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Fruit Flies of Economic Importance: From Basic to Applied Knowledge

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New Egg Shipping Container for Decentralized Medfly Sterile Male Pupae Production

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ABSTRACT: This paper describes a new double-walled container with an internal shelf system for the shipment of medfly eggs. It allows the eggs to be packed in plastic bags for shipment without the addition of any transportation medium. The size of this container can be adapted to the volume of eggs required. This container can maintain a constant internal temperature for 72 hours. Simulation of shipping conditions for 72 hours found no negative influence on the quality of eggs. The cost of the new container is comparable or even lower than the cost of commonly available commercial containers. The main advantages of this egg shipment container are: a) good thermo-insulation properties, b) variable size and c) low cost.

Key Words: Medfly, egg transportation, egg shipment container, decentralized sterile male pupae production.

INTRODUCTION

The sterile insect technique (SIT) is an area-wide method to control major insect pests. Large numbers (frequently more than 50 million/week) of the target insects are produced in specially designed mass-rearing facilities. These insects are sterilised by gamma radiation and released into the target area. Mating between the factory-reared sterile males and wild females produce no offspring. Thus, if sufficient sterile males are introduced into the target area on a regular basis there is high probability that fertile wild females will mate with sterile males (Hendrichs *et al*. 1995).

The genetic sexing *tsl* strain developed in the IAEA laboratories in Seibersdorf and introduced into mass-rearing facilities promoted the further evolution of the SIT against Medfly. Heat-treatment of the eggs kills the female offspring early in the mass-production process due to the presence of the recessive temperature lethal gene on the X chromosome that exerts its dominance in the female. The *tsl* generates some savings in the production process, but the benefits generated in the field greatly outweigh these gains as induced sterility has been shown to be 3-4 times greater than standard (male-female) strain releases (Tween 2002).

Feasibility studies and small scale trials in several countries in the Mediterranean basin show that the SIT can be used effectively in European fruit production although some technical and practical obstacles hinder its widespread use in Europe. An effective solution to this problem can be the development of decentralized production technology. The production processes in a decentralized system include egg production from a "core" egg producing facility which will supply several pupae facilities producing sterile male pupae for a series of eclosion facilities where sterile male adults are emerged and prepared for release. To use the decentralised production and release system, an effective egg shipping container has to be developed.

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In this paper, a new double-walled egg shipment container with an internal shelf to hold eggs packed in plastic bags and an effective cooling system to maintain the required internal temperature is described.

MATERIAL & METHODS

In the experiments, the *tsl* Vienna 7 – Toliman strain of Medfly *Ceratitis capitata* (Wiedemann) originating from Moscamed El Pino mass-rearing facility in Guatemala was used. Flies and pupae were maintained at 24±1°C and 60-80% RH in a photoperiod of 12/12 L/D. Larvae were maintained in a separate room at 23±1°C and 80% RH in a photoperiod of 12/12 L/D. Eggs used for the experiments were 24 hours old eggs and had been heat treated at 34°C for 12 hours.

Two types of commercially available containers were used in this study. A thermo-insulating container used for transporting of deep-frozen samples with dry ice was purchased from TNT (U.K.). The second container was obtained from KULI (Germany).

To measure the thermo-insulating properties of tested containers, Temp-tale data loggers (U.K.) were used to monitor three thermal parameters: the external temperature, temperature of the coolant and the temperature inside the container. Temperature was recorded every 15 minutes for 72 hours starting immediately after loading the container with the coolant and egg samples.

To evaluate the influence of low temperature on the quality of eggs, experiments simulating the shipment conditions were set up. Samples of 10 ml of eggs sealed in plastic bags were placed for 72 hours together with coolant in containers. The eggs were loaded into the bag either in water or agar solution (0.5%) in ratio 1 : 1 or without any medium only on wet filter paper. The egg hatchability was used to quantify the quality of eggs after the shipment simulation experiments.

RESULTS

The concept of double-walled container. To improve thermo-insulating properties, a new double walled thermo-box (box in box concept) was designed as a new egg shipment container. This arrangement of space allows improved control of the internal temperature conditions. Contrary to previously used single-walled egg shipment containers, double-walled containers offer several advantages: a) better thermo-insulating properties than single-walled containers, b) an indirect cooling system can be used (eggs are not in direct contact with the cooling medium), c) improved protection of eggs from mechanical damage and d) a choice of container size depending on the volume of eggs to be shipped. Additional requirements for a new container were that it a) was made of recyclable material, b) be light weight, c) has an acceptable cost, d) reduce the demands for storage space.

