT THAT COULD RESULT IN THIS HAZARD |
|---|---|
| 1. Outbreak of target insect pest in a new area | Faulty sterilization |
| 2. Increase of fitness of local pest population | Faulty sterilization |
| 3. Unnecessary regulatory action initiated | Faulty ID of sterile insect |
| 4. Introduction of exotic (new) contaminant organisms | Presence of hitch-hikers in shipments |

The first two scenarios require failure of the sterilization treatment as the first event. This could mean absolute failure (i.e. the shipment was not treated) or that the treatment was less than necessary to meet the required specifications for sterility.

The second event that must occur in the first two scenarios is a breach of the package to allow for spillage or escape. It is assumed that in most situations this will be under adverse conditions (e.g. airport cargo handling environment). As a result, the pest must not only be liberated (event c), but it must also survive to escape into a favourable environment (event d). Finally, it must mate and reproduce for either hazard 1 or 2 to occur. However, in the case of hazard 2, the scenario recognizes that the introduction of new genetic material in itself does not present a risk unless an undesirable genetic trait is expressed and also has a selective advantage to become established in the population (event e).

The situation in hazard 3 is not related to biological consequences but rather based on regulatory actions (e.g. delimiting survey) that may be unnecessarily taken by the country where the pest is detected but not recognized as sterile. Adverse phytosanitary measures may be put in place by trading partners based on reporting the detection without distinguishing the pest as sterile.

Hazard 4, the introduction of exotic contaminating organisms, was not characterized in the same way as the other three hazards because it is a complex set of sub-scenarios depending on the nature of the contaminant organisms (e.g. parasitoids, virus, etc). This hazard is also different because it is not unique to sterile insects. Similar hazards exist with shipment of biological control agents and to some extent with any shipment. In fact, the sterile insect mass rearing process virtually eliminates any parasitoids.

In each of the three scenarios (hazards 1, 2 and 3) for which independent events were identified, the likelihood of each event occurring is represented by rough estimates of the probability (a point estimate). The product of the estimates for independent events in each scenario gives an overall estimate for the probability of the hazard occurring. It is noted that the mathematical relationship of these events means that where any event in a scenario is zero, the probability for the entire scenario is also zero.

The estimates are based on data, past programme records, and experience and expert opinion, primarily as regards fruit fly and some Lepidoptera species. They involve extremely rare events for

[5] Reference for scenario analysis technique (L. Miller et. al., 1993).

which the primary source of evidence is the substantial history of experience with SIT shipments since 1954 and detailed knowledge of the technical/scientific aspects of the technology.

This approach was used to allow the comparison of risk levels between events and hazards associated with the transboundary shipment of sterile insects. It was not intended to be quantitatively precise, but more importantly to clarify the relative differences in magnitude. It is also useful to facilitate the comparison of phytosanitary risks associated with the transboundary shipment of sterile insects with those associated with other transboundary shipments (e.g. biological control agents).

The scenario analysis process is limited to characterizing direct phytosanitary hazards associated with the range of insect plant pests historically and currently controlled by SIT for phytosanitary applications. It should be noted that the scenarios are useful for pest risk management to the extent that they help to distinguish control points where risk-reducing measures may be applied.

The process does not consider indirect hazards or evaluate the risks against the benefits (e.g., increased pesticide use without SIT). In particular, it should be recognized that although the level of risk for any particular hazard may be the same for an importing and transit country, the transit country does not benefit to the same degree as the importing country from accepting this risk. In any case measures decided by either importing or transit countries should be technically justified (based on risk analysis or an international standard).

