Guidelines for

Mature Tsetse Sterile Male Pupae Packaging for Long Distance Shipment

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

TSETSE AREA-WIDE INTEGRATED PEST MANAGEMENT

Sterile insect technique: Mature sterile male pupae packaging protocol

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Introduction

Tsetse flies (Glossinidae) transmit trypanosomes which cause human African trypanosomosis and African animal trypanosomosis, debilitating diseases of humans (sleeping sickness) and livestock (nagana), respectively. The sterile insect technique (SIT) is one of four environmentally and economically acceptable methods that are currently used in the context of area-wide integrated pest management (AW-IPM) approaches to manage populations of tsetse flies. The application of the SIT requires mass-production of sterile males of good biological quality. The size of the project area will, in most cases, determine whether it is more cost effective to produce the sterile flies locally (and invest in a mass-rearing facility) or import the sterile flies from a mass-rearing facility that is located in another country.

Tsetse colony management requires the retention of a large portion of the females in each generation to maintain the colony. At present there is no effective way to separate male from female pupae before emergence so it is necessary to wait for all females to emerge and then chill the remaining, predominantly male, pupae to prevent emergence so they may be irradiated and shipped. Emergence can be halted at temperatures below 13°C (Pagabeleguem et al., 2015) but both mechanical disturbance and temperatures above this threshold result in the initiation of emergence.

The aim of this document is to present a standard packing and transport protocol for the international transport of mature, sterile male tsetse pupae to stakeholders for tsetse area-wide integrated pest management programmes that include an SIT component. Adherence to this protocol will prevent emergence of the male pupae inside the insulated box during transport, will reduce vibration that has a negative impact on male fly quality and will allow sufficient oxygenation of the pupae. Strict adherence to the protocol will result in an optimal sterile male fly yield and provide male flies with good sexual competitiveness.

Materials

The following materials are required for packing and shipping pupae:

1) Pupal containers:
    a) Disposable polystyrene petri dish of 5.4 cm diameter and 1.2 cm height (pierced with holes for air circulation)
    b) Disposable polystyrene petri dish of 8.8 cm diameter and 1.5 cm height (pierced with holes for air circulation)
    c) Pesticide free carton of 12.4 x 8.2 x 2 cm (pierced with holes for air circulation)

2) Packing material for inside the pupal containers:
    a) humidified saw dust (72% rH)
    b) cotton wool
    c) cotton lint
3) Phase change material packs for temperature stabilization, e.g. S8 (http://www.pcmproducts.net/Salt_Hydrate_PCMs.htm) or C7 (http://climator.com/product-data-sheets/?lang=en)
4) Humidity control packs, e.g. Boveda 72% 60g packs (http://www.bovedainc.com/store/tobacco/cubes/)
5) Vacuum insulated transport boxes (various sizes of AcuTemp, CSafe Global, 2900 Dryden Road, Dayton, Ohio 45439, http://csafeglobal.com/products-packaging/)
6) Packing material for in the transport box: Expanded polystyrene cut to size.

Number of pupae to be packed

A petri dish or carton can be used to pack the mature pupae. To adequately oxygenate the pupae, and allow air to circulate between the petri dishes or cartons that contain the pupae, the amount of pupae should be adapted to the size of the petri dishes and the cartons.

We recommend the following pupal packing densities for *Glossina palpalis gambiensis* (numbers may be adapted for other species according to their pupal size, which is 2.60 to 3.25 mm for *G. p. gambiensis*):

- a maximum of 200 male pupae for a petri dish of 5.4 cm diameter and 1.2 cm height (pierced with holes for air circulation);
- a maximum of 500 male pupae for a petri dish of 8.8 cm diameter and 1.5 cm height (pierced with holes for air circulation);
- a maximum of 1500 male pupae for a carton of 12.4 x 8.2 x 2 cm (pierced with holes for air circulation) (Feldmann et al., 1992; Pagabeleguem et al., 2015; Seck et al., 2016).

Pupae packing

Provisions should be made to reduce the vibrations and shocks received by the pupae during transport, i.e. a single layer of pupae should be placed on a layer of cotton and mosquito netting that are placed on the bottom of the petri dish or the carton, and covered by a second layer of cotton and mosquito netting (Figure 1). The carton or petri dish should be closed with adhesive tape, taking care not to cover any aeration holes (Figure 1). The order of layers needs to be respected e.g. the second layer of cotton must be in direct contact with the pupae, in order to prevent their accumulation on one side of the box should the transport box not be placed upright during transport. Enough cotton should be placed in the petri dish or cartons so that a small pressure is exerted on the pupae that will stabilize them during transport. The saw dust can be used instead of the cotton to reduce vibration effects.

Once the pupae are placed in these cartons, they are placed in an insulated transport box (25.5 x 21.5 x 26.5 cm; the size of the insulated box depends on the number of cartons or petri dishes that contain the pupae) as follows (Figure 2):

1) one S8 pack is placed on the bottom of the insulated box, and two packs are placed on the sides;
2) bubble wrap or another shock absorber film is placed between the lateral S8 packs and the cartons or petri-dishes with the pupae, to reduce as much as possible vibrations. Circular pieces of sponge could be also used but these must be glued to the cartons at least 48h before transport (to avoid potential solvent residues) or alternatively, using double sided adhesive tape (not shown on the figure);
3) pieces of expanded polystyrene (or similar) are to be placed between the S8 packs and the walls of the transport container (top, bottom and sides) to immobilize the packs so that they cannot vibrate against the cartons or Petri dishes;

