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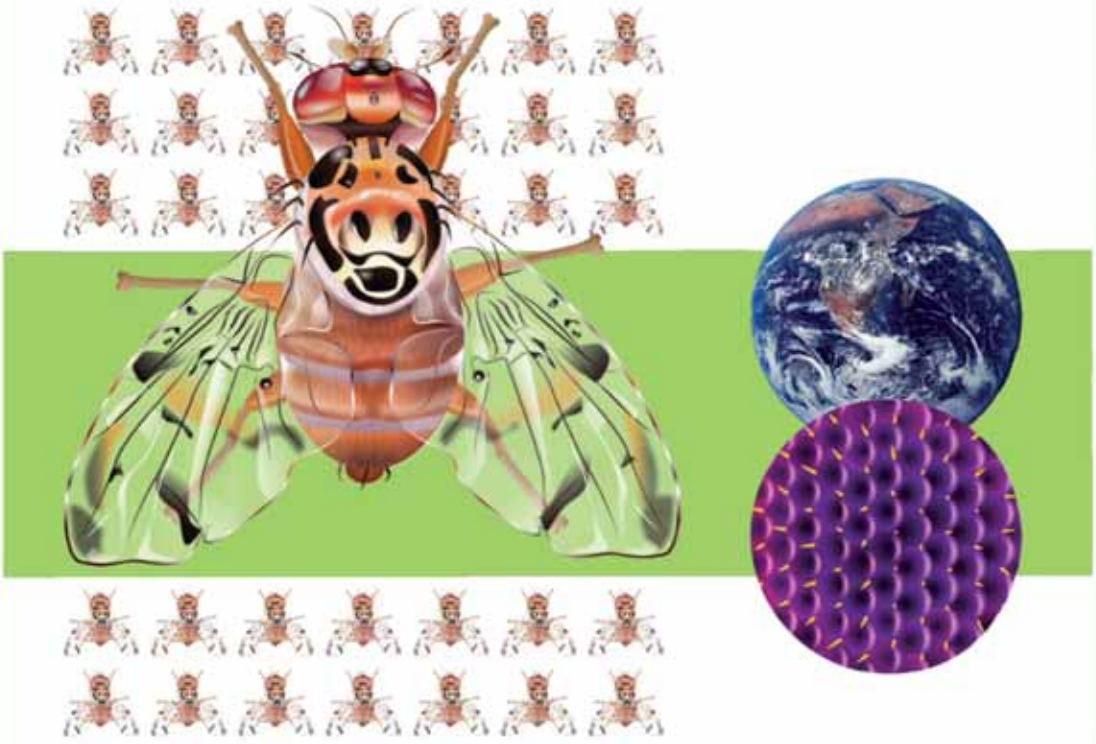
Guidance for packing, shipping, holding and release of sterile flies in area-wide fruit fly control programmes



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

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Edited by
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Joint FAO/IAEA Programme of Nuclear Techniques
in Food and Agriculture

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Foreword

The International Plant Protection Convention (IPPC) is the international treaty under which the International Standards for Phytosanitary Measures (ISPM) are adopted. ISPMs enable the development of technically justified measures for imported plants and plant products, and are intended to harmonize phytosanitary measures applied in international trade. These standards are the accepted reference under the World Trade Organization (WTO) Agreement on Sanitary and Phytosanitary Measures (SPS Agreement). The use and transboundary shipment of sterile insects was not part of ISPM No. 3, "Code of Conduct for the Import and Release of Exotic Biological Control Agents" adopted in 1995, because biological control agents had been defined as *self-replicating* organisms. Since the implementation of the Sterile Insect Technique (SIT) has largely been dominated by the public sector, this did not represent a problem for the transboundary shipment of sterile insects. However, the lack of regulatory framework did discourage private investment in the production and shipment of sterile insects.

Over the last three years (2002–2005) ISPM No. 3 has undergone a major revision to update and broaden its scope. In particular, we have been involved in explicitly including sterile insects as *beneficials* in the revised standard.

The revised ISPM No.3 "Guidelines for the Export, Shipment, Import, and Release of Biological Control Agents and Other Beneficial Organisms" was drafted in 2004 and submitted for country consultation. The revised ISPM No. 3 was adopted by the governing body of the IPPC, the Interim Commission for Phytosanitary Measures (ICPM), in April 2005 at FAO headquarters in Rome. Thus sterile insects are considered in parallel to other beneficial organisms by the IPPC through the adoption of the revised ISPM No. 3 and this should facilitate their use, especially in terms of commercialisation of the SIT and international trade of sterile insects.

In view of these developments, there is the need for harmonized guidelines and standard operating procedures for the various post-production processes and procedures involved in SIT application, so that they can be used in relation to the above mentioned revised ISPM No. 3 and other relevant ISPMs on fruit flies, such as ISPM No. 26 "Establishment of Pest Free Areas for Fruit Flies (Tephritidae)". Under the leadership of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, some guidelines related to the SIT such as the Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies and the Gafchromic® Dosimetry System for the SIT already exist or are being developed (FAO/IAEA/USDA 2003, FAO/IAEA 2000). On the other hand there has been little harmonization for the processes involved in the handling and release of sterile insects after the production in mass rearing facilities. There is no harmonized guidance available to transfer this technology to FAO or IAEA Member States that want to embark on SIT activities. There is also increased interest by the private sector in investing in sterile insect production and/or other SIT activities, and this harmonized guidance on the post-production phase will facilitate SIT application and foster the commercialisation of the SIT.

This guidance resulted from two FAO/IAEA consultants meetings with representatives of relevant SIT programmes, the first held in Sarasota, Florida (April 2004) and the second in Vienna (August 2005) (list of contributors to this guidance, see Appendix 1). It has identified a number of gaps in knowledge as well as procedures that are often based on conventional wisdom, but which need scientific verification or optimisation. A 5-year FAO/IAEA coordinated research project on "Improving Sterile Male Performance in Fruit Fly SIT Programmes" has been initiated in 2004 to address these gaps in post–factory processes and to develop procedures to improve sterile male performance through improved handling and the use of nutritional, hormonal and semiochemical supplements. The findings resulting from this R&D will be incorporated into future updated versions of this guidance document.

The officer responsible for this publication was W. Enkerlin of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture.

Table of contents

1. Introduction

1.1 SCOPE

This guidance represents the recommendations, reached by consensus of an international group of experts, on the standard procedures for the packing, shipping, holding and release of mass reared and sterilized tephritid flies that are to be used in area-wide programmes that include the Sterile Insect Technique (SIT). The majority of the procedures were initially designed specifically for the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (or Medfly), but they are applicable, with minor modifications, for other tephritid species such as those in the genera *Anastrepha*, *Bactrocera* and *Dacus*. The guidance is designed to be a working document that can be subject to periodic updates due to technological developments and research contributions. Future editions will endeavour to include more specific recommendations for other species of fruit flies as the relevant data become available.

The procedures described in this guidance will help ensure that released sterile fruit flies will be of optimal quality and that the resulting field density of these flies will be as closely aligned to the individual programme needs. It is hoped that this guidance will help to quickly identify and correct problems in programme effectiveness, resulting from less than optimal emergence and release conditions.

The procedures in this guidance are presented following a logical flow of activities in operational programmes from packing after pupal irradiation to field release of sterile flies (*FLOW CHART*, see **Appendix 2**).

1.2 BACKGROUND

The SIT relies on the release of thousands of insects per unit area to reduce the reproductive potential of a specific target pest. The release of insects is the process by which sterile insects are delivered into a target area to allow them to compete with their wild counterparts. Prior to their release, sterile insects are shipped and handled, emerged from their puparia, matured and are 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.

The SIT for fruit flies has developed in parallel for several pest species in different countries and action programmes (**Table 1.1**). Programmes integrating the SIT have been conducted with great success and have developed information during their activities that, although it may not be peer reviewed and published, offers a successful guide for specific pest problems. With this information of practical implementation, action programmes have developed operational guidance that summarize their approach to solve their local needs, however, this information is generally only available to the respective programme.

Interactions among some programmes have allowed new procedures and technology to be developed. Researchers have also contributed by experimentally determining the most appropriate approach to specific problems.

A quality control manual has also been developed as a contribution to understanding what makes a successful sterile insect. This manual describes the standard evaluations required to determine the quality of mass reared sterile insects (FAO/IAEA/USDA 2003). Most of these tests can also be applied to measure the integrity of the processes that are used to release sterile insects. These parallel developments have produced a wealth of information and technology that now need to be summarized into manuals to provide guidance. New area-wide programmes utilizing SIT technology will benefit from this compilation in order to implement their activities using state of the art technology.

An SIT programme can be clearly divided into two areas of activity. The mass rearing of insects is a specialised activity and minor variations in rearing procedures can have a significant impact on the quality of reared flies. Rearing and irradiation are carried out in strictly controlled environments prior to insect release.

The post-production process, involving the packing, shipping, handling, emergence, holding and release of sterile flies, is also a specialised procedure and requires similar but different skills. 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 factory based stages and are generally held for shorter periods (several days) compared with weeks at the production facility.

New World Screwworm flies *Cochliomyia hominivorax* (Coquerel) have now been eradicated from the continental USA. as well as from Mexico and Central America using aerial release. This technology was initially also used for fruit fly aerial release but has been superseded by new technology developed over the last 25 years in the different fruit fly programmes (**Table 1.1**). The need for increased numbers of insects in large scale programmes has led to the development of standardized conditions for all the processes from 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.

1.3 STERILE INSECT TECHNIQUE (SIT) APPLICATIONS

The Food and Agriculture Organization of the United Nations (FAO), through its International Plant Protection Convention (IPPC) whose standards are accepted by the signatory countries of the SPS Agreement of the World Trade Organization (WTO) defines "control" of a given plant pest (FAO 2006) as encompassing: suppression, containment or eradication of a pest population.

These three strategies would apply to most area-wide integrated pest management (AW-IPM) programmes with a sterile insect technique (SIT) component, including those against insect pests of medical and veterinary importance. However, the most efficient and cost-effective "control" programme is the one that aims at preventing the entry of a pest (movement of a pest into an area where it is not yet present (FAO 2006, Enkerlin 2005). This is preferable to dedicating resources to suppress, eradicate or contain an introduction (the entry of a pest resulting in its establishment (FAO 2006)) once it has occurred (Knipling 1979). On this basis, the fourth control strategy is "prevention". **Table 1.1**, summarizes all the current fruit fly programmes releasing sterile insects and their current strategic objective(s).

TABLE 1.1
Countries where SIT is being integrated into are-wide fruit fly control

Country	Fruit fly species	Objective
Argentina	Mediterranean fruit fly (or Medfly) (*Ceratitis capitata*, Wiedemann),	Eradication
Australia	Queensland fruit fly (or Qfly) (*Bactrocera tryoni*, Froggatt)	Prevention, eradication
	Medfly	Prevention
Brasil	Medfly	Suppression
Chile	Medfly	Prevention
Guatemala	Medfly	Containment, eradication
Israel	Medfly	Suppression, eradication
Japan, Okinawa	Melon fly (*B. cucurbitae*, Coquillett)	Prevention
Jordan	Medfly	Suppression, eradication
Mexico	Medfly	Eradication
	Mexican fruit fly (or Mexfly) (*Anastrepha ludens*, Loew)	Prevention, suppression
	West Indian fruit fly (*A. obliqua*, Macquart)	Prevention, suppression
Peru	Medfly	Suppression, eradication
	South American fruit fly (*A. fraterculus*, Wiedemann)	Suppression
Portugal, Madeira	Medfly	Suppression
Philippines	Philippine fruit fly (*B. philippinensis*, Drew & Hancock)	Suppression
South Africa	Medfly	Suppression
Spain	Medfly	Suppression
Tunisia	Medfly	Suppression
Thailand	Oriental fruit fly (*B. dorsalis*, Hendel)	Suppression
	Guava fruit fly (*B. correcta*, Bezzi)	
USA, California	Medfly	Prevention
USA, Florida	Medfly	Prevention
USA, Hawaii	Melon fly	Suppression
USA, Texas	Mexfly	Suppression, eradication

1.3.1 Eradication

In the past, most AW-IPM programmes integrating the SIT aimed at eventual eradication of the target population, and high densities of sterile insects were often released only during the last phase of the programme. The eradication strategy is applied mainly in the following two situations (Hendrichs *et al.* 2005):

- Eliminating an established pest population, e.g. the tsetse fly Glossina austeni in Unguja Island (Zanzibar) (Vreysen *et al.* 2000)
- Eliminating outbreaks of an exotic invasive species before full establishment can occur, e.g. the painted apple moth in New Zealand (Suckling 2003)

The second situation is likely to increase, with more pest introductions due to globalization, and the growing awareness by governments of the need for monitoring networks for early detection to facilitate eradication. Once the target pest has been eliminated from a given area, it is imperative to maintain this area pest free. This will require efficient, permanent, and stringent quarantine procedures to preclude reinvasion.

For eradication, two very important concerns (which have significant economic implications) have to be addressed: (1) the period of time in which releases of sterile insects should continue after the last wild insect has been detected (Vreysen 2005), and (2) the duration of continued monitoring after releases have stopped, to be able to declare with sufficient confidence the status of eradication (Barclay 2005).

The eradication of the Medfly in Chile (SAG 1996) opened trade opportunities annually worth several hundred million USD, and the eradication of the Mexican fruit fly (or Mexfly) and the West Indian fruit fly in north-western Mexico allows fruit trade with the USA without the need for costly postharvest treatments (Reyes *et al.* 2000; Enkerlin 2005).

1.3.2 Suppression

A suppression strategy requires continuing low to medium density releases of sterile insects to maintain the low population level. Permanent application of a suppression strategy, including continuing releases of sterile insects, could be considered disadvantageous when compared with the sustainable elimination of a pest from an area. However, this permanent need for sterile insects could stimulate and promote investment in, and the commercialization of, the mass production of sterile insects (Hendrichs *et al.* 1995, Enkerlin and Quinlan 2004). For some key fruit fly and moth pests of major agricultural crops, using sterile insects as part of a suppression strategy has become cost-competitive with conventional or other population reduction methods, e.g. Mediterranean fruit fly in Israel and Jordan (Cayol *et al.* 2004) and in South Africa (Barnes *et al.* 2004), Oriental fruit fly in Thailand (Enkerlin *et al.* 2003), and codling moth in British Columbia, Canada (Bloem and Bloem 2000).