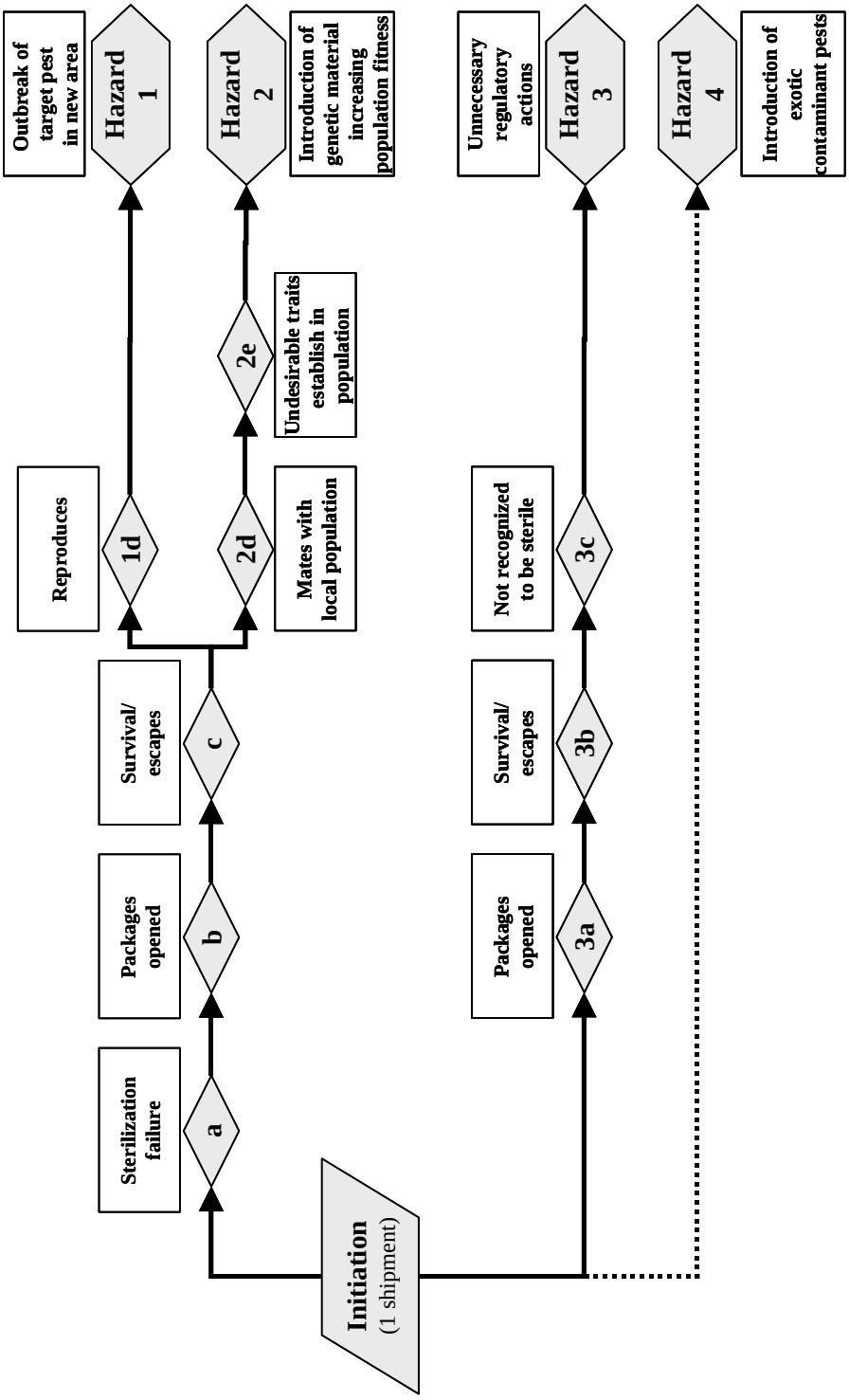



**Figure 1**. Hazard Scenarios for Transboundary Shipment of Sterile Insects

## IV. LIKELIHOOD OF THE EVENT

*IV-A Hazard 1: Outbreak of the target insect pest in a new area*

**Event *a*: Sterilization failure**

An estimated 12,000 ground and air shipments of sterile insects have occurred since 1954 and two instances of partial failure to sterilize (1 confirmed and 1 unconfirmed) have been reported. The confirmed incident occurred in 1982 in a shipment of medflies from Costa Rica to Guatemala (S. Sanchez, personal communication, 1982) and the unconfirmed incident with a shipment of medflies from Peru to California, USA, in 1980 (Rohwer, 1987). Since then, international quality control standards were put in place and there have been no sterilization failures despite the significant increase in the use of SIT.

Current safeguards to prevent sterilization failure:

- Modern production facilities employ failsafe irradiation systems (i.e. physical and/or procedural) to prevent this.
- Each treated container has a dosimetry device that assures the container was irradiated.
- Minimum dosage received by all the insects far exceeds the dosage required to sterilize the females.
- Irradiators are equipped with automatic exposure settings that are tamper-proof.
- Procedures are observed for routine calibration of the equipment.
- Packages are clearly labelled as containing irradiated insects.
- A sample of insects from each shipment is bio assayed for sterility at factory and release site for quality control.

*The likelihood was estimated by the consultants group to be an **extremely rare event** with an estimated probability of **$0.5 \times 10^{-6}$***

**Event *b*: Packages open**

In addition to the above event, it would be unlikely for the packages carrying the fertile insects to open because:

- From tens of thousands of containers shipped since 1954 there has been no documented case of breakage of shipping package.
- Using one of the longest routes (i.e. Guatemala City-Miami-Frankfurt-Tel Aviv) from 1998 to 2001, 1 out of over 400 shipments was never recovered. In this event, due to the length of time involved, highly perishable material (i.e. sterile insects) would not survive.
- Current safeguards to prevent mishandling leading to breakage of package include:
    - All consignments are double packaged, some triple packed, and then sealed.
    - Consignments are closely tracked with commercial motivation for rapid transit of highly perishable material.
    - Rapid feedback from receiver when the package is delayed.
    - Size and weight of package designed to minimize breakage.
    - All packages are appropriately labelled (e.g. fragile, biological material) and numbered.
- Content of package does not attract theft.