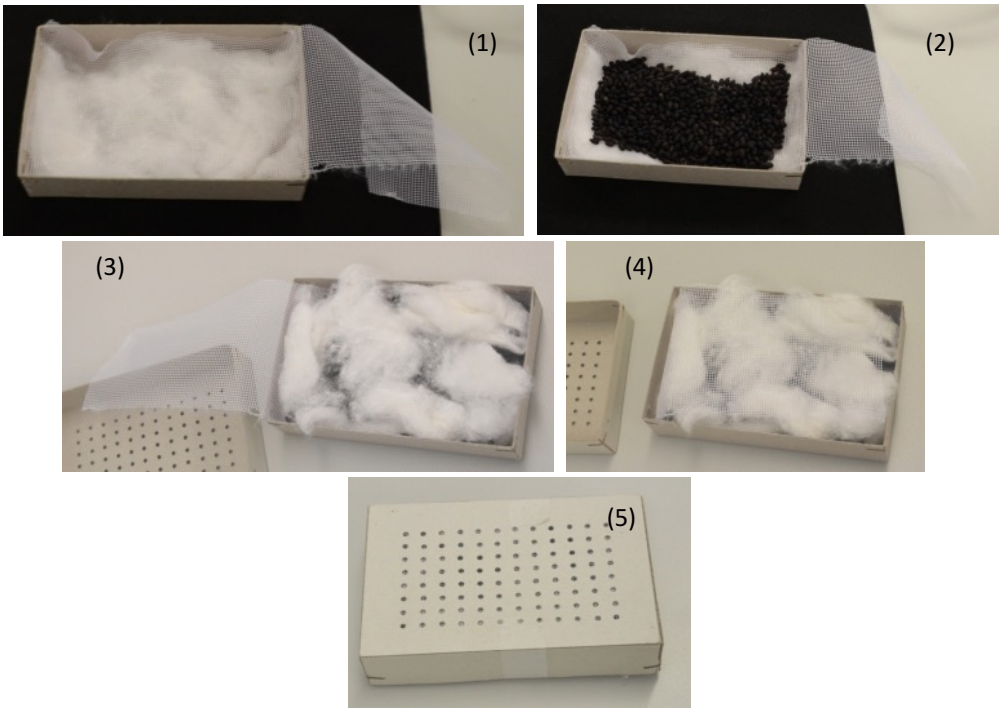

Figure 1: Packing of mature pupae in a carton (photos J. Bouyer).

4) a data logger is placed inside the insulated transport box to record temperature and relative humidity during transport;
5) the top expanded polystyrene piece is placed to cover the content of the container and the transport container is closed.

One S8 pack on the bottom of the transport container is held by 10 mm of EPS and a piece of 20 x 25 x 160 mm EPS at the side.

Two S8 packs placed on each long side of the transport box and held by 20 mm of EPS at each end of the transport box. Three or four cartons with pupae are put inside with air space. A data logger place inside the insulated box to record temperature and humidity.

Final S8 pack put on top of 20 mm pieces of EPS and held by 20 x 27 x 130 mm piece of EPS at the side.

An expanded polystyrene sheet covers the final S8 pack. Box lid sits on top.

Figure 2: Packing of the cartons holding the pupae in the transport container (photos J. Bouyer). Cartons can also be placed horizontally.

Cooling system

Four S8 packs can maintain the temperature inside the insulated box of 25.5 x 21.5 x 26.5 cm at 10 °C for up to 4 days.
Following the above mentioned guidelines, an insulated transport container of 25.5 x 21.5 x 26.5 cm can hold a maximum of 7500 *G. p. gambiensis* pupae. In case the duration of the transport is reduced to 2 days, one S8 pack can be replaced by 2 pupal cartons, increasing the maximum number of pupae per insulate container to 10,500.

Precautions have to be taken to avoid any temperature increase during the irradiation of the pupae, as this will cause premature emergence. To maintain the temperature, the cartons containing the pupae can be placed in between two S8 packs during irradiation.

When pupae are irradiated in a facility other than the rearing facility, it is important not to break the cold chain to avoid premature emergence of the pupae. The transport box must be opened in a cold room at 5 °C (a chiller for example), where the cylinder used in the irradiator (Figure 3) is stored, together with three C7 packs.

Figure 3: Irradiation conditions used to maintain the cold chain during irradiation (photos J. Bouyer).

Pupae transport

Pupae can be transported either by air or by surface transport. Transport by road is only advised if the transport takes less than 24 hours. Transport by air using courier services or air freight is recommended if the mass-rearing facility and target areas are remote. Irrespective of the transport method chosen, total pupal transport time should not exceed 4 days. The number of S8 packs should be adapted to the expected transport time and the ambient environmental condition to maintain inside temperatures between 7 and 13°C, the range of temperatures allowing to maximize the male yield (Pagabeleguem et al., 2015; Seck et al., 2016). It is recommended to maintain the relative humidity inside the box between 70 and 95% (Pagabeleguem et al., 2015; Seck et al., 2016); this can be obtained using humidity control packs.

Each consignment should be accompanied by proper documents that should be attached to the outside of the transport box. Documents generally required are export permits, import permits, and certificates that certify that the content of the package does not pose a health hazard and has no commercial value. Other valuable data that should accompany each shipment: the number of pupae in the consignment, larviposition date, start of cooling of the pupae, irradiation date and time, radiation dose, the shipping date and miscellaneous comments.

Learn more

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Cette fiche est destinée aux programmes nationaux de lutte contre les tsé-tsé (PATTEC), aux responsables et techniciens entomologistes.

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