1.3.3 Containment

Containment programmes are adopted to avoid the spread of invading exotic pests that have become established, or to consolidate progress made in an ongoing eradication programme (Hendrichs *et al.* 2005). In areas where pest levels are too high for sufficient numbers of sterile insects to be released, they have to be integrated with other population reduction tools where, as in low pest prevalence areas with remaining pest remnants or incursions, high density releases of sterile insects are particularly effective. In adjacent areas that already are largely pest free, but that are subject to regular pest entries (FAO 2005), low density releases of sterile insects are effective as insurance in a buffer zone, over parts of the contiguous pest free areas to which the pest may occasionally be moved by the transport of infested host material. An example is the Queensland fruit fly Tri-State Fruit Fly programme, which has operated since 1988 in eastern Australia to protect a quarantine area called the Fruit Fly Exclusion Zone (FFEZ). This region contains much of the horticultural production areas of southern New South Wales, northern Victoria, and eastern South Australia (Jessup *et al.* 2004) and its fruit fly free status results in enhanced access to domestic and export trade. Other examples are the Moscamed Programme which has operated since 1983 at the Guatemala–Mexico border to protect northern Guatemala, Mexico and the USA, and the Mediterranean fruit fly programme which has operated since 1996 at the Peru–Chile border to protect Chile's multibillion export horticultural industry. Some of these programmes are stationary and thus become permanent containment efforts, whereas others successfully advance or gradually retreat and eventually collapse (Hendrichs *et al.* 2005).

1.3.4 Prevention

Preventive release has been applied where the invasion pressure is very high, and quarantine activities are not sufficient to maintain the area pest free. Permanent low density releases of sterile flies are required. An example is the permanent release of sterile melon flies over the Japanese islands closest to Taiwan (Kuba *et al.* 1996) and the preventive release of sterile Medflies in California and Florida, USA (CDFA 2002). *Sequential* or *serial* eradication approaches are probably more viable economically in situations where the invasion risk is not very high (Hendrichs *et al.* 2005).

It should be noted that all preventive and containment programmes use eradicative releases, as needed, to augment their regular releases in case of an outbreak.

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2. Packing at mass rearing facility

STEP I OF PROCESS IN FLOW CHART IN APPENDIX 2

After irradiation has been carried out, sterile pupae should be adequately packed for transportation to the 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 and FAO/IAEA 2001, FAO/IAEA/USDA 2003). 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**). 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.

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 Hussman irradiators is constructed of double-walled corrugated cardboard of 74 × 34 × 34 cm with a top and bottom full overlap. Inside the box, a central compartment, 46 cm long, is lined with additional layers of

FIGURE 2.1
Plastic containers used to sterilize and transport medfly pupae in Mexico

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

FIGURE 2.3
Sealed boxes used for shipping sterile medfly pupae from Guatemala Moscamed rearing facility

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 15–20°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**). (FAO/IAEA/USDA 2003).

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**).

2.3 LABELLING

All boxes are properly labelled with the words: "Fragile" and/or "Biological Material". The words "Live 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 international standards. 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.

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 2003).

FIGURE 2.4
Three labels placed on boxes containing sterile medfly pupae shipped from Argentina (Mendoza rearing facility) to Spain (region of Valencia)

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3. Transportation to emergence and release centre (pupae) and rearing facility (eggs)

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

3.1. PUPAE

During transport, boxes containing pupae should not be handled roughly or be subjected to excessive stocking 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 contaminating pests in shipments (hitch-hikers).

Ideally, boxes of pupae should be held at or slightly below 20°C during transportation. In all cases, the containers must not be held below 0°C or spend more than a few minutes at temperatures above 30°C. Conditions such as prolonged exposure to direct sunlight, would create 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 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 important to verify the condition of the irradiation indicators attached to each pupal 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 2003). 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 pupal 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 to satellite rearing facilities that do not need to invest in maintaining large adult colonies and mother stocks. 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 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 1–12 hours after oviposition, should be dipped in a chlorine solution (200 ppm) for 10 minutes and then bubbled in a water bath at room temperature (24ºC) for 24 hours. Eggs for male only production should be incubated for an additional period of 12 hours at 34ºC 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 5ºC and stored in the appropriate container for transportation.

It has been demonstrated that eggs collected between 0 to 12 hours after oviposition and pre incubated at 25ºC for 12 hours and then stored between 10 to 15ºC 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:

- Plastic bags: For short transportation time between 24–48 hours, 0.5 to 1 litre of egg solution (1 vol eggs: 1 vol transportation medium) are sealed within polyethylene "Zyploc" 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 15ºC 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.

- 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 litre 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.

- 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, eggs should be re-rinsed in chlorine solution 200 PPM (Veloran) 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 eggs are bubbled for around 12 hours to allow the embryos to finish their development. Eggs for male only production, which were not heat-treated in the egg producing facility, should be heat-treated immediately after initiation of bubbling at 34°C for 12 hours.

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. Subsequently, additional quality control test should be conducted as specified in the Manual for Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies (FAO/IAEA/USDA 2003).

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4. Long distance (transboundary) shipment

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

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 2003). 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 almost half a century, and over 300 billion sterile pupae involving tephritid fruit fly pests (History of Transboundary Shipments of Sterile Tephritid Fruit Flies, see **Appendix 4**), 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 5**) if procedures outlined in this guidance 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 guidance, in conjunction with ISPM No.3 (FAO 2005), will assist national authorities, factories or any other organization shipping sterile insects. This document will outline standard operation procedures to follow, thus helping to assure safe shipments 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

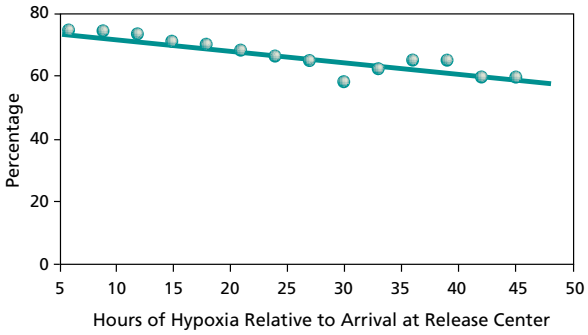

FIGURE 4.1
Detrimental effects on flight ability from prolonged hours in hypoxia

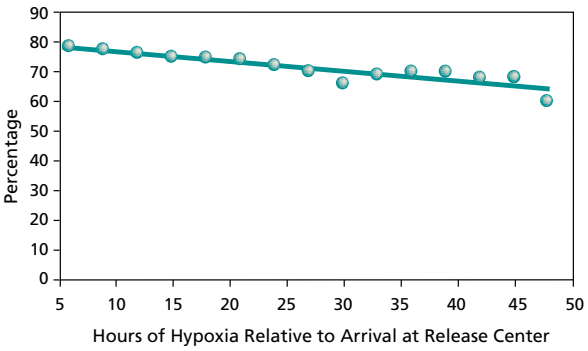

FIGURE 4.2
Detrimental effects on emergence from prolonged hours in hypoxia

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**).

4.1 OPERATIONAL PROCEDURES (SEE ALSO SECTIONS 2 AND 3 OF THIS GUIDANCE)

4.2 NORMATIVE PROCEDURES

This section provides guidance 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 6). It covers shipment of sterile, mass reared insects, including those developed through traditional selection and mutation breeding.

It is suggested that the National Plant Protection Organization (NPPO) of each country designates 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 Actions 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 recommended actions of the producer and shipper are:

Make sure that sterile insects conform to international accepted quality control standards and operation procedures (FAO/IAEA/USDA 2003), 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 guidance 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 Actions of the authorities prior to export

The recommended actions of the authorities of the exporting country are:

- 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 2003) or other procedures developed by the Joint FAO/IAEA Programme in cooperation with national and/or local governments.
- Verify that the shipment complies with the necessary documentation for safe transport.
- Issuance of Phytosanitary Certificate, issued under the authority of the NPPO in accordance with import requirements specified by the importing NPPO for the shipment may also be extended.

4.2.3 Actions of the authorities upon import (final or transit)

The recommended actions of the authorities of the importing country are:

- 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.
- Seek to verify that the packages have not been breached, and/or there is living material spilled in or on the packages.
- 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.
- Take phytosanitary action if an exotic contaminant species of quarantine concern is detected in or on the packaging of a consignment of sterile insects.
- 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 The recommended actions of the importer

The importer may be the NPPO, a regional authority, a research centre, or a private organization. For the purposes of this manual, 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

It is recommended that packages be accompanied by the necessary documentation to guarantee timely and safe delivery. Shippers may be vigilant of the following:

- Documentation may 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**).
- Documents may 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.

FIGURE 4.3.
"Transit" documents for shipment of sterile medfly pupae from Guatemala to Israel through the Netherlands

- The documentation may indicate that package content is perishable and therefore rapid transit of the material should be allowed.
- The receiver may have the necessary documentation to provide rapid feedback when the package is delayed.
- The receiver might request data on the quality of the sterile insects being reared.
- The receiver may request, for each consignment, a datasheet with a minimum of information as shown in **Appendix 3**.
- Documents may 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.

4.2.6 Traceability

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

4.2.7 Recommended actions in case of non-compliance

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

- Detection of a listed quarantine pest associated with sterile insect consignments for which it is regulated.
- Evidence of failure to meet prescribed requirements (including bilateral agreements or arrangements, or import permit conditions) such as treatment and laboratory tests.

- 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.
- Invalid or missing required documentation.
- Prohibited consignments or articles.
- 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 Recommended emergency action

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

- In consignments for which phytosanitary measures are not specified.
- In regulated consignments or other regulated articles in which their presence is not anticipated and for which no measures have been specified.
- As contaminants of conveyances, storage places or other places involved with imported commodities.
- Emergency actions should result in destruction of consignment in cases where the authorities considers the consignment cannot be otherwise handled. If destruction is required it must be done at least under supervision of the end user.

4.2.9 Records

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

- Records of inspection, sampling and testing.
- 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 may want to ensure that there are communication procedures to contact:

- Producer/end user and appropriate industry representatives.
- National authorities (including NPPOs if applicable) of exporting/transit/importing countries.
- 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 IN APPENDIX 2

5.1. RECEPTION AND UNPACKING OF PUPAE

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

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

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

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

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

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

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

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

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

5.2 PROCEDURES FOR CHILLED ADULTS

5.2.1 Setting up for fly emergence

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

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

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

FIGURE 5.1
Towers used to emerge and hold sterile flies

FIGURE 5.2
PARCs used to emerge and hold sterile flies

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

5.2.2 Food preparation and feeding

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

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

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

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

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

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

TABLE 5.2
Diet preparation

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

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

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

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

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

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

5.2.3 Emergence and holding

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

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

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

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

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

5. 3 PROCEDURES FOR ADULTS PACKED IN PAPER BAGS

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

5.3.1 Bagging procedures

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

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

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

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

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

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

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

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

5.3.2. Setting up for emergence

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

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

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

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

5.3.3 Food preparation and feeding in paper bags

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

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

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

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

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

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

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

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

The materials required are:

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

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

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

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

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

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

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

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

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

5.4 ENHANCING PERFORMANCE OF RELEASE STERILE MALES

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

5.4.1 NUTRITIONAL SUPPLEMENTS

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

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

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

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

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

5.4.2 Semiochemical supplements

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

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

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

5.4.3 Hormonal supplements

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

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

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

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6. Preparation of adults for release

STEP IV OF PROCESS IN FLOW CHART IN APPENDIX 2

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 needs to be investigated (IAEA 2004).

6.1 CHILLING OF ADULT FLIES IN PARC BOXES

Procedures are as follows:

- 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.
- The required amounts for a day's release of stacked PARCs are moved from the emergence areas to a cold rooms for immobilization by exposure to temperatures in pre-cooled cold rooms in the range of 3 to 5°C for 10 to 30 minutes.
- The aerial release box is also pre-chilled at this time in the same room.
- 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.
- 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.
- 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 TOWERS

Procedures are as follows:

- In the tower system, the ventilation fan is removed and the towers are moved into the cold rooms.
- A "knock down" fan (high volume movement fan) is placed on top of each tower to facilitate the movement of air through the towers.
- After about of 10 to 30 min., 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.
- The towers are positioned under the vacuum and processed from the top down.

FIGURE 6.1
Loading of release box into a truck for transportation to the airport.

FIGURE 6.2
Loading of a release box into a fixed-wing aircraft.

Steps are:
Food is removed and discarded; puparia are vacuumed from the edges of the tower tray; flies are removed by tapping each tray on the cross bars of the collection hopper (care should be taken that the trays are horizontal when tapped on cross bars).

- Flies are then dumped into the collection hoppers that are in turn used to load the release box.

No statistical difference in terms of quality of the sterile flies has been found between emerging and holding sterile medflies in PARCs and in Towers. Eclosion towers save space and labour (Shelly *et al.* 2006).

6.3 LOADING AND TRANSPORTATION OF RELEASE BOXES WITH CHILLED ADULTS FOR AERIAL RELEASE

Procedures are as follows:

- The release box must be inspected to ensure the slide on the bottom is in place prior to loading.
- The release boxes are weighed prior to and after loading to determine the weight of flies to be released.

FIGURE 6.3
Loading of paper bags into a fixed-wing aircraft.

FIGURE 6.4
Paper bags inside a fixed-wing aircraft.

- Flies are collected (3 to 5 g samples) prior to release from each shipment for quality control tests as well as a means of determining the individual fly weights.
- The number of flies per release box is calculated by dividing the total fly weight by the individual fly weight.
- 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).
- 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.
- The slide is then removed from the release box enabling flies to drop onto the screw augers.

6.4 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).

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7. Aerial sterile fly release

STEP V OF PROCESS IN FLOW CHART IN APPENDIX 2

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.

Sterile flies are released using fixwing aircrafts or helicopters. It has been shown that sterile medflies released by helicopter disperse throughout a narrower band than those released at higher altitudes by the standard airplane method. Helicopters appear to be well suited for sterile fly release in mountainous areas where terrain and unpredictable weather conditions are unsuitable for airplanes (Vargas *et al.* 1995).

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 11).

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**).

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.