*The likelihood was estimated by the consultant group to be an **extremely rare event** with an estimated probability of 1 **$\times 10^{-5}$***

**Event *c*: Survives/escapes**

In addition to the above events, the fertile insects would be unlikely to survive and disperse to a favourable habitat because:

- Immediate in-transit area is inhospitable (i.e. lack of water, food, wrong temperature, no host, concrete/asphalt substrate). Presence of insecticide/toxicants at airports.
- Airport security prevents unauthorized removal of packages from the airport.
- Limited survival from pupal to adult stage, and even lower chance to survive to sexual maturity and disperse because of high predation, desiccation, starvation, drowning, temperature stress, etc.

*The likelihood was estimated by the consultant group to be a **fairly unlikely event** with an estimated probability of 1* $\boldsymbol{x\ 10^{-3}}$

**Event *d*: Reproduces**

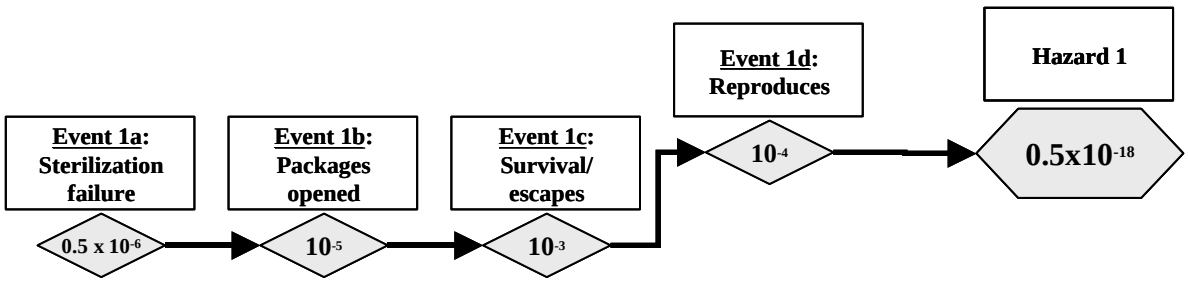
In addition to the above events, reproduction by the escaped insects would be unlikely because:

- Event may occur during seasonally inhospitable period.
- Climatic factors not suitable for establishment.
- Factory strain has lower fitness for survival in nature.
- Too few survivors to disperse and find suitable environment, mating partners and hosts.

*The likelihood was estimated by the consultant group to be a **rare event** with an estimated probability of* $\boldsymbol{1\ x\ 10^{-4}}$

***For the scenario for hazard 1 the likelihood of all four events occurring was estimated as a negligible risk with a probability of*** $\boldsymbol{0.5\ x\ 10^{-18}}$

**Summary of hazard 1: Outbreak of the target insect pest in a new area**




*IV-B Hazard 2: Increase of fitness of the local pest population through introduction of genetic material from the escaped insects*

For this scenario to take place, events 2a, 2b and 2c must occur. These have the same values as 1a, 1b and 1c. In addition, events d and e must occur:

**Event d: Escaped insects reach sexual maturity and mate with local population**

In addition to the above events, the escaped insects would be unlikely to reach maturity and mate. This event is very similar to *1d* but assumes that an established pest population exists in the area and that wild mates are receptive to mating.

*The likelihood was estimated by the consultants group to be a **fairly unlikely event** with an estimated probability of* $\boldsymbol{1\ x\ 10^{-3}}$.

**Event e: Undesirable traits established in the population**

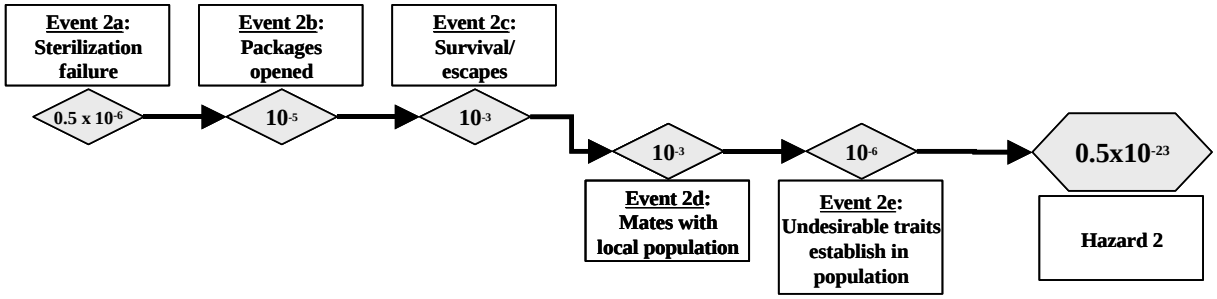
In addition to the above events, the escaped insects would have to possess traits that convey a selective advantage leading to increased fitness. Furthermore, these traits would have to become established in the population. However, this is extremely unlikely because:

- Most introductions of genetic material have neutral or even a detrimental effect on the population. Furthermore, because of the small numbers of escaped insects, it is unlikely that these traits would become established in the wild population.
- Under mass rearing conditions over many generations, all laboratory strains are known to loose their fitness to survive under natural conditions, therefore they are highly unlikely to carry genetic traits that would increase the fitness of the wild population.
- In addition, the only known traits that have been introduced into mass reared strains through traditional selection and mutation breeding (i.e. markers and sexing features) are detrimental (e.g. temperature sensitive lethal).