Selection of material. A broad spectrum of materials with good thermo-insulating properties is commercially available. The material used for the box should fulfil some additional parameters such as being of sufficient firmness, be low weight, have the highest possible incombustibility and have an acceptable cost. Bearing this in mind various materials were evaluated and four were selected for more detailed testing: a) expanded polystyrene, b) extruded polystyrene foam STYROFOAM, c) extruded polystyrene URSA FOAM and d) extruded polystyrene STYRODUR.

The evaluation of technical parameters presented in Table 1 led to the selection of STYROFOAM as the material most suitable for the construction of the container prototype. The STYROFOAM plates used were supplied by DOW Ltd, Germany.

The design of the egg shipment container. Using a double walled container allows the creation of a more homogenous internal environment to house the eggs, and substan-

Table 1. Physical properties of the materials tested for the construction of a double walled egg shipment container.

	STYROFOAM	STYRODUR	URSA FOAM	EXP POLYSTYRENE
Volumetric weight (kg/m3)	28	30	35	20
Coefficient of therm. conductivity (W/m.k)	0.035	0.037	0.034	0.039
Firmness in pressure (Mpa) (10% pressing)	0.25	0.25	0.30	0.10
Coefficient of diffusion	100	100 - 160	80	
Absorbency (%)	0.2	0.3	0.5	0.4
Combustibility (STN)	C1	C1	C1	C1
Cost (€/m2)	3.63	3.48	3.48	3.27

tially increase their protection of eggs from mechanical injury during shipment.

The entire container consists of (from the outside inward): a) a cardboard external box, b) an external thermo-box, c) an internal thermo-box and d) a shelf system for storing the egg packages.

The cardboard box is the outermost part of the whole system, covering external thermo-box during the shipment. The prototype cardboard box was constructed from a double-layer cardboard paper supplied by MTM – Packing Ltd, Slovakia. This cardboard has a high firmness level and a low cost. The size of cardboard box can be changed according to the size of the external thermo-box. The cardboard box is sealed using plastic adhesive tape as soon as the egg shipment container is loaded with eggs and coolant.

The external thermo-box (Figure 1) contains the cooling system and internal thermo-box with eggs. The inner edges of the walls of both thermo-boxes were folded allowing a firm and impermeable structure. The walls were glued together by a ZWALUW silicone glue, supplied by Den Braven Sealants Ltd. The size of both boxes was designed to transport 1 litre of eggs. The outer dimensions of external thermo-box were 450 x 380 x 370 mm.

The internal thermo-box was constructed of the same material as the external thermo-box. The outer dimensions of internal thermo-box of the prototype were 320 x 250 x 240 mm. Inside the internal thermo-box, shelf system was inserted for the loading of egg packs, PCM (Phase Change Material) coolant and a temperature monitoring logger.

Additional insulation. To increase the insulation capacity of both thermo-boxes, their internal sides were covered by insulation foil, THERMOREFLEX, consisting of a layer of aluminium foil covering 4 mm broad polystyrene. The foil reflects almost 90% of heat and