FIGURE 7.1
Typical chute used in aircraft for paper bag aerial release.

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 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 500 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 80% emergence)
1.3 Number of sterile flying adults per bag = 5,120 (6,400 adults × 80% fliers)
1.4 Total number of effective (flying) sterile adult flies per load = 1,536,000 (5,120 sterile flies × 300 bags)
1.5 Required sterile fly density = 2000 sterile flies per hectare
1.6 Total release area:

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

2. To determine **length of flight lanes** of release area
2.1 Total square area (from 1.5) = 7.7 km² (~ 2.77 km × 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:

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

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:

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

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 11).

According with the longevity of the insect (measured as specified in the quality control manual, FAO/IAEA/USDA 2003) 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 (See Section 13).

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°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.

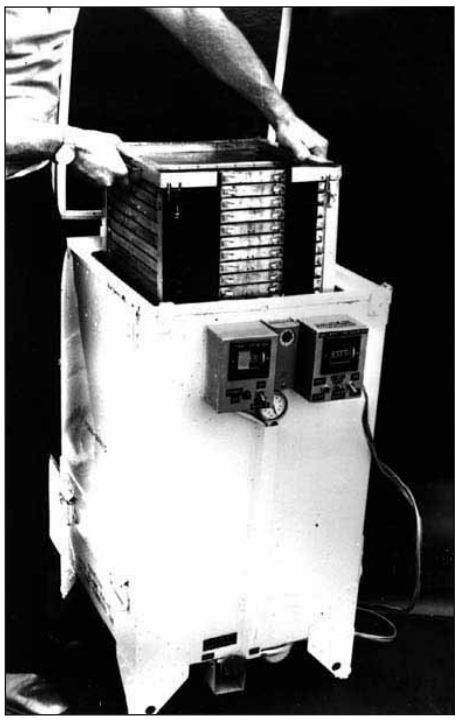

FIGURE 7.2
Release machine with capacity of 5 million sterile flies per load used for medfly release in Southern California in 1975.

FIGURE 7.3
Release machine with a capacity of 10 million sterile flies per load.

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.

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
Screw auger system for chilled adult release.

FIGURE 7.5
Release system installed inside a Cessna 206 aircraft.

The most current design was developed by the USDA, APHIS in Guatemala for the large scale Moscamed Program in Guatemala and southern Mexico. This design replaces the release box with a cylindrical container to hold and convey the chilled flies toward the release chute. Mechanical refrigeration is replaced with frozen carbon dioxide (CO_2) through the use of ducts and heat exchangers. Some of the advantages of this design include much larger load capacity, less electric wiring required in aircraft, reduced ferrying time between flights, better moisture removal from the flies and programmable fly release rates directly linked to GPS-GIS system (**Figure 7.5**) (Tween 2007).

7.2.2 Aircrafts and chilled fly release machines

Aircraft and chilled fly release machines used for the different programmes often vary. Both single and twin engine aircraft, gas and turbine are utilized (**Tables 7.1 and 7.2**). All release systems use 32 amp/24 volt electrical systems. Aircraft that operate with 12 volts will need to be converted to 24 volts.

7.2.3 Quality control of chilled adult release process

There is a new quality control system called MACX, used in Mexico for assessing the release process of *Anastrepha* spp. 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, analyzed, translated and re-transmitted to a web-site where it is available to supervisors and programme managers.

This system has been developed to work together with the Mubarqui 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 determining 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(s) equipped with the MACX system include(s) a transmitter linked to the sensor devices of the Mubarqui chilled adult release machine 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.

TABLE 7.1
Machines for chilled adult release.

Frut fly species	Type of Machine[1]	Type of Aircraft	Capacity (million sterile flies/load)
Medfly (*C. capitata*)	USDA chilled release machine	Cessna Grand Caravan	40 million
Medfly	Cylindrical box with frozen carbon dioxide	Cessna Grand Caravan	15 million
Medfly	USDA chilled release machine	Cessna 2007	2.5–3.5 million
Medfly	USDA chilled release machine	Beechcraft King Air 90 Norman Islander	5 million
Mexfly (*A. ludens*), West Indian fruit fly (*A. obliqua*)	Mubarqui, MACX SYSTEM	Cessna 206 Maule Cherokee	5 million
Mexfly, West Indian fruit fly	USDA chilled release machine	Cessna 205 Cessna 207	5 million

[1]For suppliers of chilled adult release machines see **Appendix 7**.

TABLE 7.2
Common aircraft and release systems used.

Fruit fly species	Type of release system	Aircraft	Programme location
Medfly (*C. capitata*)	Paper bags Chilled adult	Cessna 172	Chile
Medfly	Paper bags	Pipper PA-28	Chile
Medfly	Chilled adult	Cessna Grand Caravan 310	Guatemala, Mexico, USA
Medfly	Chilled adult	Beechcraft King Air 90	Guatemala, Mexico, Madeira, Portugal, USA
Medfly	Paper bags	Helicopter Bell 206 Helicopter Bell 212	Mexico
Medfly	Paper bags Chilled adult	Cessna 206 Aerocommander	USA, Mexico
Medfly	Chilled adult	Cessna 207	South Africa
Medfly	Chilled adult	Norman Islander	Israel
Medfly West Indian fruit fly (*A. obliqua*)	Paper bags Chilled adult	Cessna 207	Guatemala, Mexico
Medfly Mexfly *A. ludens*) West Indian fruit fly	Paper bags Chilled adult	Cessna 208	Guatemala, Mexico
Mexfly	Chilled adult	Maule	Mexico
Mexfly	Chilled adult	Cessna 205	Mexico
West Indian fruit fly	Chilled adult	Cherokee	Mexico

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 must 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.

There are many factors that need to be considered to assess the altitude of releases. Some of these are; 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 to include the flight dispersal of the insect being released, influence from other agencies to include governmental regulations on aviation, flight restrictions (no fly areas) also require the releases to vary in altitude.

For example, in most fruit fly release programmes in the USA, the altitude used for release is 610 – 762 m (2000 – 2500 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 warming up. 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 762 m (2500 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 = $M \times A \times V \times Z$

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 144 km/h with 268 m (880 feet) width of lane spacing.

$$\textit{Flies per second} = M \times A \times V \times Z;\ 5{,}000 \times 268 \times 144 \times 0.0000278$$

$$5{,}000 \times 268 = 1{,}340{,}000 \times 144 = 192{,}960{,}000 \times 0.0000278$$
$$= 5{,}364 \textit{ flies per second}$$

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(km²) divided by 268 m (lane spacing) = 0.373 linear km/km² 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 Pre-and post-release control of fly quality

The degree of damage to the flies caused by the release machine can be assessed by collecting samples of the flies and determining flight ability at three points in the release process:

- 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.
- Post-release: The third sample is collected from the runway and is a measure of damage caused by the release chute and wind shear as the flies exit the release aircraft. This is typically performed by the aircraft making several low passes over the runway with the machine dispensing flies at a high rate of release. The immobile flies are carefully aspirated into containers. A minimum of three samples of 100 flies each should be collected at each of the three points.

This last test should only be conducted when ambient temperatures are low enough (12°C) that the released flies do not become active before being collected. Otherwise, a disproportionate number of damaged flies will be collected, thus biasing the results.

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 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 (FAO/IAEA/USDA 2003).

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°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

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 airconditioned 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.

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.

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 excretia may pool in the bottom of any container, particularly bins, and adversely effect adults emerging in the bottom of the container (see Sections below).

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 holg 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**).

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 10 cm width × 35 to 45 cm height and contain about 4,000 to 8,000 pupae with an expected 80 to 85% emergence.

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.

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 × 0.7 m width × 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.

Distribution can be improved by the use of smaller cages (50 cm length × 50 cm width × 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.

Another similar method is the nylon mesh bag. These bags (~90 cm length × 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.

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,

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.

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.

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.

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 11).

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 8.7
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.

In the PARC box or plastic bin releases where larger numbers of pupae are involved, unemerged pupae should be returned to the vehicle for re-use and possibly a subsequent second release however this does not occur with bag releases. Unemerged 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. Unemerged 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.

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 >4/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°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°C) up to the point of release. After release from the aircraft, adult flies quickly warm up and fly to trees.

Both these approaches have some general limitations. High temperatures (>30°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):

- **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 × 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.

- **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.

- **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 × 85% emergence × 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).

- **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.

- **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

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.

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).

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.

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.

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).

Beds may be protected from ant predation by placing the material and pupae in a tray, bin, box or other container. These containers frequently have lids or covers to shelter the pupae from the sun and to minimise bird predation on the unemerged pupae and adults (**Figure 8.9**).

The ideal container appears to be a white styrene foam box (commonly used to supply vegetables to markets — 30 cm × 58 cm × 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 × 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.

FIGURE 8.10
Foam box for release being used in late winter.

FIGURE 8.11
Bucket release

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**).

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.

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 IN APPENDIX 2

9.1 FACTORS TO CONSIDER FOR ESTABLISHMENT OF 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.

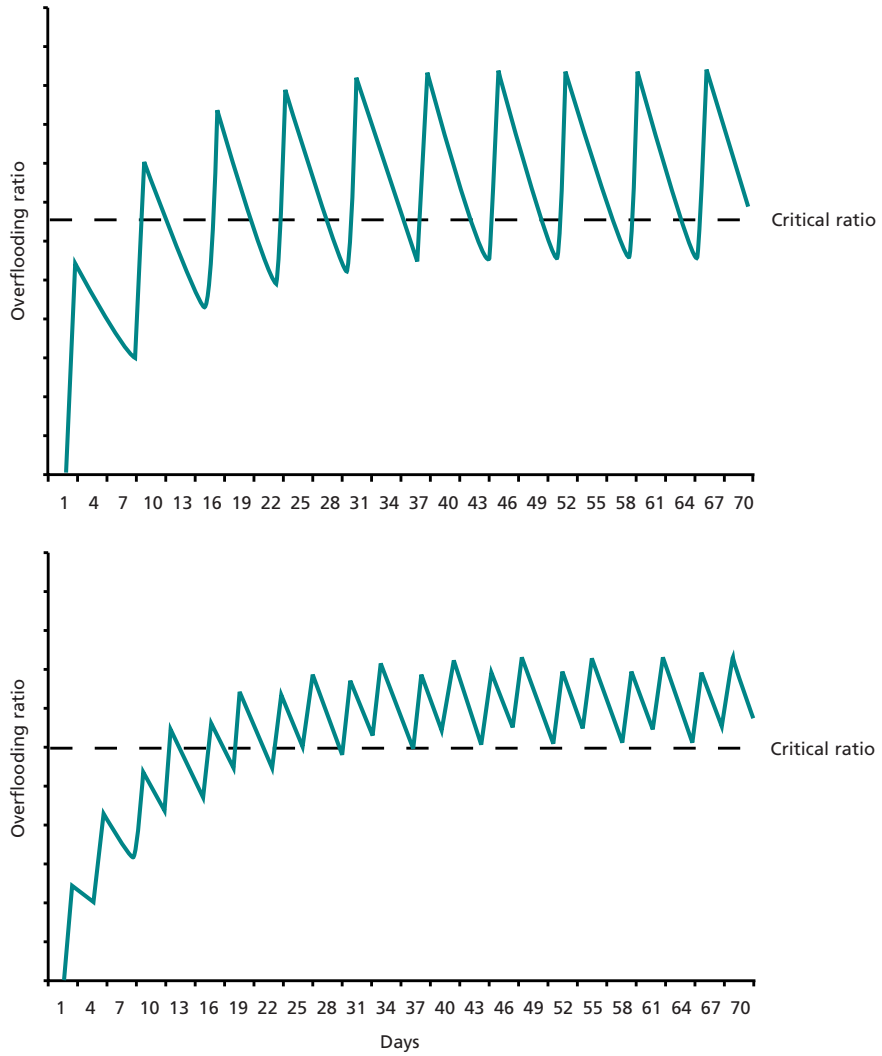

FIGURE 9.1
Effect of sterile insect longevity (assume daily mortality rate of sterile males is 0.1) on sterile to wild over-flooding ratio. Upper: Due to only weekly releases, sterile insect population routinely decreases significantly below the critical over-flooding ratio; Lower: twice-a-week releases 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

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 reduceddue 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.

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:

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

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

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

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 5).

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).

Example:

Assuming that 5 traps in 1km² (100 ha) exposed in the field for 7 days captured 3 wild flies, then:

a) $FTD_{fertile}$ *= 3 flies/(5 traps × 7 days) = 0.085*
b) The same calculation using $FTD_{sterile}$

Assuming 1,000,000 sterile flies were released in the same 1 km² area and that 3,000 flies were recaptured.

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

*c) Current sterile:f*ertile 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 km²)

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)*

TABLE 9.2
Release densities for different fruit fly SIT programmes and their respective programme objectives.

Country	Fruit fly species	Objective	Aerial Release Density (Male Flies[1]/Ha)	Main Host and Area Characteristics
Argentina	Medfly (C. capitata)	Eradication Prevention	500–3,000 250–1500	Stone and soft fruit (peaches, plums, apples and others)/Oasis–Valleys with extreme high/low temperatures.
Australia	Qfly (B. tryoni)	Prevention Eradication	1,000 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 Eradication	1,500–2,500 >3,000	Guava, mangoes/isolated valleys surrounded by mountains and desert.
Guatemala	Medfly	Containment Eradication	5,000	Continuos coffee, mixed host rural areas/ coastal, valley and mountanous area.
Israel	Medfly	Eradication 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	5,000	Continuos coffee, mixed host rural areas/ coastal, valley and mountanous area.
	Mexfly (A. ludens)	Suppression	2,500	Citrus, Guava, mangoes production areas/ coast, oasis, mountainous area.
	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
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 Eradication	250 1,000	Urban (Jungle) fruit and vegetables. Variable climate and topography.
USA Florida	Medfly	Prevention Eradication	500 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

[1]Adjusted for percent emergence, however, not for flying males.

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 km² 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 $FTD_{fertile}$ constant (**Figure 9.2**).