*The likelihood was estimated by the consultants group to be an* ***extremely rare event*** *with an estimated probability of* $\mathbf{1 \times 10^{-6}}$.

***For scenario 2 the likelihood of all five events occurring was estimated as a negligible risk of 0.5 x*** $\mathbf{10^{-23}}$

**Summary of hazard 2**: **Increase of fitness of the local pest population through introduction of genetic material from the escaped insects.**




*IV-C Hazard 3: Unnecessary regulatory actions initiated due to failure to recognize the detected insect as sterile*

Event 3a (i.e. packages opened) is identical to event 1b. Event 3b (i.e. survives and escapes) is the same as event 1c.

**Event c: Not recognized to be sterile**

In addition to the above events, the escaped insects would have to be detected and not recognized as sterile.

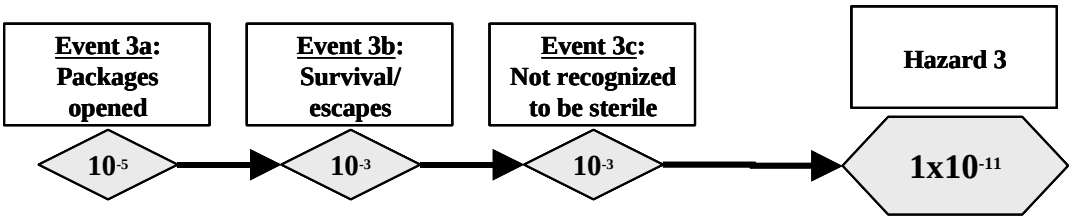
For this to occur the insect must be of regulatory significance:

- The plant protection authorities would have to be conducting detection surveys.
- The plant protection authorities would have to fail to recognize that this could be a sterile insect, which is an unlikely event. Those countries that are most likely to take a regulatory action have standard operation procedures that recognize the possibility of capturing sterile insects.
- The sterile insect marking process and cytological identification for sterility would have to fail.

*The likelihood was estimated by the consultant group to be a **fairly unlikely event** with an estimated probability of **1 x $10^{-3}$**.*

***For scenario 3 the likelihood of all three events occurring was estimated as a negligible risk of 1 x $10^{-11}$.***

**Summary of hazard 3: Unnecessary regulatory actions initiated due to failure to recognize the detected insect as sterile**




*IV-D Hazard 4*: Introduction of exotic (new) contaminant organisms

The introduction of exotic contaminant organisms was characterized in a different way because of the complexity of the sub-scenarios involved depending on the nature of the contaminant organisms (e.g. parasitoids versus micro-organisms). This hazard is also different because it is not unique to sterile insects. Similar hazards exist with shipment of biological control agents and to some extent with any shipment. Therefore it was compared to the risks from the shipment of biological control agents, which is widely practiced.

The risk of sterile insect shipments introducing exotic organisms were estimated to be considerably smaller based on the following considerations:

- There is no documented evidence that such an event has occurred during the past 46 years of sterile insect shipping.
- The items being shipped undergo sterilization. This would effectively reduce the risk of introducing unwanted parasitoids.
- Wild-collected organisms are never shipped for SIT purposes. The product is mass reared over many generations under quality control procedures aimed at eliminating unwanted organisms.
- The standard operating procedures for insect mass rearing specifically provide mechanisms to prevent unwanted organisms.
- Biological control agents are sometimes shipped with live hosts or prey. Sterile insects are not.

***For scenario 4, the consultants estimated that this risk would be many-fold less likely than the risk of introducing exotic organisms involved when moving biological control agents.***

## V. CONSEQUENCES IN CASE THE IDENTIFIED HAZARDS OCCURRED

Assuming that the identified hazards have occurred, the expert group described the following potential consequences:

### Hazard 1: Outbreak of the target insect pest in a new area

The consequence of this hazard is the incursion or establishment of a serious insect plant pest. Negative impact of the new pest could include:

- Decrease in production of crops.
- Reduction in quality.
- Increase in production costs.
- Impact on trade.
- Impact on the environment.

These consequences apply to both incursions and establishment. In the case of incursions, the negative impact would be limited in scope and duration. This is because for an incursion, the conditions would not be suitable for permanent pest establishment (e.g. pest not able to survive winter or summer temperatures). However, in the event of pest establishment, eradication would be an option since SIT and other eradication tools are available for the species that are currently shipped as sterile insects.

**Hazard 2: Increase of fitness of the local pest population through introduction of genetic material from the escaped insects.**

The consequences of the existing local pest population could increase as a result of the introduction of new genetic material. This negative impact could be:

- Decreased production on already affected crops.
- Increased cost on already affected crops.
- Losses on other crop species.
- Environmental impact.
- Impact on trade.

