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).

3.3. REFERENCES CITED

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

Cáceres C., D. Mcinnis, T. Shelly, E. Jang, A. Robinson, and J. Hendrichs. 2007b. Quality management systems for fruit fly (diptera: tephritidae) sterile insect technique. Florida Entomologist 90: 1–9.

FAO/IAEA/USDA. 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.

James, D. J. 1993. Evaluation of the sterile insect technique as a management tool for Queensland fruit fly (*Bactrocera tryoni*). Final report. Project H/0116/RO. Horticultural Research and Development Corporation.

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