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

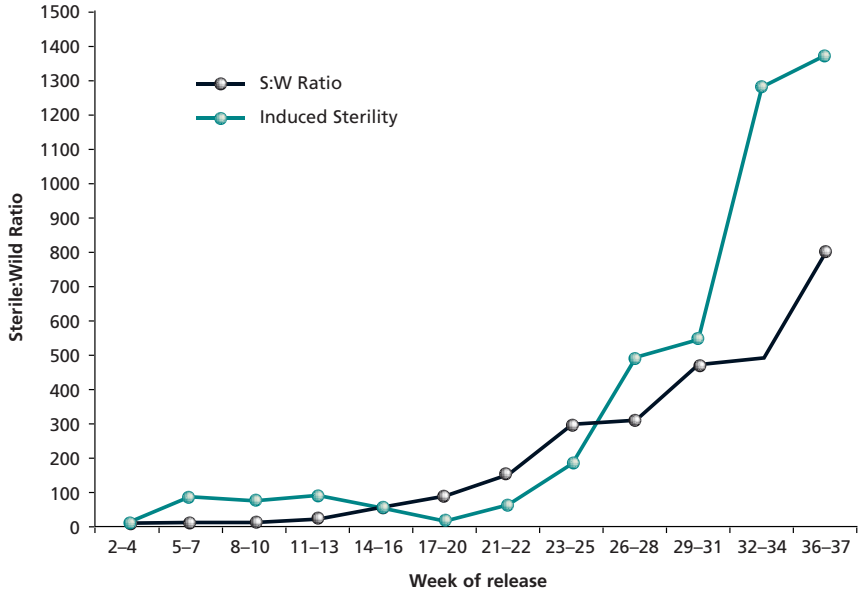

FIGURE 9.2
Increased S:W ratio as result of SIT control.

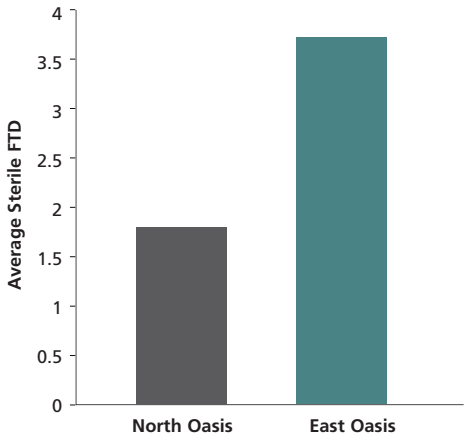

FIGURE 9.3
Effects of release densities on the number of sterile flies per trap per day (FTD) using the bag release system in the North (500-1000 sterile flies/ha) and East Oasis (1000 sterile flies/ha) in the Province of Mendoza, Argentina, 2004 – 2005.

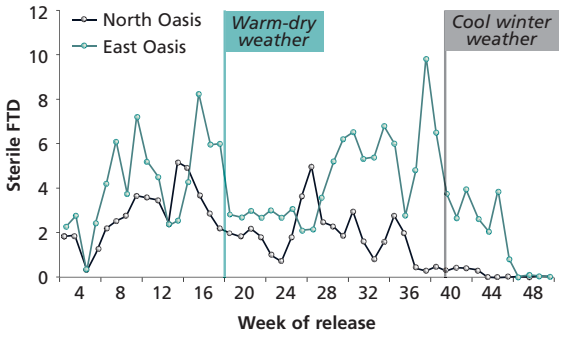

FIGURE 9.4
Fluctuations of sterile flies per trap per day due to changes in the climatic conditions in the North and East Oasis in the Province of Mendoza, Argentina, 2004 – 2005.

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.

The list of existing SIT programmes, their objective and actual sterile insect release densities are shown in **Table 9.2**. New prgrammes should determine their required

release densities considering the conditions under which activities will be conducted, objectives of the programme 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).

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10. GPS–GIS support to sterile release programmes

STEP V OF PROCESS IN FLOW CHART IN APPENDIX 2

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 (Latitude/Longitude and/or Universal Traverse Methods), altitude flown, speed of aircraft, lane numbers of release, 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, actual maps are normally collected 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 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 printed, these systems can still be utilized.

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.

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.
- Software designed for parallel offset in increments equal to the assigned swath width of the application aircraft.
- 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 one second 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 flight data log software shall be compatible with DOS compatible PC computers, dot matrix/laser/inkjet printers and plotters.
- 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.
- The software must generate the map of the entire flight within a reasonable time. System that require more than one minute to generate a map for a three hour flight on a PC (minimum 486 microprocessor with 16 MB of RAM) are unacceptable. 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. 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.
- 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 a standard 3.5" high density diskette or if another means is used, a downloading devise to enable programme personnel the ability to retrieve information must be provided.

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).

10.4 REFERENCES CITED

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11. Quality control post-irradiation

PART OF STEPS IIIb, IIIc, and STEP V OF PROCESS IN FLOW CHART IN APPENDIX 2

Routine and periodic quality control tests are required to determine the effect of radiation, handling, shipment duration, holding and release, as well as to verify that the sterile insect received fulfil minimal requirements as specified in the Manual for Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies, (FAO/IAEA/USDA 2003).

The following specific laboratory quality control tests should be conducted during different steps of the process at the emergence and release centre:

11.1 AFTER RECEPTION OF PUPAE

After unpacking, a sample of pupae should be taken to conduct quality control tests which should be done routinely (R), periodically (P) or occasionally (O). Tests include:

- Percent of emergence (R)
- Percent of flyers (R)
- Emergence peak (grids) (R)
- Stress test (longevity) (R)
- Sex ratio (R)
- Pupal weight (pupal density) (O)
- Irradiation verification (R)

11.2 PRIOR TO PREPARATION FOR RELEASE

Prior chilling of adults or bag release, a sample of adult specimens should be taken to conduct periodically the following quality control tests:

- Percent flyers (P)
- Stress test (longevity) (P)
- Fly weight (P)
- Mating competitiveness (O)
- Mating compatibility (O)
- Sterility test (% egg hatch) (O)

11.3 PRIOR TO RELEASE

After chilling of adults, or after packing and handling of bags for release, a sample of adult specimens should be taken to conduct periodically the following quality control tests:

- Percent flyers (P)
- Stress test (longevity) (P)
- Mating competitiveness (O)
- Mating compatibility (O)

11.4 AFTER RELEASE

After release, a sample of adult specimens (see Section 7.2.6) should be taken to conduct periodically the following quality control tests:

- Percent of flyers

11.5 FIELD AND FIELD CAGE QUALITY CONTROL TEST

A comprehensive list and description of this required test in a confined semi-natural environment in field cages to measure mating performance of the sterile males when competing against wild males for mating with wild females plus the methodology to perform open field dispersal and longevity test are described in the same quality control manual (FAO/IAEA/USDA 2003).

Test frequency should be determined in order to ensure that the sexual behaviour of the released sterile insect of a given fruit fly species is similar with that of the target wild population.

11.6 REFERENCES CITED

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

12. Adult processing and identification of recaptured sterile flies

STEP VI OF PROCESS IN FLOW CHART IN APPENDIX 2

12.1 OBJECTIVE

The objective of this process is two fold: (1) monitor sterile fly densities, and (2) identify fertile/sterile flies. The first provides feedback on the effectiveness of the release procedures in attaining the desired sterile fly density and sterile:wild ratio. The second is important in evaluating the effectiveness of the release in terms of reducing or eliminating fertile fly populations and also when the identification of fertile flies is a trigger for implementing suppression or eradication procedures.

12.2 TRAPPING SYSTEMS AND COLLECTION PROCEDURES

Released sterile flies are re-captured in the same traps that are used for detection of the wild population. For example, the most common trap and lure types used for Medfly detection are Jackson traps with a male specific lure (Trimedlure) and Multilure traps with a female biased lure (Biolure) (**Figure 12.1**). The densities of the traps vary depending on the programme objectives. Trapping recommendations for this fly species and others are detailed in Trapping Guidances for Area-Wide Fruit Fly Programmes (IAEA 2003), although some trap types, such as the Lynfields (Cowley *et al.* 1990, Dominiak *et al.* 2003) used in Australia, are not included in this document.

In an area subjected to sterile fly releases, the vast majority of flies caught will be sterile. Typically the flies are collected during the normal servicing of traps and brought from the field at the end of each day. When wet traps (e.g. Multilure and McPhail) are checked for fly catches in the field, samples are stored in a suitable preservative solution such as 70% isopropyl alcohol. In the case of dry traps such as Jackson or Open Bottom Dry Trap (OBDT) flies caught are left on the sticky insert and transported to the identification centres. In general flies are examined the following day.

FIGURE 12.1
Jackson (left) and Multilure (right) traps (Courtesy CDFA).

12.3 MARKING SYSTEM

When identifying sterile insects it is important to have a rapid method of distinguishing them from fertile flies. The only marking system currently used for fruit flies involves the application of a fluorescent dye onto the surface of the pupae, which is then transferred to the teneral adult upon emergence. Steiner (1965) first reported this method of identifying large numbers of released sterile flies. This method initially used oil soluble dyes and required crushing the head and extracting the dye. Holbrook *et al.* (1970) reported on the use of fluorescent dyes and the use of ultra-violet light. This was subsequently improved to increase the accuracy of discriminating between unmarked wild and dye-marked released flies (Enkerlin *et al.*1996).

The amount of Day-Glo dye applied to Medfly pupae ranges between 3.0 – 4.0 grams per kg of pupae (1.5 – 2 grams per litre of pupae). This dose may vary depending on the fly species and the needs of the individual programme. Many dyes are hydrophilic and excess amounts of dye may cause dehydration of pupae: higher body weight is an important parameter contributing to other quality parameters (Dominiak *et al.* 2002). Some dyes contain levels of deleterous chemicals such as formalin. Most dyes are manufactured in different particle sizes with smaller particles potentially clogging respiratory passages (Dominiak *et al.* 2000, Weldon 2005) A crucial element to this process is to have the pupae clumped together to increase the amount of dye covered surface area with which the emerging flies come into contact. Various colours are used depending on individual programme preferences, with the most commonly used one being red-orange. The dyes are visible under white light as dull colours, but they become most visible when viewed under ultraviolet light because the dye colour brightens and fluoresces. Some dyes reduce emergence or may interfere with dispersion ability and recapture rates (Dominiak *et al.* 2000, 2003, Jackman *et al* 1996).

The most useful area to find dye on the fly body is a membranous pocket in the head capsule called the ptilinum. This membrane is used by the fly during emergence from the pupa to break open the hardened puparium surrounding the pupa. Haemolymph is pumped into it to force it out of the head and enlarge it to an extent that it breaks the puparial shell. Shortly after the fly has emerged from the puparium, the ptilinum is retracted back into head and is not exposed again for the life of the fly. During the brief period when the ptilinum is exposed it typically becomes covered with dye dust from the outside of the puparium. Unlike the other parts of the body, the amount of dye on the ptilinum does not decrease as the fly ages because it is withdrawn within the head capsule (**Figure 12.2**).

FIGURE 12.2
Freshly emerged adult fruit flies with pink dye clearly showing in the ptilinum. It is essential for dye to be retained in the ptilinum to ensure correct identification of wild and sterile flies.

Research is ongoing to develop genetic and biochemical markers which confer identifying characteristics, such as bioluminescence or pigment changes, to the flies, but none of these are currently used in operational programmes (for more information see Sections 12.7 and 12.8).

FIGURE 12.3
Ultraviolet light and dissection microscope combination for examining dyed fruit flies (Courtesy USDA and FDACS).

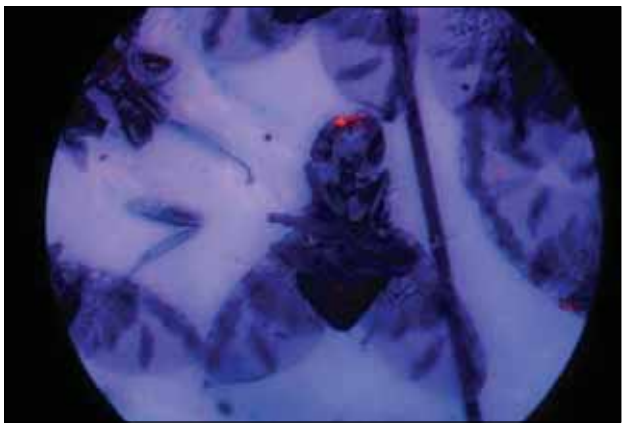

FIGURE 12.4
Sterile *C. capitata* showing marking dye on ptilinum underneath ultraviolet light (Courtesy USDA and FDACS).

12.4 INTERPRETATION OF STERILITY VIA EXAMINATION FOR DYE PRESENCE

The dye is most visible when viewed under ultraviolet light in a darkened environment. A current setup used in the Florida Preventive Release Programme uses an ultraviolet ring light attached to a dissecting microscope (**Figure 12.3**). The dye can be observed either on the external surface of the fly and/or on the ptilinum within the head capsule (**Figure 12.4**). Flies displaying the dye in the manner detailed below are considered to be sterile. In Australia, a blue light is used in a darkened room to minimise any health concerns associated with ultra violet light. If no dye is found under blue light, then flies are examined under ultra violet light.

Dye in the ptilinum is the definitive method to determine if flies are sterile and programmes should aim at 99.5% of recaptured flies having dye in the ptilinum. Dye on the ptilinum can often be seen around the edges of the ptilinal fissure and through the frons. In those instances when it can not be reliably seen, the ptilinum can be exposed in one of two ways. One is to mimic the original method of exposure, namely forcing liquid into the ptilinum. This can be accomplished by collapsing the head capsule, thereby forcing liquid forward into the ptilinum. The easiest way to do this is to lay the head on its side and gently press down. The ptilinum can also be exposed by using forceps to press against both eyes. This latter method should not be used in conjunction with examination for dye with a white light because reddish retinal tissue exiting the eyes can be mistaken for reddish dye. Alternately, the ptilinum can be physically pulled out of the head capsule by pulling on the frons just below the antennae.

Ptilinum dye is the most reliable method of identifying sterile flies. However a small number of flies may not pick up dye in the ptilinum and other tests must be used to subsequently determine if flies are wild or poorly marked sterile flies. Other parts of the body must be examined for the presence of dye. Dye that initially adheres to hardened surfaces is more likely to be removed prior to recapture than dye that collects on softer membranous surfaces. Exposed dye gradually falls off, is removed by flies during grooming, and/or can be washed off in a liquid based trap. A caution is that dye can also transfer from one fly to another in a trap on these exposed areas. Therefore, the best areas to look for dye on the fly's body are on membranous areas between the sclerotized portions of the body, especially underneath the wings and at the leg joints

and in the base of the neck. The most reliable pattern of dye is a scatter pattern similar to that made by the discharge of a shot gun. Dye in these body crevices is not easily preened off by flies and is a reasonable secondary indicator.