With the existence of a local population, however, control practices may already be in place that will effectively manage the fitter pest. This may reduce the consequences.

**Hazard 3: Unnecessary regulatory actions initiated due to failure to recognize the detected insect as sterile**

This would apply only to pests subjected to an active surveillance programme. The detection and failure to recognize the insect as sterile could trigger several different actions:

- An increase in trapping (i.e. delimiting trapping) to assess the status of the detection.
- The initiation of an emergency programme for eradication.
- Disruption of internal movement and marketing by domestic regulatory actions.
- Prohibition of host product by a trading partner.

The implementation of these actions could have significant short-term financial implications.

**Hazard 4: Introduction of exotic (new) contaminant organisms**

The introduction of an exotic organism into a new ecosystem can have the following negative impacts:

- Direct damage on agricultural crops if the introduced organism is an exotic plant pest.
- Indirect damage on agricultural crops if introduced organism has a negative impact on beneficial organisms (pollinators, predators and parasites).
- Change in biodiversity and natural ecosystem.

This hazard is not unique to the shipment of sterile insects, and therefore should be considered in comparison to or in the context of the same hazard associated with shipments of other commodities, including non-biological shipments.

## VI. ASSESSED RISK

Risk is the product of the likelihood of the hazard times the consequences. The potential consequences from the identified hazards could be significant. However, the extremely low likelihood of the hazards occurring indicates an overall negligible risk.

## VII. CONCLUSIONS

The Consultants held detailed discussions and reviewed reference documents taking into consideration the scientific, technical and operational aspects of the Sterile Insect Technique (SIT) as applied to plant protection. Potential biological hazards and associated risks were identified for transboundary shipment of sterile insects for use in SIT programmes.

The consultants concluded the following:

**A.** Evidence indicates that SIT is likely to become more widely used. There is also a shift from government to private responsibility for certain aspects of the technology. This will require a more formal approach to activities involving more than one country. This is particularly relevant to production that results in transboundary shipments of the sterile insects.

**B.** The SIT has been used for nearly 50 years against insect pests of plants and animals. During this time, standard operating procedures have been developed by most individual programmes. In some cases, international standards have been developed and are in use worldwide. For fruit fly species, the most important of these are the quality control and dosimetry manuals[1] (FAO/IAEA/USDA, 1998 and FAO/IAEA, 2000). The proper application of these manuals precludes the hazards identified by the Consultants Group from occurring.

**C.** There is a need for an internationally accepted code of conduct (or similar document) relating to transboundary shipments of sterile insects for use in SIT programmes. The International Plant Protection Convention (IPPC) is the international standard setting body for phytosanitary measures. Since the SIT is also used against insect pests of veterinary and medical importance, livestock insect pests and insect vectors of medical importance should be considered by the appropriate bodies in the near future.

**D.** The Consultants Group identified the hazards and assessed the risks associated with the transboundary shipment of sterile insects for SIT programmes. Both the likelihood and the consequences were considered for each of the hazards identified. A series of sequential events would be required for any of these potential hazards to occur. None of the events alone would constitute a hazard (refer to Figure 1).

**E.** The hazards identified, potential consequences and likelihood of the hazards occurring were:

1. Failure of sterilization, either total or partial, resulting in the target insect becoming an established pest in a new area, with the likelihood of $0.5 \times 10^{-18}$.
2. Introduction of new (intra-specific) genetic material into an established pest population by the "sterile insects", resulting in a more damaging insect pest, with the likelihood of $0.5 \times 10^{-23}$.
3. Failure to recognize a detected insect as sterile, resulting in an unnecessary and perhaps costly regulatory action, with the likelihood of $1 \times 10^{-11}$.
4. Introduction of an exotic contaminant organism, resulting in a new pest becoming established, was estimated to involve many folds less risk than from the movement of biological control agents, a risk already widely accepted.

**F.** Because of the sequence of events required for any of the above hazards to occur, the Consultants Group concluded that transboundary shipment would result in negligible risk with the use of FAO/IAEA operating procedures[6] regarding sterilization, handling/packaging and shipment of sterile insects.

[6] Comprehensive FAO/IAEA standard operating procedures exist for fruit fly species. For other plant pest species controlled by SIT, best practices are in place and standard procedures will be harmonized internationally over time.

## VIII. RECOMMENDATIONS

The Consultants Group recommends that this discussion paper be sent to the IPPC Secretariat for consideration by the ICPM as the basis for a standard. The Group also recommend that this standard be separate from the International Standard for Phytosanitary Measures number 3 on biological control agents.

Furthermore, the consultants recommend that the appropriate international bodies should assess the risks from transboundary shipment of insect pests of livestock and insects of medical importance controlled through SIT, and develop harmonized guidance.

## IX. REFERENCES


***Relevant guidelines for SIT***

**American Society for Testing and Materials (ASTM)** (1999) Standard Guide for Irradiation of Insects for Sterile Release Programs. Designation: ASTM E 1940 - 98. 11 pp.