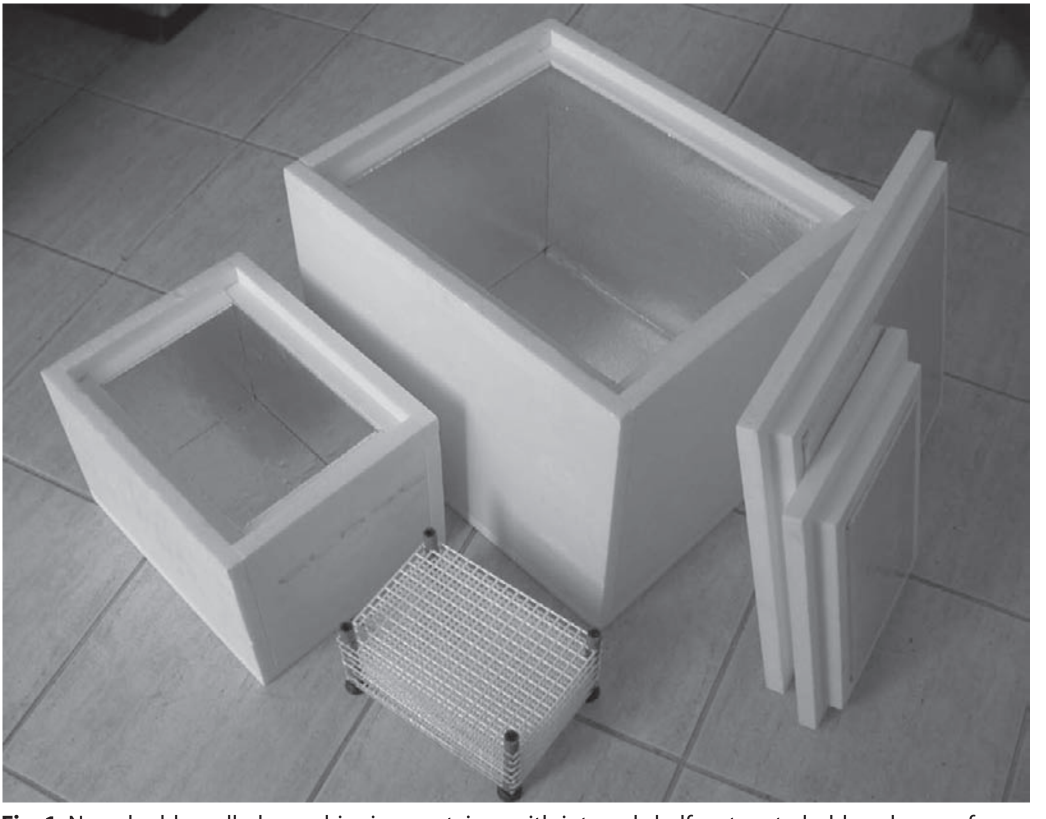

Fig. 1. New double walled egg shipping container with internal shelf system to hold packages of eggs with phase change coolant on the top and bottom grids. The shelf is placed into the smaller box which is placed into the larger cooler box. The gap between the two boxes is filled with cooler blocks. The inner walls of both boxes are lined with aluminium foil.

substantially increases the insulative properties of both thermo-boxes.

Shelf system for storage of egg packages. The shelf system for transportation of eggs packed in plastic bags provides sufficient protection of eggs against mechanical damage, increase the effectiveness of egg shipment, and allow more precise temperature control all at an acceptable cost. The size of completed shelf system for storage of egg packages is related to the internal size of the inner thermo-box. The shelf system is constructed before placing it into the inner thermo-box. The base is a metal grid plate with four aluminium rods plugged into each corner. The base of each rod is closed by a rubber stopped. More grids are added with the distance between individual metal grids set by rubber rings on the rods. Packages with Medfly eggs are placed between the grid plates. The lowermost and uppermost grids are filled with PCM coolant, which help maintain the temperature in the internal thermo-box. The central grids are used for placing the data logger measuring the temperature during the shipment. When the grid is loaded with the egg packages, PCM coolant and the data logger, the whole shelf system is secured by a final grid and the end of the rods closed with the rubber stoppers. The completed shelf system is then loaded into internal thermo-box.

Cooling system. One of the features of the double-walled container cooling system is to separate the coolants from the egg packages by thermo-insulating material (wall of internal thermo-box) and provide homogenous distribution of the coolant around the whole surface of the internal box. As the coolant is frozen at -20°C, direct contact with the egg packages would have a fatal effect on the eggs. The gap between walls of the internal and external thermo-boxes is filled with coolant. The coolant is then sufficiently insulated from the external environment and well separated from the egg packages. The temperature of the coolant increases very slowly because of the double insulation and the eggs inside the internal thermo-box are well protected against the negative influence of the coolant. The homogenous distribution of the coolant and effective thermo-insulation maintains the temperature inside the internal thermo-box in the optimal range during the entire transportation period. In addition the temperature inside the internal thermo-box is buffered during the shipment by blocks of PCM coolant.

Coolants. Several materials were tested during the development of new egg shipping container; water, glycerol, dioxane, water mixtures of both these compounds, PCM and some others. From practical reasons, commercially available cooling blocks were selected as the external coolants and PCM cooling blocks as the internal coolants to buffer the temperature inside the internal thermo-box. Commercially available cooling blocks are commonly used as a cooling material for portable thermo-boxes and are easily available in different sizes. Cooling blocks are frozen for -15 to 20°C overnight before use.

The PCM Climacell 7 packs were supplied by CLIMATOR Ltd, Sweden. They were used to maintain the temperature in the range 0 to 15°C. Climacell 7 blocks were loaded together with egg packages inside the internal thermo-boxes. Monitoring the temperature in the internal thermo-box revealed that the cooling system maintained the temperature in the desired range 10 to 15°C for 72 hours.

Monitoring the temperature inside the internal thermo-box during the shipment. The temperature inside the internal thermo-box was monitored by TempTale (U.K.) data loggers. The same data loggers were used for recording the external and internal temperature during tests of thermal capacity of the new container.

Simulation of egg transport conditions. The shipment simulation experiments showed that there was no difference in hatchability of eggs that had been stored up to 72 hours at 10°C either in water or 0.5% agar solution when compared with the control eggs (Figure 2).