The collection method used within a trap can contribute to transfer of dye from a sterile fly to a fertile fly. For example, traps using sticky boards that rely on the sticky substance to entrap and kill the flies can have dye embedded in the sticky substance because captured sterile flies may struggle for some time before death. Dye that has become embedded in the sticky substance can then potentially be transferred to fertile flies subsequently landing next to the entrapped sterile. In addition, traps using liquids as a killing agent result in dye particles washing off into the liquid which can potentially be transferred to fertile flies caught in the same trap. In dry non sticky traps such as Lynfield traps, malathion causes the dying flies to buzz and convulse. These actions may cause some dye particles to be transferred to a wild fly. Therefore a high level of ptilinal dye is important to confidently and accurately identify sterile flies.

Some programmes process the flies prior to dye examination (Enkerlin *et al.* 1996, Programa Regional Moscamed 2003). In these programmes the flies are removed from the trap and placed onto a gridded sticky board. The flies are then examined for dye. For those flies where no dye is seen on the external surface, the heads are removed and placed onto a similarly gridded sticky board, crushed and then examined for dye. Some programmes only look for dye on the ptilinum (Guillen Aguilar 1983), in which case all heads are routinely taken off all of the flies, lined onto gridded paper, crushed, and examined for dye. Acetone has been used to wash the dye from the head but results may be variable for different dye formulations.

To increase accuracy in discriminating between sterile marked flies and wild flies an epi-fluorescent compound microscope (Nikkon Model Y2B-EFD-3, 1990; objective CF ACHRO 10, 20, 30, 40 and 100x; oil iris diaphragm; fluorescent filter B-24 and Epi-fluorescent accessories EPI-FIELD) can be used (Enkerlin *et al.* 1996). The epi-fluorescent microscope is more powerful than the conventional ultraviolet lamp normally used. The amount of dye used to mark the flies can be reduced if a more powerful tool for detecting marked sterile flies such as the mentioned microscope is available. Excess dye in the sterile flies has substantial detrimental effects on quality including survival and flight ability. Weldon (2005) reported that light wavelength was an important contributor to making dyes more visible, with a light filter in the blue range (467 nm) being optimal for the dye evaluated. Some programmes use different colours to evaluate different treatments. However some colours such as Deep Green and Chartreuse were highly visible under blue light but not visible under green light (511 nm) or yellow light (563 nm). Lilac was more visible under green and yellow light but less visible under blue light. Programmes need to carefully match their laboratory identification services with the dyes used in the field.

12.5 INTERPRETATION OF STERILITY VIA EXAMINATION OF REPRODUCTIVE ORGANS

Flies dislike the dye particles and expend energy to preen dust from their bodies and wings. Excess dye may result in excess preening and subsequently low energy for searching for food and shelter. The determination of the sterile/wild status based on ptilinal dye is quick and cost effective. Using other techniques, such as examining the deep body crevices for dye, using DNA, or examination of sperm (all described below) are much more time consuming and expensive. They should be used only after the examination for ptilinal dye has failed to detect dye.

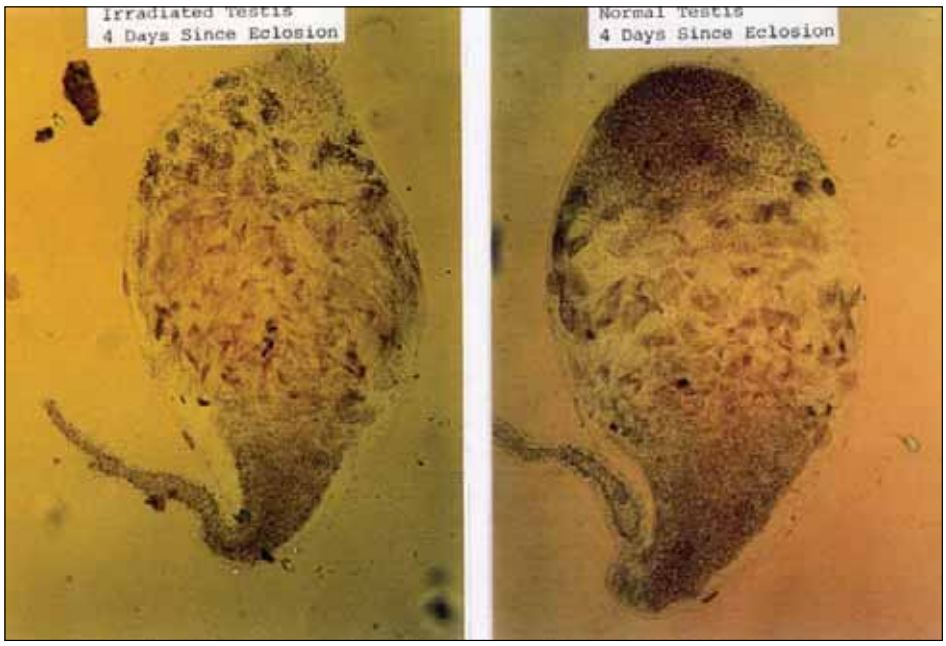

FIGURE 12.5
Testes from irradiated (left) and non-irradiated (right) male *C. capitata* four days post emergence (Courtesy CDFA).

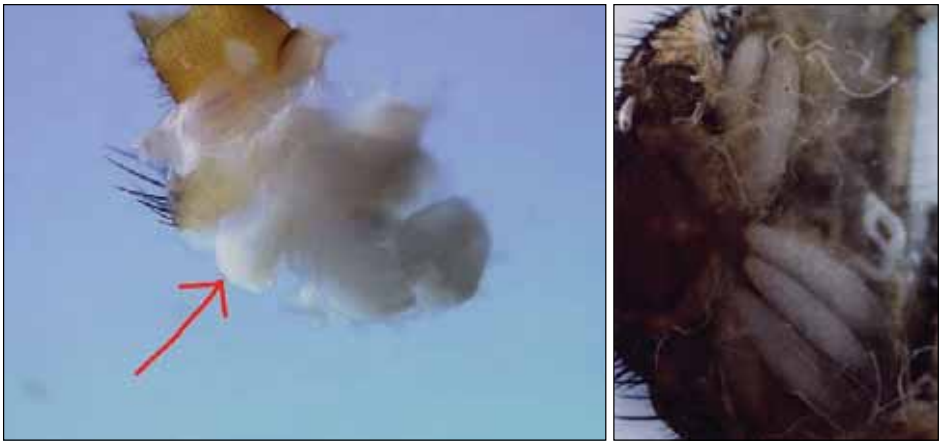

FIGURE 12.6
Ovaries from irradiated (left) and non-irradiated (right) sexually mature female *C. capitata* (Courtesy CDFA).

Occasionally recaptured flies will show no definitive evidence of having been dyed. In these cases the reproductive organs can be examined to determine whether the fly had been irradiated (Guillen Aguilar 1983). Typically, the percentage of irradiated flies requiring this level of examination should be extremely low, e.g., in the neighbourhood of 0.004% of the recaptured sterile Medfly for the California PRP.

The damage to the reproductive organs caused by irradiation results in a cessation of sperm and egg production by killing the reproductive cells. In males, this damage occurs after some sperm is already in production, so an irradiated male will have a certain amount of sperm. However, the DNA in the sperm is damaged so that the fly is functionally

sterile. Production of new sperm is prevented by the death of the reproductive cells. In females, irradiation prevents the production and maturation of eggs**.**

The damage to the reproductive cells can be seen by microscopic examination. For males, the testes must be slide mounted and examined in a 2% aceto-orcein dye under a compound microscope. Female ovaries can be examined directly beneath a dissecting microscope. An excellent day-by-day chronology of development in irradiated and non-irradiated Medfly can be found in the work by Guillen Aguilar (1983).

The testes of a sterile male show a progressive deterioration with age. The germarial cells die from the bottom to the tip. The cells undergo pycnosis where they collapse into themselves. This is seen visually by numerous dots surrounded by empty space (**Figure 12.5**). Sperm may migrate up into the germarium through these spaces. Also, spermatid production is halted so the characteristic "strawberry"-shaped spermatids disappear. In contrast, the germarium of fertile flies consists of closely packed, well defined cells and the spermatids are present in the zone below the germarium.

The ovaries of a sterile female are present as translucent sacs that can be examined visually without slide mounting (**Figure 12.6**). The ovaries of fertile flies have eggs in various stages of development.

12.6 INTERPRETATION OF FEMALE MATING STATUS VIA EXAMINATION OF REPRODUCTIVE ORGANS

While not an indicator of irradiation exposure, it is often of use to programme managers to determine the mating status of captured fertile females. This is accomplished by removing the spermathecae and slide mounting them in aceto-orcein. The spermathecae are then crushed by gently pushing down on the cover slip. This exposes any sperm inside, which then can be seen as a tangled mass in oval spermathecae from *Anastrepha*

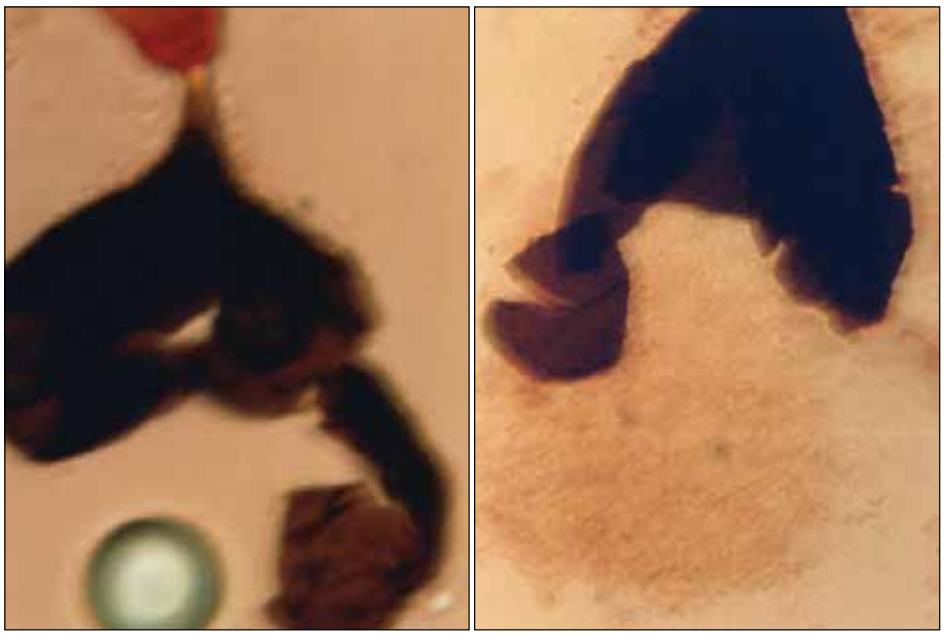

FIGURE 12.7
Spermatheca from unmated (left) and mated (right) female *C. capitata* **(Courtesy CDFA).**

and *Ceratitis* (**Figure 12.7**) or as an ordered rope-like mass in coiled spermathecae from *Bactrocera* (**Figure 12.8**).

A question often asked by programme managers is whether a mated fertile female was mated with a sterile male. This is useful information because it provides feedback on the success of the releases and it can identify localities that may require more intensified efforts. Currently, this can only be answered to some extend for one species, Medfly, by using a method of sperm head measurement (McInnis 1993). Research is underway to develop genetic and/or biochemical sperm markers to address this problem, but none of these systems have been incorporated into large scale release programmes.

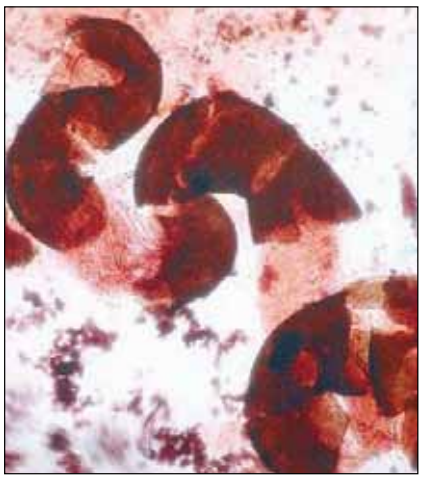

FIGURE 12.8
Spermatheca from mated female *Bactrocera oleae* (Gmelin) (Courtesy CDFA).

12.7 MITOCHONDRIAL DNA ANALYSIS

Molecular markers can be very useful in order to differentiate released flies from wild flies and endogenous populations from invasions. A class of markers that are in use for Medfly are derived from the mitochondrial DNA (mtDNA) molecule using restriction site analysis. In order to identify a marker in a release strain it is important to know the genetic variation (haplotypes) in this molecule in the target field population (IAEA 2004). The same applies to populations from different geographical regions for which haplotypes can be determined and used to compare with those of endogenous populations. This technique is routinely being used in some prevention programmes to identify possible sources of incursion of exotic flies (Yu *et al.* 2001, Sved *et al.* 2003) and in others it has been used to provide assurance to programme managers that unmarked fly finds are not unsterilized flies from the mass rearing facility (Barnes *et al.* 2004).

Another approach to marking flies for release is to transform them with a genetic construct that expresses a fluorescent protein in different body parts or in the sperm. A genetic construct has been introduced into the Medfly VIENNA 8 genetic sexing strain for evaluation (IAEA 2004).

12.8 MORPHOLOGICAL MARKERS

A dominant mutation called *Sergeant* Sr^2 could be used as a visible marker for Medflies released into the field. This mutation has been incorporated into the VIENNA 8 only male strain. The marker consists of three abdominal bands instead of the two bands that the normal medfly strain has. Releasing Medfly only male strain with the addition of a visible marker would very much simplify the discrimination between sterile released males and wild males caught in traps. Initial work on the fitness of the mutation in terms of mating behaviour showed no negative effects on the strain carrying the mutation. Furthermore, the VIENNA 8 strain with the visible marker has very similar quality profile compared with the normal VIENNA 8 strain (Niyazi *et al.* 2005). This is very encouraging for any eventual use of the strain in an operational SIT programme. However, an open field evaluation should be conducted before any decision is made on the use of the strain.