**FAO/IAEA** (2000) Gafchromic® Dosimetry System for SIT, Standard Operating Procedure. Joint FAO/IAEA, Division of Nuclear Techniques in Food and Agriculture. Vienna, Austria, 42 pages.

**FAO, IAEA and United States Department of Agriculture (USDA)** (1998) Product Quality Control, Irradiation and Shipping Procedures for Mass-Reared Tephritid Fruit Flies for Sterile Insect Release Programs. Recommendations reached by consensus by an international group of fruit fly quality control experts. 52 pages.

***Other references***

**Food and Agriculture Organization of the United Nations (FAO)** (1992) The new world screwworm eradication programme-North Africa 1988-1992. 192 pages.

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## ANNEX 1. LIST OF PARTICIPANTS IN THE CONSULTANT'S MEETING ON "TRANSBOUNDARY SHIPMENTS OF STERILE INSECTS"


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# APPENDIX 5
# LIST OF SUPPLIERS FOR AERIAL RELEASE SERVICES

(List not comprehensive)

| Name of Company | Contact Address |
|---|---|
| Shickel Corporation | 115 Dry River Road Bridgewater Virginia 22812; phone: 540-828-2536<br>Tel: (540) 828-2536<br>Fax: (540) 828-4781<br>E-mail: shickel@shickel.com<br>www.Shikel.com |
| USDA Aircraft and Equipment Operations | Plant Protection and Quarantine (PPQ)<br>Mission, Texas<br>USA<br>E-mail: APHIS.Web@aphis.usda.gov<br>www.aphis.usda.gov/ppq/ispm/aeo/ |
| K&K Aircraft, Inc. | Post Office Box 7<br>1402 Airport Road<br>Bridgewater Airport/VBW<br>Bridgewater, Virginia 22812<br>USA<br>Tel: (540) 828-6070<br>Fax: (540) 828-4031 |
| Servicios Aereos Biologicos Y Forestales Mubarqui | Blvd. Enrique Cardenas Gonzalez 1359<br>Fracc. Los arcos 87040 Cd.Victoria<br>Tamaulipas<br>Mexico<br>Tel/Fax. 52-834-3164921<br>E-mail: rlmubarqui@yahoo.com.mx |
| Air Sal Leasing (Global ASL) | 14005 SW 127th St<br>Miami, FL 33186<br>United States of America<br>Tel: (305) 251-1982<br>Fax: (305 251 1966<br>E-mail: airsal@bellsouth.net |

# APPENDIX 6
# GLOSSARY OF RELEVANT TERMS

| | |
|---|---|
| Area | An officially defined country, part of a country or all or parts of several countries [ISPM 5, FAO 2005] |
| | |
| Area-wide integrated pest management (AW-IPM)* | IPM against an entire pest population within a delimited geographic area, with a minimum size large enough or protected by a buffer zone so that natural dispersal of the population occurs only within this area. |
| | |
| Absorbed dose | Quantity of radiation energy (in gray) absorbed per unit of mass of a specified target. [ISPM 18, FAO 2005] |
| | |
| Classical biological control | The intentional introduction and permanent establishment of an exotic biological agent for long-term pest control [ISPM 3 1996, FAO 2005] |
| | |
| Commodity | A type of plant, plant product, or other article being moved for trade or other purpose. [FAO 1990; revised ICPM 2001] |
| | |
| Compliance procedure (for a consignment) | Official procedure used to verify that a consignment complies with stated phytosanitary requirements. [CEPM 1999] |
| | |
| Contaminants | For purpose of this document, any impurities in a consignment. |
| | |
| Contaminating pest | A pest that is carried by a commodity and, in the case of plants and plant products, does not infest those plants or plant products. [ISPM 5 2005; FAO 2005] |
| | |
| Control (of a pest) | Suppression, containment or eradication of a pest population. [ISPM 5 2005; FAO 2005] |
| | |
| Consignment | A quantity of plants, plant products and/or other articles being moved from one country to another and covered, when required, by a single phytosanitary certificate. (A consignment may be composed of one or more commodities or lots.) [FAO 1990; revised ICPM 2001] |
| | |
| Consignment in transit | A consignment that is not imported into a country but passes through it to another country, subject to official procedures which ensure that it remains enclosed, and is not split up, not combined with other consignments nor has its packaging changed. [FAO, 1990; revised CEPM 1996, CEPM 1999, ICPM 2002 formerly *country of transit*] |
| | |