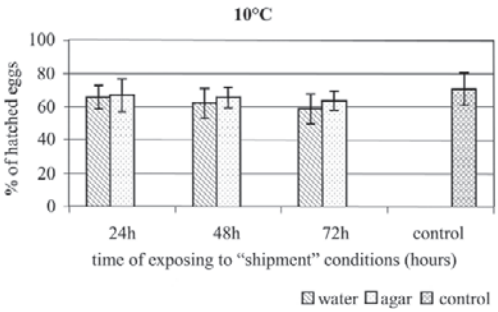

Fig. 2. Hatchability of eggs exposed to the conditions simulating egg transport. The eggs were loaded into the bag either in water or agar solution (0.5%) in ratio 1 : 1 or without any medium only on wet filter paper.

DISCUSSION

Systems currently used for transporting Medfly pupae and eggs are described in the IAEA document "Guideline for packing, shipping, emergence and release of sterile fruit flies in area-wide control programmes". Two types of egg transportation are described depending on the shipping time. For short shipping times (not exceed 48 hours) polyethylene "Zyploc" bags are recommended for shipping up to 1 litre of eggs mixed with the same volume of transportation medium. Bags are placed in insulated shipping boxes filled with coolant to maintain the temperature between 5 to 15°C. For long-distance shipping either sealed insulated metal or plastic thermos-bottles are recommended. Each thermos is filled with 0.5

litres of eggs and agar solution (0.1-0.2%) in a 1:1 ratio. A number of thermos' are placed into insulated boxes and placed into cardboard boxes. The thermos should be maintained at room temperature during shipping and the time of shipping should be as short as possible and should not exceed 72 hours (Enkerlin in press).

Several Medfly egg shipment experiments completed demonstrated that the shipment of Medfly eggs over long distances is feasible. Medfly eggs seem to be much less sensitive to long term cooling and anoxia than pupae and are more suitable for long distance transportation. Keeping eggs in 10°C for 72 hours has no significant influence on their quality, hatchability and effectiveness of pupae production. Eggs have also been shipped in a 0.1% agar solution in thermos flasks, to provide a homogenous distribution of eggs in suspension during the shipment and to avoid sedimentation and subsequent damage.

This method of egg shipment is acceptable for experimental purposes but for routine shipments of large volumes of eggs, a new cost effective egg shipping container as well as egg shipping technologies had to be developed. New egg shipping technology should create optimal conditions for Medfly egg transportation over long distances, even if the transportation time is prolonged for several days. Apart from the development of technical components such as an egg shipping container, coolants and a packing system, more information is needed about the thermal biology of the egg of the Medfly *tsl* strain.

To eliminate the shortcomings of the existing commercially available thermo-boxes, a new Medfly egg shipping container was designed and a prototype manufactured and tested. The new container consists of a double-walled thermo-box (box in box approach) with an internal shelf system for storing of egg packages during the shipment.

The new developed double-walled Medfly egg container allows transportation of Medfly eggs from a core egg producing facility to satellite pupae production facilities. An optimal environment for eggs can be maintained and the temperature monitored during the entire period. The size of the container can be tailored to the volume of eggs needing to be shipped. The cost of the new container is comparable to or even lower than the cost of commercially available containers.

The new cooling system consists of two components. Firstly, blocks of coolant placed in the gap between the external and internal thermo-boxes maintaining the temperature inside the internal thermo-box between 10 to 15°C. Secondly, PCM (Phase Change Material) is placed on the bottom and top grid of the shelf system inside the internal thermo-box to help buffer any changes of internal temperature.

The temperature during the shipment will be continually monitored by a data-logger placed inside the internal thermo-box. These records will be evaluated after the delivery of the egg shipment to the pupae production facility.

The new double-walled container increase the chances of the safe delivery of high quality eggs as a result of a stable internal environment due to the high thermo-insulting properties and a two-component cooling system, all at a cost comparable with common commercially available containers. This double walled container will become a valuable component of the Medfly egg shipment system for the purposed of decentralized sterile Medfly male pupae production.

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1 st	1982	Athens, Greece
2 nd	1986	Crete, Greece
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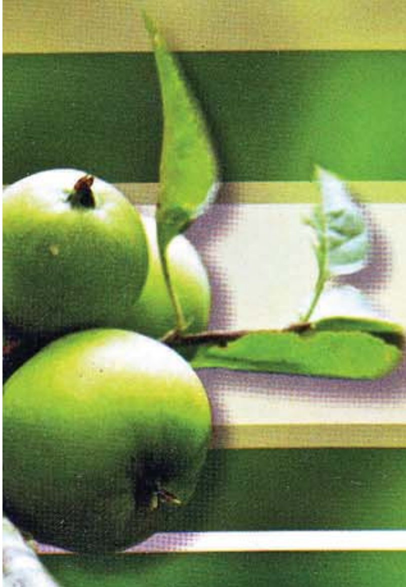
4 th	1994	Florida, USA
5 th	1998	Penang, Malaysia
6 th	2002	Stellenbosch, South Africa
7 th	2006	Salvador, Brazil



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