This will have to include a trapping component to assess if the marker is useful when the sterile flies are trapped and exposed to weathering in the field (Robinson and Hendrichs 2005).

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13. Interpretation of sterile fly recapture

STEP VII OF PROCESS IN FLOW CHART IN APPENDIX 2

13.1 BACKGROUND

Application of the SIT against fruit flies was first attempted at least 45 years ago (**Table 13.1**). 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.

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

TABLE 13.1
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	Timeframe	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

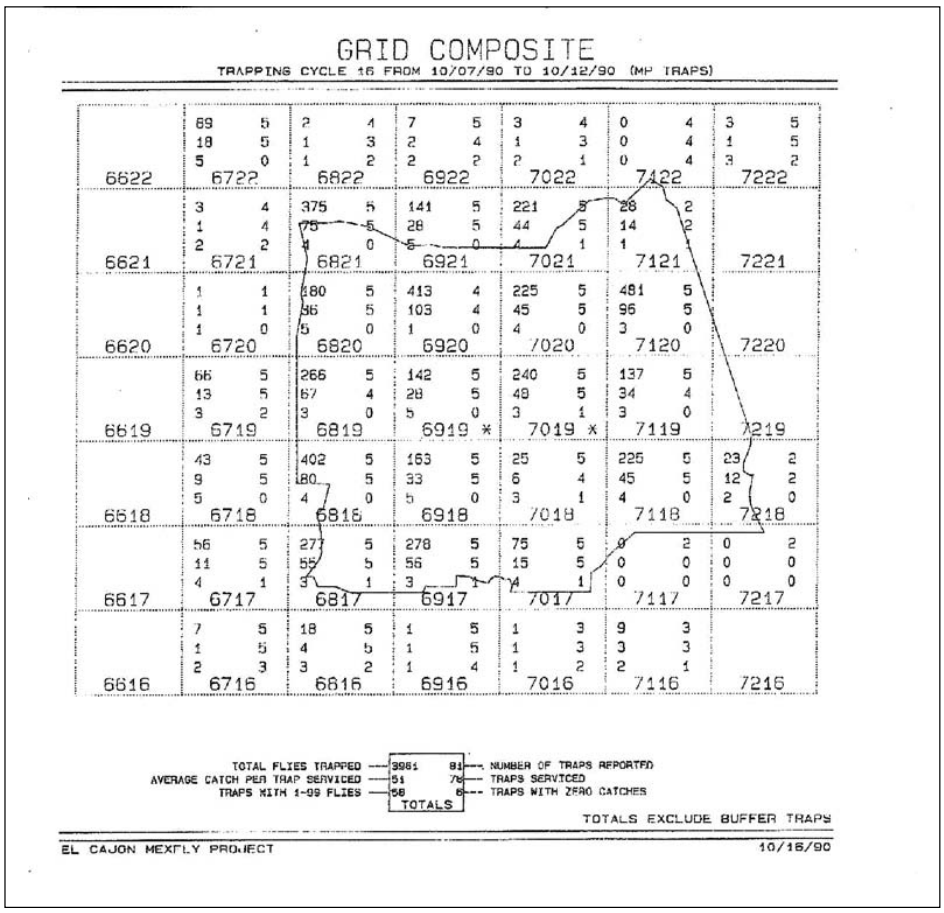

FIGURE 13.1
Cunningham report.

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 13.1**).

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.

13.2 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:

- Sterile fly distribution in the field (percentage of traps with capture)
- Sterile fly/trap/day (FTD) as a measure of sterile fly relative abundance and survival
- 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.

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

- Egg sterility measurements
- Determining larval infestation levels in the preferred host in the area
- 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.

13.2.1 DESCRIPTION OF RECAPTURE INDICES

- 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.

- 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 2003) is relevant to define if additional releases are needed and when they are needed to ensure sterile fly availability in the field.

- 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.

13.2.2 Description of evaluation parameters

- 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 2003). This test would be difficult to implement under high availability of fruit and very low population levels inherent to eradication programmes.

- 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).

- 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.

- 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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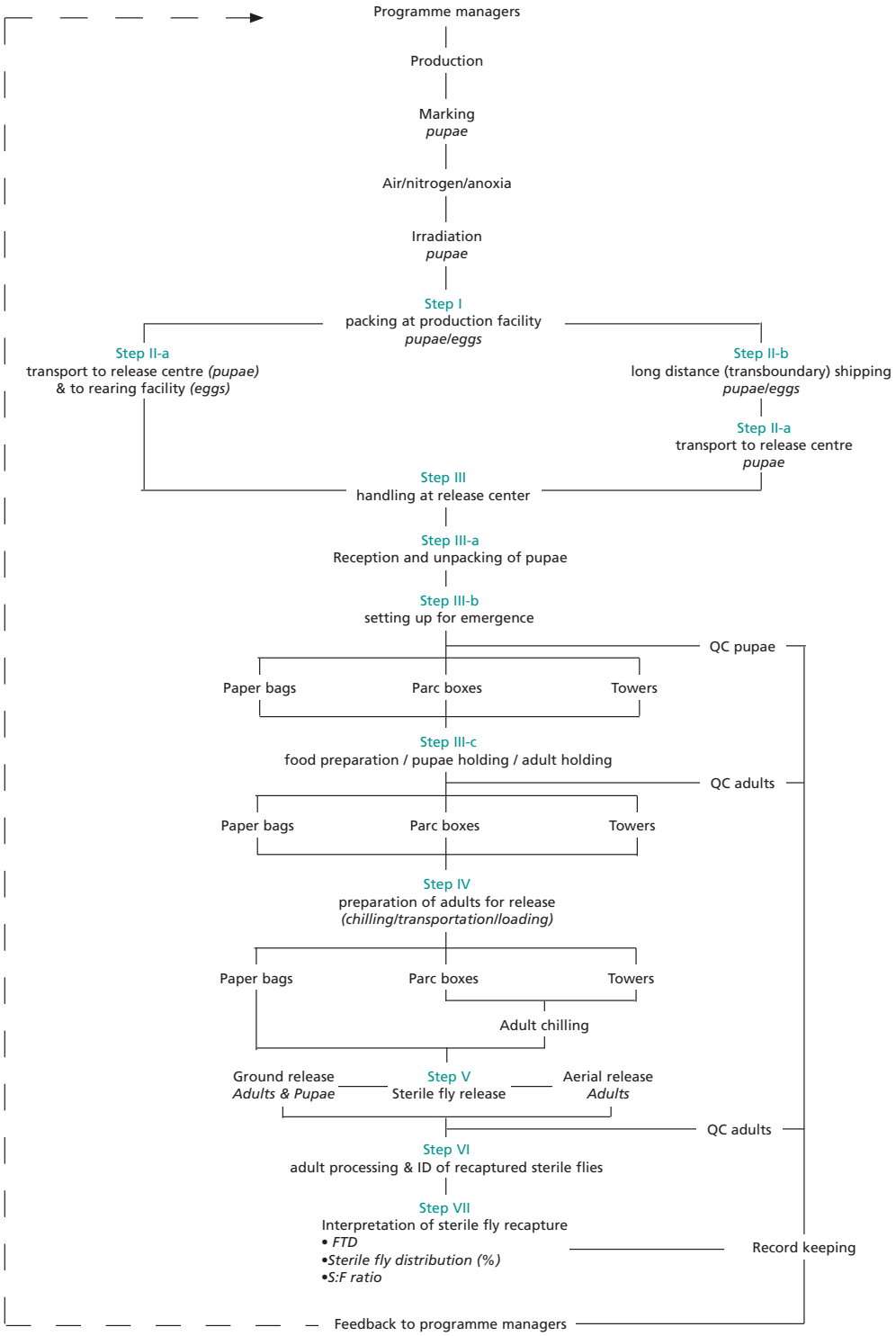
APPENDIX 2
Flow chart of sterile fly release process



Appendix 3
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:

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 4
History of transboundary shipments of sterile tephritid fruit flies

Year	Tephritid species	Site of production	Amount 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
1979-2000	Mediterranean fruit fly	Chiapas, Mexico	280,000	Guatemala	Biweekly transboundary shipments have been carried out for the past 21 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
1994-2001	Mediterranean fruit fly	El Pino, Guatemala	51,800	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
1997-2000	Mediterranean fruit fly	El Pino, Guatemala	1,000	Israel	In support of pilot suppression programme
1998-2001	Mediterranean fruit fly	El Pino, Guatemala	19,500	Florida, USA	To assist the State of Florida in eradication of medfly outbreaks
1999-2000	Mediterranean fruit fly	El Pino, Guatemala	600	South Africa	In support of pilot suppression programme
TOTAL			**363,201**		

Appendix 5
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[1] from transboundary[2] 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)

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

[2] "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.

regarding the use of sterile insects for control of human or animal diseases.

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:
  - Outbreak of the target pest in a new area, where it does not already occur.
  - 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.
  - Unnecessary regulatory actions being initiated following false identification of captured sterile insects and conclusion that it is a quarantine threat.
  - 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. 0.5×10^{-18}
  2. 0.5×10^{-23}
  3. 1×10^{-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.

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:

- Sterile insects are not self-replicating and cannot become established in the environment.
- Autocidal control is by definition intraspecific.
- 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[3]. 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

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

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 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).

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.

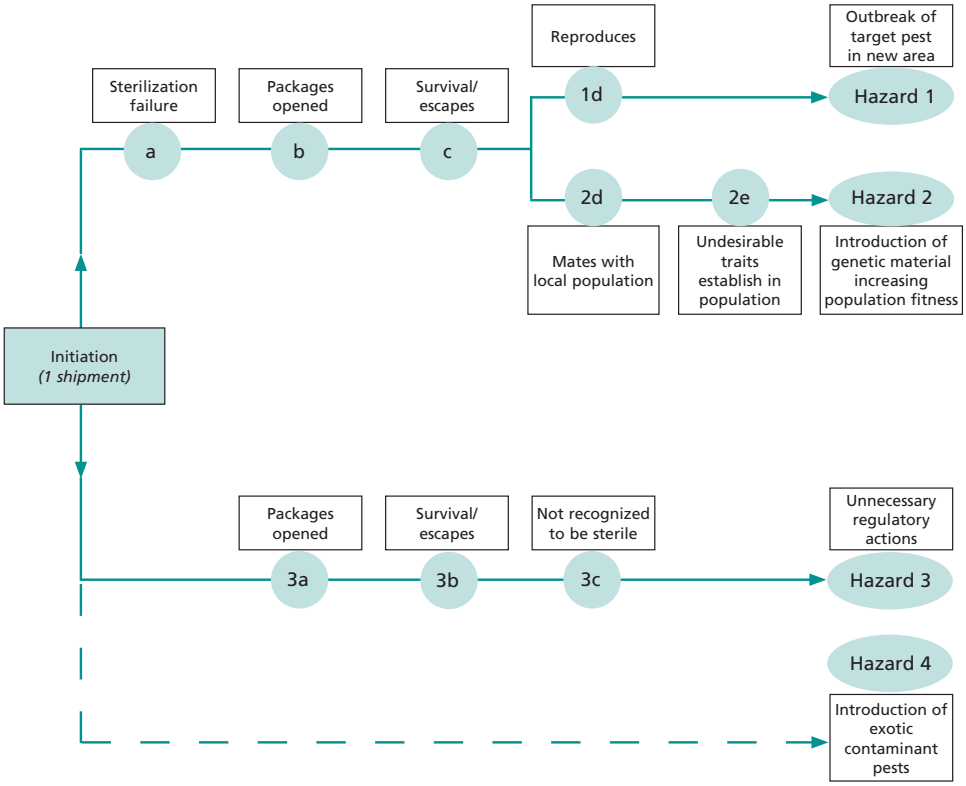


FIGURE 1
Hazard Scenarios for Transboundary Shipment of Sterile Insects

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* 0.5×10^{-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* 1×10^{-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* 1×10^{-3}

Event d: Reproduces

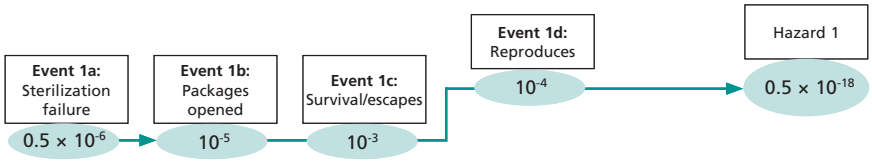
In addition to the above events, reproduction by the escaped insects would be unlikely because:

- 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* 1×10^{-4}

For the scenario for hazard 1 the likelihood of all four events occurring was estimated as a negligible risk with a probability of 0.5×10^{-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* 1×10^{-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.

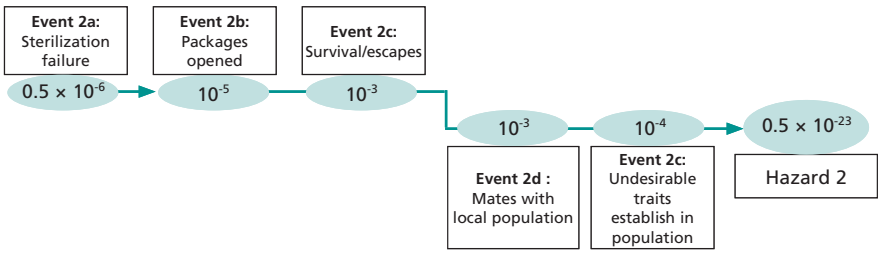
- Under mass rearing conditions over many generations, all laboratory strains are known to loose their fitness to survive under natural conditions, therefore they are highly unlikely to carry genetic traits that would increase the fitness of the wild population.

- 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* 1×10^{-6}.

For scenario 2 the likelihood of all five events occurring was estimated as a negligible risk of 0.5×10^{-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.

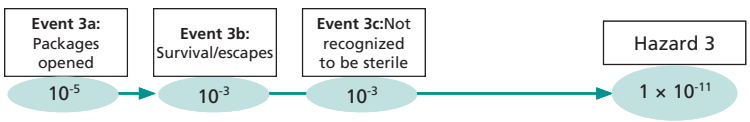
For this to occur the insect must be of regulatory significance:

- 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 × 10⁻³****.*

For scenario 3 the likelihood of all three events occurring was estimated as a negligible risk of 1 × 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:

- 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.