| | |
|---|---|
| Data sheet* | Document that shows production facility and contact information, species (and where available strain identification), estimated insect count and weight, consignment number, bill-of-lading, etc. |
| | |
| Detection survey | Survey conducted in an area to determine if pests are present. [FAO 1990, revised FAO, 1995] |
| | |
| Detention | Keeping a consignment in official custody or confinement for phytosanitary reasons. (See quarantine) [FAO 1990, revised FAO 1995, CEPM 1999] |
| | |
| Dispersion* | The act or an instance of dispersing; the process of being dispersed. . [Oxford Dictionary 1990] |
| | |
| Eclosion* | The emergence of an insect from a pupa-case or of a larvae from an egg. [Oxford Dictionary 1990] |
| | |
| Emerge* | Come up or out into view, especially when formerly concealed. [Oxford Dictionary 1990] |
| | |
| Emergence (adult emergence)* | The escape of the adult insect from the cuticle of the pupa. |
| | |
| Emergency action | A prompt phytosanitary action undertaken in a new or unexpected phytosanitary situation. [ICPM 2001] |
| | |
| Environmental data logger* | A device used to monitor and record environmental conditions within a consignment. |
| | |
| Entry (of a consignment) | Movement through a point of entry into an area. [FAO 1995] |
| | |
| Eradication | Application of phytosanitary measures to eliminate a pest from an area. [FAO 1990, revised FAO 1995] |
| | |
| Establishment* | Perpetuation, for the foreseeable future, of a pest within an area after entry. |
| | |
| Non native (previously "Exotic") | Not native to a particular country, ecosystem or ecoarea (applied to organisms intentionally or accidentally introduced as a result of human activity). As this Code is directed at the introduction of biological control agents from one country to another, the term "exotic" is used for organisms not native to a country. [ISPM 3 1996] |
| | |
| Feral | Existing in a wild or untamed state. [The American Heritage Dictionary, 2nd College Ed. 1982 Houghton Mifflin Company] |
| | |
| Gray (Gy)* | Unit of absorbed dose where one Gy is equivalent to the absorption of one joule per Kg. 1 Gy = 1 $J.kg^{-1}$. |
| | |
| Incubate* | Sit on or artificially heat (eggs) in order to bring forth young birds etc. [Oxford Dictionary 1990] |
| | |

| | |
|---|---|
| Incubation* | The act of incubating. [Oxford Dictionary 1990] |
| | |
| Incursions | An isolated population of a pest recently detected in an area, not known to be established, but expected to survive for the immediate future (FAO 2005). |
| | |
| Infestation (of a commodity) | Presence in a commodity of a living pest of the plant or plant product concerned. Infestation includes infection. [CEPM 1997, revised CEPM 1999] |
| | |
| Inspection | Official visual examination of plants, plant products or other regulated articles to determine if pests are present and/or to determine compliance with phytosanitary regulations. [FAO 1990; revised FAO 1995; formerly *inspect*] |
| | |
| Inspector | Person authorized by a National Plant Protection Organization to discharge its functions. [FAO 1990] |
| | |
| Intended use | Declared purpose for which plants, plant products, or other regulated articles are imported, produced, or used. [ISPM 16 2002] |
| | |
| Interception (of a consignment) | The refusal or controlled entry of an imported consignment due to failure to comply with phytosanitary regulations. [FAO 1990, revised FAO 1995] |
| | |
| Introduction | The entry of a pest resulting in its establishment. [FAO 1990, revised FAO 1995, IPPC 1997] |
| | |
| Ionizing radiation | Charged particles and electromagnetic waves that as a result of physical interaction create ions by either primary or secondary processes. [ISPM 18 2003] |
| | |
| Irradiation | Treatment with any type of ionizing radiation. [ISPM 18 2003] |
| | |
| Irradiation certificate* | Document that verifies that the sterile insects in the consignment were irradiated in accordance with approved procedures. It includes the name of the production facility and contact information, date of treatment, number of packages treated, consignment number, and signatures of two authorized officials. |
| | |
| Irradiation indicators (radiation-sensitive indicator)* | An indicator that verifies that sterile insects were exposed to ionizing radiation. |
| | |
| Labelling* | A small piece of paper or cloth attached to an article to designate its origin, owner, contents, use, or destination. |
| | |
| MACX | The MACX system is a conjunction of virtual and physic elements which make a fit up package for supervision and quality control requirements that ensures a fine development and performance at all levels of the packing, holding and release of sterile flies. |
| | |

| | |
|---|---|
| Medfly* | Mediterranean fruit fly. |
| | |
| Mexfly* | Mexican fruit fly. |
| | |
| Minimum absorbed dose (Dmin) | The localized minimum absorbed dose within the process Load. [ISPM 18 2003] |
| | |
| Official | Established, authorized or performed by a National Plant Protection Organization. [FAO 1990] |
| | |
| Packaging | Material used in supporting, protecting or carrying a commodity. [ISPM 20 2004, FAO 2005] |
| | |
| Parasite | An organism which lives on or in a larger organism, feeding upon it. [FAO 2005] |
| | |
| Parasitoid | An insect parasitic only in its immature stages, killing its host in the process of its development, and free living as an adult. [FAO 2005] |
| | |
| Pathogen | Micro-organism causing disease. [FAO 2005] |
| | |
| Pest | Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products. [FAO 1990, revised FAO 1995, IPPC 1997] |
| | |
| Pest status (in an area) | Presence or absence, at the present time, of a pest in an area, including where appropriate its distribution, as officially determined using expert judgement on the basis of current and historical pest records and other information. [FAO 2005] |
| | |
| Phytosanitary measure | Any legislation, regulation or official procedure having the purpose to prevent the introduction and/or spread of pests. [FAO 2005] |
| | |
| Phytosanitary procedure | Any officially prescribed method for implementing phytosanitary regulations including the performance of inspections, tests, surveillance or treatments in connection with regulated pests. [FAO 2005] |
| | |
| Point of entry | Airport, seaport or land border point officially designated for the importation of consignments, and /or entrance of passengers. [FAO 1995] |
| | |
| Point of transhipment* | The place where consignment is transferred from one conveyance to another before proceeding on to final point of entry. |
| | |
| Preventative release* | Continued release of low density sterile insects over a delimited area to prevent introduction of fruit fly populations. |
| | |
| Prevention* | Application of phytosanitary measures in and/or around a pest free area to avoid the introduction of a pest. |
| | |
| Progeny* | The offspring of a particular mate, or of a particular individual in the case of asexual reproduction. |