- 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[4] (FAO/IAEA/USDA, 1998 and FAO/IAEA, 2000). The proper application of these manuals precludes the hazards identified by the Consultants Group from occurring.

- 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.

- 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).

- The hazards identified, potential consequences and likelihood of the hazards occurring were:
  - Failure of sterilization, either total or partial, resulting in the target insect becoming an established pest in a new area, with the likelihood of 0.5×10^{-18}.
  - 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 0.5×10^{-23}.
  - Failure to recognize a detected insect as sterile, resulting in an unnecessary and perhaps costly regulatory action, with the likelihood of 1×10^{-11}.
  - 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.

- Because of the sequence of events required for any of the above hazards to occur, the Consultants Group concluded that transboundary shipment would result

[4] 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. The Consultants Group believes that the risk will be negligible from transboundary shipment of these other species as well, when best practices are applied.

in negligible risk with the use of FAO/IAEA operating procedures[5] regarding sterilization, handling/packaging and shipment of sterile insects.

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.

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ANNEX 1
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Appendix 6

IAEA-314-D4-04CT03139
LIMITED DISTRIBUTION

WORKING MATERIAL

Guidelines for export and import of sterile insects for the implementation of sit programmes against endemic/invasive crop pests

REPORT OF THE CONSULTANTS MEETING

SARASOTA, FLORIDA, USA.
11–15 MAY 2004

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Vienna, Austria 2004

TABLE OF CONTENTS

PREAMBLE

A Consultants Group Meeting was held to develop guidelines, in support of the SIT aspects of a revised International Standard on Phytosanitary Measures ISPM No. 3 and the discussion paper on *Transboundary Shipment of Sterile Insects*, that can be used to harmonize and standardize processes to promote and facilitate the use of sterile insects for current and new SIT programmes against crop pests. This meeting took place in Sarasota, Florida, USA at the USDA–APHIS–PPQ Sterile Insect Facility, from 11–15 May 2004 (Annex 1). Consultant's names are listed in Annex 2.

Harmonized guidance regarding regulation of consignments of sterile insects will facilitate transboundary trade while addressing concerns with regards to consignments in relation to possible phytosanitary risks. This document was developed as a set of technical support guidelines for consideration by the Interim Commission on Phytosanitary Measures (ICPM), the governing body for the International Plant Protection Convention (IPPC).

One possible result of consideration of these guidelines will be the development of an international standard providing guidance on measures pertaining to consignments of sterile insects. 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.

1. INTRODUCTION

The integrated use of the sterile insects technique (SIT) provides an effective and environmentally sound alternative to conventional control practices (insecticides, etc.) for an increasing number of key insect pests. Two major reasons for using SIT are that the released sterile insects are not self-replicating, intra-specific in their effect, and incapable of introducing an exotic species into the environment (see chapter on *Transboundary shipment of sterile insects for pest control programmes In: Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies*, Version 5.0, May 2003).

The release of sterile insects targets a population of the same species and serves as a form of "birth control". The sterile insects mate with the wild population but fertilization results in no viable offspring. It is only through repeated releases of sterile insects on an area-wide basis that a reduction occurs in a pest population. Technological changes are occurring resulting in increased efficiency in mass rearing, sterilization and release, making SIT more affordable than in the past. More production facilities exist today than any time previous and consignments to destinations around the world have increased significantly.

For these reasons, officials, producers and end users need guidance for dealing with packing and transport of these organisms from the production facilities to their final destination. This document also serves to support SIT aspects of a revised International Standard on Phytosanitary Measures ISPM–3.

2. SCOPE

This document provides guidance on information for export and import including: first importation, production procedures, sterilization, packaging, transportation, receipt procedures, and release of sterile insects used for prevention, containment, suppression/eradication. The document aims to address key issues related to export/import processes. Insect strains produced artificially by genetic engineering or other modern biotechnology methods are not covered under this document.

3. REFERENCES

Agreement on the Application of Sanitary and Phytosanitary Measures, 1994. World Trade Organization, Geneva.

Code of conduct for the import and release of exotic biological control agents, 1996. ISPM No. 3, FAO, Rome.

Export certification system, 1997. ISPM No. 7, FAO, Rome.

Glossary of phytosanitary terms, 2004. ISPM No. 5, FAO, Rome.

Guidelines for the notification of non-compliance and emergency action, 2001. ISPM No. 13, FAO, Rome.

Guidelines for regulating wood packaging material in international trade, 2004. ISPM No. 15, FAO, Rome.

Guidelines for the use of irradiation as a phytosanitary measure, 2003. ISPM No. 18, FAO, Rome.

International Plant Protection Convention, 1997. FAO, Rome.

FAO/IAEA/USDA. 2003. Manual for Product Quality Control and shipping Procedures for Sterile Mass-Reared Tephritid Fruit flies, Version 5.0. International atomic Energy Agency, Vienna, Austria. 85pp.

Lindquist, D. 2000. Pest Management Strategies: Area-wide and Conventional pp. 13–19. *In* Area-wide control of fruit flies and other insect pests. Ed. K.H. Tan. Penerbit Universiti Sains Malaysia.

Standard Guide for Irradiation of Insects for Sterile Release Programs. Document Number ASTM E1940–98, 11 pages, 1998 ASTM International.

4. DEFINITIONS

Area-wide control	Control measures applied against a given plant pest over a geographically defined area that includes all known or potential hosts with the objective of preventing pest build-up while minimizing damage to commercial host. Control actions are conducted whenever and wherever the target pest exists regardless of host seasonality.
Absorbed dose	Quantity of radiation energy (in gray) absorbed per unit of mass of a specified target. [ISPM 18].
ASTM	American Society for Testing and Materials
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.
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.
Detention	Keeping a consignment in official custody or confinement for phytosanitary reasons. (See quarantine) [FAO, 1990; revised FAO, 1995; CEPM, 1999]
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].
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 $J.kg^{-1}$
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 No. 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]
ICPM	International Commission on Phytosanitary Measures
Ionizing radiation	Charged particles and electromagnetic waves that as a result of physical interaction create ions by either primary or secondary processes. [ISPM No. 18, 2003]
IPPC	International Plant Protection Convention, as deposited in 1951 with FAO in Rome and as subsequently amended. [FAO, 1990; revised ICPM, 2001]
Irradiation	Treatment with any type of ionizing radiation. [ISPM No. 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.
ISPM	International Standard for Phytosanitary Measures [CEPM, 1996; revised ICPM, 2001]

Labelling	A small piece of paper or cloth attached to an article to designate its origin, owner, contents, use, or destination.
Minimum absorbed dose (Dmin)	The localized minimum absorbed dose within the processLoad. [ISPM No. 18, 2003]
NPPO	National Plant Protection Organization. [FAO, 1990; ICPM, 2001]
Official	Established, authorized or performed by a National Plant Protection Organization. [FAO, 1990]
Quality control procedures	For purposes of this document, standardized testing procedures for assessing product, process and production controls in mass-rearing of insects.
Packaging	Material used in supporting, protecting or carrying a commodity. [ISPM No. 20, 2004]
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]
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.
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]
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. [IPPC, 1997]

RNQP	Regulated non-quarantine pest. [ISPM No. 16, 2002]
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.
SPS	Sanitary and phytosanitary Standards
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 No. 15, 2002; ISPM No. 18, 2003]

5. OBJECTIVE

The objective of this document is to provide guidelines in support of the SIT aspects of a revised ISPM No. 3 that can be used to harmonize and standardize packing, shipment and release activities to facilitate internationally the use of sterile insects for current and new SIT programmes against crop pests.

6. OUTLINE OF REQUIREMENTS

6.1 International agreements, principles and standards

National governments have the sovereign right to regulate imports to achieve their appropriate level of protection, taking into account their international obligations. Rights, obligations and responsibilities associated with international agreements as well as the principles and standards resulting from international agreements, in particular the IPPC (1997) and the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (WTO–SPS Agreement), affect the structure and implementation of import regulatory systems. These include effects on the drafting and adoption of import regulations, the application of regulations, and the operational activities arising from regulations.

In particular, the phytosanitary procedures and regulations should take into consideration the concept of minimal impact and issues of economic and operational feasibility in order to avoid unnecessary trade disruption.

6.2 Producer/End User

6.2.1 Prior to first importation, the importer should prepare dossiers with information on the proposed sterile insect

- Accurate identification or, where necessary, sufficient characterization of the agent to allow its unambiguous recognition.

- A summary of all available information on its origin, distribution, biology, natural enemies and impact in its area of distribution.
- Full documentation of novel importations and their release programme as to identities, origins, numbers/quantity released, localities, dates, and any other data relevant to assessing the outcome, and maintenance of records of appropriate information with regard to other repeated releases of the same species.

6.2.2 Measures for consignments to be imported

The importer should indicate to the exporter/producer measures with which exported consignments should comply. These measures may be general, applying to all types of commodities, or the measures may be specific, applying to specified commodities from a particular origin. Systems approaches may also be used when appropriate.

Measures required from the producer (production facility) include:

- Inspection prior to export — ensure that the correct species is being shipped; minimize possible contaminants.
- Treatment prior to export — with ionizing radiation.
- Testing prior to export — routine quality control procedures in place to ensure that the product has received the required minimum absorbed dose and that the insects are marked to differentiate them from the wild insects (see FAO/IAEA/USDA *Product quality control and shipping procedures for sterile mass-reared tephritid fruit flies*, Version 5.0, May 2003).
- Maintenance of consignment integrity — placement of commodity in sealed primary and secondary packaging, use of radiation detectors inside primary packaging.
- Appropriate certification/documentation in place. (For an example see Annex 2 – Radiation Certificate).
- Additional requirements specified by end user.
- Accreditation procedures – personnel appropriately trained in irradiation procedures.

Measures required during consignment include:

- Use of the most direct route/method of transportation available.
- Avoid exposure to temperature extremes and direct sunlight.
- Maintain consignment integrity:
  - Primary packaging should be escape proof, tamper proof, transparent so that radiation indicator inside packaging can be clearly viewed without opening or compromising the integrity of the primary packaging.
  - Secondary packaging should be sufficiently sturdy to withstand stacking, crushing and sufficiently tamper proof to withstand the perceived shipping processes.
    i) Wood packaging material is not recommended because of issues related to ISPM–15.
- Consideration should be given to the use of environmental data loggers for monitoring temperature and humidity conditions during transport (see FAO/IAEA/USDA *Product quality control and shipping procedures for sterile mass-reared tephritid fruit flies*, Version 5.0, May 2003).
- All secondary packaging to be properly labelled with the words: “Fragile” and “Perishable”. In some cases, the mention “Live Insects” and some indication of the storage conditions (“This Side Up”, “Handle with Care”) are also present

on the secondary packaging. To facilitate tracking of consignments, these should have complete information on the location of the addressee, a consignment number and in addition, secondary packaging for each consignment should be numbered consecutively in large, clear writing on the outside of the container; e.g., "Consignment #, Box 3 of 24". Affix permits to secondary packaging as required.

Conveyances and Transport:

- For local transportation, air-conditioned or refrigerated vehicle should be used if ambient conditions are likely to result in overheating of material.
- For long-distance consignment, material is typically transported by commercial aircraft in a portion of the cargo hold where temperature and air pressure are held at cabin levels. Airline routing should be selected to minimize transshipment points and overall transit time.
- For misdirected/lost consignments, producer and end user should work with carrier to locate and forward the consignment to its intended destination. End user is responsible for final disposition (use or destruction).

Measures required at the point of entry include:

- Upon arrival at point of entry ensure appropriate procedures to clear consignment by required authorities.
- Consignment details should be verified with corresponding documentation.
- Documents should also include clear instructions to officials at transshipment or entry points on how a misdirected/lost consignments that are found are to be handled.
- If integrity of packaging is breached, containment actions must be taken (e.g., organisms immediately immobilized, collected and/or destroyed as appropriate for the commodity).

Measures required for transportation to processing/release facility:

- Where possible, transport vehicle should be secure and climate controlled.
- Procedural instructions should be in place to address transportation problems to the processing facility (e.g., break downs, traffic accidents).

Measures required after entry include:

- Inspection and verification of receipt of correct species, treatment certificate and radiation indicator labels. Receiver must carefully check the documentation that accompanies the consignment and verify that:
  - Documentation has been signed by the shipper.
  - Consignment contents match the information reported on the documentation. Any discrepancy in consignment contents should immediately be reported to the producer and a decision on final disposition of the consignment should be made. If there is no certificate in the consignment that verifies that the material was correctly irradiated, contact producer to ascertain if proper documentation can be obtained. If certificate cannot be obtained then affected insects should be destroyed.
  - Verification of the condition of the radiation sensitive indicators attached to each primary package. The indicators must clearly show that they have been exposed to the minimum absorbed dose (Dmin).

Inspection of packaging integrity.

- Examination of each primary package: The insects contained in the package must be destroyed if the radiation-sensitive indicator is missing, underexposed or partially exposed or if the packaging is ruptured or broken.

Other measures that may be required:

- Licences or permits.
- Limitations on points of entry for specified commodities (sterile insects).
- Advance notification of arrival of specified consignments.
- An audit of procedures in the exporting country.

6.2.3 Consignments in transit

Consignments in transit are not imported (see ISPM No. 5). However, the import regulatory system may be extended to cover consignments in transit and to establish technically justified measures to prevent the introduction and/or spread of pests (Article VII.4 of the IPPC, 1997).

Measures may be required to track consignments, to verify their integrity and/or to confirm that they leave the country of transit.

Producer and end user must take into consideration that in transit countries may:

- Establish points of arrival in transit country.
- Determine routes within the in transit country.
- Determine conditions for transportation and time spans permitted within their territories.

6.3 Phytosanitary Measures

6.3.1 Procedures in the production facility

Import regulations often include specific requirements that should be done in the country of export, such as production procedures (usually during the growing period of the commodity concerned) or specialized treatment procedures. In certain circumstances, such as in the development of a new trade, the requirements may include, in cooperation with the NPPO of the exporting country, an audit in the exporting country by the NPPO of the importing country such as:

- Production Systems (e.g., Standard Operation Procedures Manual and Quality Control Manual).
- Treatments (e.g., ionizing radiation).
- Inspection Procedures (e.g. product integrity, radiation sensitive indicators).
- Testing Procedures (e.g., process and quality control routine tests).
- Packaging.
  - Primary packaging holds the product that is escape proof, tamper proof and transparent (so that irradiation indicator inside packaging can be clearly viewed without opening or compromising the integrity of the primary packaging).
  - Secondary packaging that is sufficiently sturdy and tamper proof to withstand the perceived shipping processes. Wood packaging material is not recommended because of issues related to ISPM–15. Consideration must be given to official

inspection processes when secondary packaging is being designed so that primary packaging can remain intact whilst allowing organisms to be viewed.

6.3.2 Shipping concerns

- Labelling:
  - All secondary packaging to be properly labelled with the words: "Fragile" and "Perishable". In some cases, the mention "Live Insects" and some indication of the storage conditions ("This Side Up", "Handle with Care") are also present on the secondary packaging. To facilitate tracking of consignments, these should have complete information on the location of the addressee, a consignment number and in addition, secondary packaging for each consignment should be numbered consecutively in large, clear writing on the outside of the container; e.g., "Consignment #, Box 3 of 24". Affix permits to secondary packaging as required.

- Documentation should:
  - Conform to relevant regulations of exporting and importing countries especially concerning import permit, national transit permit, phytosanitary certificate, irradiation certificate, labelling and notification.
  - Include clear instructions to handlers and officials at point of embarkation, transhipment or entry on how consignment should be treated to avoid damage to contents and on action to be taken if consignment is breached (e.g., containment actions such as organisms immediately immobilized, collected and/or destroyed, as appropriate for the commodity).
  - Indicate that consignment is perishable and, therefore, rapid transit of material should be allowed.
  - Provide rapid feedback to end-user when consignment is delayed.
  - Provide relevant data to end user on quality of sterile insects in consignment.
  - Include clear instructions to officials at transhipment or entry points on final disposition if a lost consignment is found.

6.3.3 Compliance checking at point of entry

Some of the basic elements to compliance checking are:

- Advance notification and documentation of consignment specifying arrival information.
- Verification that required clearances for consignment have been obtained.
- Documentary checks (e.g., product contents correspond with documentation).
- Consignment integrity checks.

Compliance checking at point of entry of consignments may be required to:

- Determine compliance with phytosanitary regulations.
- Check that phytosanitary measures are effective in preventing the introduction of quarantine pests and limiting the entry of RNQPs.
- Detect potential quarantine pests or quarantine pests whose entry with that commodity was not predicted.

Phytosanitary inspections should be carried out by or under the authority of the NPPO.

6.3.4 Inspection

Inspections may be done at point of entry, transhipment, destination or other locations provided that phytosanitary integrity is maintained and appropriate phytosanitary procedures can be carried out. By agreement or other arrangement, they may also be done in the country of origin as a part of a pre-clearance programme in cooperation with the NPPO of the exporting country.

Phytosanitary inspections may be applied:

- To all consignments as a condition of entry.
- Where the level of monitoring (i.e. the number of consignments inspected) is established on the basis of predicted risk.

Inspection and sampling procedures may be based on general or specific procedures to achieve pre-determined objectives.

6.3.5 Transport to processing/release facility

- Where possible, transport vehicle should be secure and climate controlled.
- Procedural instructions should be in place to address transportation problems to the processing facility (e.g., break downs, traffic accidents).

6.3.6 Testing

Testing may be required for:

- Verification of the declared product — e.g., correct identification of species.
- Verification of product integrity.
- Audit or monitoring.

Testing should be performed by persons experienced in the appropriate procedures and, if possible, following internationally agreed protocols. Cooperation with appropriate academic and international experts or institutes is recommended when validation of test results is needed.

6.3.7 Action in case of non-compliance

Examples where phytosanitary action may be justified regarding non-compliance with import regulations include:

- Detection of a listed quarantine pest associated with consignments for which it is regulated.
- Evidence of failure to meet prescribed requirements (including bilateral agreements or arrangements, or import permit conditions) such as treatment and laboratory tests.
- 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.
- Required documentation e.g., invalid or missing.
- Prohibited consignments or articles.
- Failure to meet 'in-transit' measures.

Type of action will vary with circumstances and should be 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.

6.3.8 Emergency action

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

- In consignments for which phytosanitary measures are not specified.
- In regulated consignments or other regulated articles in which their presence is not anticipated and for which no measures have been specified.
- As contaminants of conveyances, storage places or other places involved with imported commodities.

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.

6.4 Documentation

6.4.1 Procedures

Procedures to be documented include:

- Inspection, sampling and testing methodology (including methods for maintaining sample integrity).
- Action on non-compliance, including treatment.
- Notification of non-compliance.
- Notification of emergency action.

6.4.2 Records

Records should be kept of all actions, results and decisions including:

- Records of inspection, sampling and testing.
- Non-compliance and emergency action (in accordance with ISPM No. 13: *Guidelines for the notification of non-compliance and emergency action*).

6.5 Communication

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

- Producer/end user and appropriate industry representatives.
- NPPOs of exporting/importing countries.

ANNEX 1
AGENDA

Tuesday 11 May

08:30	09:00	Welcome / Administration	J. Stewart / C. Cáceres
09:00	09:30	Objectives of the meeting	C. Cáceres
09:30	10:30	Current technology for fruit fly SIT release programmes in Guatemala/Mexico	P. Gomes
10:30	10:45	Coffee break	
10:45	11:15	Anastrepha SIT release programmes in the USA	J. Worley
11:15	12:15	Available and required technology for Codling moth SIT and other moth release programmes	S. Bloem
12:15	14:00	Lunch	
14:00	15:30	Current technology for fruit fly SIT release programmes in California, USA	Eileen Smith
15:30	16:00	Visit to the USDA–APHIS Sarasota sterile fly emergence and release centre	J. Steward
16:00	16:30	Review of risk assessment document on transboundary shipment of sterile insects	G. Mynard/C. Cáceres
16:30	17:30	Regulatory issues to be considered in relation to sterile insect reception, emergence and release	G. Mynard

Wednesday 12 May

08:30	10:30	Structure of the document, general discussion	P. Gomes
		Divide into groups for drafting	
10:30	10:45	Coffee break	
10:45	12:00	Drafting of document	
12:00	13:30	Lunch	
13:30	15:30	Drafting of document	
15:30	15:45	Coffee break	
15:45	17:30	Drafting of document	

Thursday 13 May

08:30	10:30	Drafting of document	G. Mynard
10:30	10:45	Coffee break	
10:45	12:30	Drafting of document	
12:30	14:00	Lunch	
14:00	15:30	Drafting of document	
15:30	15:45	Coffee break	
15:45	17:30	Drafting of document	

Friday 14 May

08:30	10:30	General discussion of document components	G. Mynard
10:30	10:45	Coffee break	
10:45	12:30	Drafting of document	
12:30	14:00	Lunch	
14:00	15:30	Drafting of document	
15:30	15:45	Coffee break	
15:45	17:30	Drafting of document	

Saturday 15 May

08:30	10:30	Drafting of document	S. Bloem
10:30	10:45	Coffee break	
10:45	12:30	Presentation of the final draft	
12:30	14:00	Lunch	
14:00	15:30	Presentation of the final draft	
15:30	15:45	Coffee break	
15:45	17:30	Presentation of the final draft	

ANNEX 2
LIST OF PARTICIPANTS

Australia

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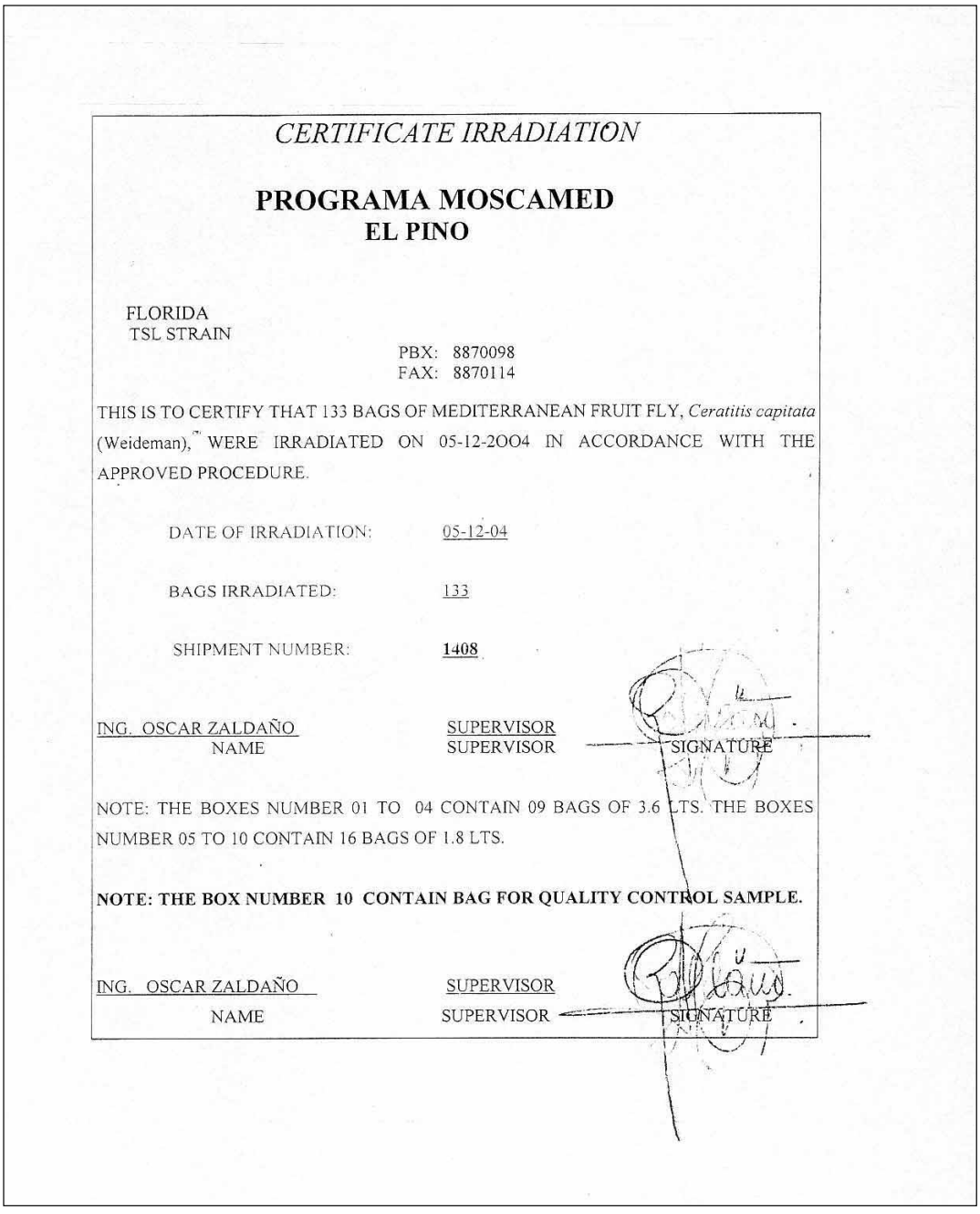
ANNEX 3
EXAMPLE OF CERTIFICATE



CERTIFICATE IRRADIATION

PROGRAMA MOSCAMED
EL PINO

FLORIDA
TSL STRAIN

PBX: 8870098
FAX: 8870114

THIS IS TO CERTIFY THAT 133 BAGS OF MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Weideman), WERE IRRADIATED ON 05-12-2004 IN ACCORDANCE WITH THE APPROVED PROCEDURE.

DATE OF IRRADIATION: 05-12-04

BAGS IRRADIATED: 133

SHIPMENT NUMBER: **1408**

ING. OSCAR ZALDAÑO — SUPERVISOR
NAME — SUPERVISOR — SIGNATURE

NOTE: THE BOXES NUMBER 01 TO 04 CONTAIN 09 BAGS OF 3.6 LTS. THE BOXES NUMBER 05 TO 10 CONTAIN 16 BAGS OF 1.8 LTS.

NOTE: THE BOX NUMBER 10 CONTAIN BAG FOR QUALITY CONTROL SAMPLE.

ING. OSCAR ZALDAÑO — SUPERVISOR
NAME — SUPERVISOR — SIGNATURE

Appendix 7
List of suppliers for aerial release machines (list not comprehensive)

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

Appendix 8
Glossary of 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.
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 $J \cdot kg^{-1}$.
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 processLoad. [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 9
Glossary of 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 in 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 laid down by Article IX of the IPPC.
SIT	Sterile Insect Technique.
SPS	Sanitary and Phytosanitary Standards.

Appendix 10
Other relevant literature

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55	Breeding for durable disease and pest resistance, 1984 (C E)
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57	Coconut, tree of life, 1984 (E S)
58	Economic guidelines for crop pest control, 1984 (E F S)
59	Micropropagation of selected rootcrops, palms, citrus and ornamental species, 1984 (E)
60	Minimum requirements for receiving and maintaining tissue culture propagating material, 1985 (E F S)
61	Pesticide residues in food 1983 – Evaluations, 1985 (E)

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63 Manual of pest control for food security reserve grain stocks, 1985 (C E)
64 Contribution à l'écologie des aphides africains, 1985 (F)
65 Amélioration de la culture irriguée du riz des petits fermiers, 1985 (F)
66 Sesame and safflower: status and potentials, 1985 (E)
67 Pesticide residues in food 1984 – Evaluations, 1985 (E)
68 Pesticide residus in food 1985 – Report, 1986 (E F S)
69 Breeding for horizontal resistance to wheat diseases, 1986 (E)
70 Breeding for durable resistance in perennial crops, 1986 (E)
71 Technical guideline on seed potato micropropagation and multiplication, 1986 (E)
72/1 Pesticide residues in food 1985 – Evaluations – Part I: Residues, 1986 (E)
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73 Early agrometeorological crop yield assessment, 1986 (E F S)
74 Ecology and control of perennial weeds in Latin America, 1986 (E S)
75 Technical guidelines for field variety trials, 1993 (E F S)
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77 Pesticide residues in food 1986 – Report, 1986 (E F S)
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83 Litchi cultivation, 1989 (E S)
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89 Vegetable production under arid and semi-arid conditions in tropical Africa, 1988 (E F)
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