| | |
|---|---|
| Primary packaging* | A sealed escape-proof container or bag for holding insects for irradiation and shipping. Irradiation indicator should be affixed on inside of the sealed container clearly visible from the exterior without need to open it. |
| | |
| Producer* | For purposes of this document, the one who produces, sterilizes and ships sterile insects for use in control/eradication. |
| | |
| Production facility* | A building designed specifically for mass-production/rearing and sterilization of insect species (single or multiple) for use in control/eradication. |
| | |
| Pre-clearance | Phytosanitary certification and/or clearance in the country of origin, performed by or under the regular supervision of the National Plant Protection Organization of the country of destination. [FAO 1990, revised FAO 1995] |
| | |
| Quality control procedures* | For purposes of this document, standardized testing procedures for assessing product, process and production controls in mass-rearing of insects. |
| | |
| Quarantine pest | A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled. [FAO 2005] |
| | |
| Regulated non-quarantine pest | A non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party. [FAO 2005] |
| | |
| Release (into the environment)* | Intentional liberation of an organism into the environment (see also introduction and establishment). |
| | |
| Release centre* | Packing, emergence and holding centre. |
| | |
| Secondary packaging* | A container sufficiently sturdy and tamper-proof to withstand stacking, crushing and other perceived shipping processes. It holds primary packaging with sterile insects to protect product integrity during consignment from mechanical damage and environmental extremes. Wood packaging material/dunnage is not recommended because of issues related to ISPM-15. |
| | |
| Sterility* (radiation induced) | A condition in which sperm or eggs from irradiated reproducing individuals do not result in fertile offspring following fertilization. |
| | |
| Suppression | The application of phytosanitary measures in an infested area to reduce pest populations. [FAO 2005] |
| | |
| Survey | An official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species occur in an area. [FAO 2005] |
| | |

| | |
|---|---|
| Test | Official examination, other than visual, to determine if pests are present or to identify pests. [FAO 1990] |
| | |
| Treatment | Officially authorized procedure for the killing, inactivation or removal of pests, or for rendering pests infertile or for devitalization. [FAO 1990, revised FAO 1995, ISPM 15 2002, ISPM 18 2003] |
| | |
| Wild* | Not domesticated or cultivated. [Oxford Dictionary 1990] |

Terms marked with * do not appear in the International Plant Protection Convention's Glossary (ISPM No. 5) and may require review by an international panel.

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# APPENDIX 7
# GLOSSARY OF ACRONYMS

**Acronyms:**

| | |
|---|---|
| ASTM | American Society for Testing and Materials. |
| | |
| FAO | Food and Agriculture Organization of the United Nations. |
| | |
| IAEA | International Atomic Energy Agency. |
| | |
| CPM | Committee on Phytosanitary Measures. |
| | |
| IPPC | The International Plant Protection Convention, as deposited 1951 with FAO in Rome and as subsequently amended. |
| | |
| NPPO | National Plant Protection Organization. |
| | |
| RNQP | Regulated non-quarantine pest. [ISPM No. 16, 2002] |
| | |
| RPPO | Regional Plant Protection Organization with the functions down by Article IX of the IPPC. |
| | |
| SIT | Sterile Insect Technique. |
| | |
| SPS | Sanitary and Phytosanitary Standards. |

# APPENDIX 8
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This guideline is an updated version of the one published in 2007. It is aimed at providing harmonized processes involved in the handling and release of sterile insects after production in mass rearing facilities to FAO or International Atomic Energy Agency (IAEA) member countries that want to embark on sterile insect technique (SIT) activities. There is also increased interest by the private sector in investing in sterile insect production and/or other SIT activities, and these harmonized guidelines on the post-production phase will facilitate SIT application and foster the commercialization of the SIT. This guideline resulted from two FAO/IAEA consultants' meetings with representatives of relevant SIT programmes, the first held in Sarasota, Florida, United States of America (April 2004) and the second in Vienna, Austria (August 2005). It also resulted from an in depth review of the first edition, conducted in 2014 and 2015 by SIT program managers and scientists working with SIT technology.

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