MASS REARING OF THE MEXICAN FRUIT FLY, *Anastrepha ludens,* AT THE FRUIT FLIES BIOFACTORY IN METAPA DE DOMINGUEZ, CHIAPAS, MEXICO

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Abstract

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A description of the present methods for mass rearing *Anastrepha ludens,* known as the Mexican fruit fly, at the Fruit Flies Biofactory in Metapa de Dominguez, Chiapas, is given. Important contributions and improvements are described for the rearing stages, e.g. egg production and incubation, larvae diets, lab conditions for the development of larvae and pupae, larvae and pupae handling and environmental control.

1. INTRODUCTION

As main part of the Mexico National Campaign against Fruit Flies, a Fruit Flies Production Biofactory was built in Metapa de Dominguez, Chiapas, Mexico, to produce flies in the genus *Anastrepha.* This biofactory was designed based on the experience obtained from Mediterranean fruit fly (*Ceratitis capitata,* Wied.) mass rearing for 14 years, transfer of lab technical aspects from the Mexican fruit fly mass rearing in Mission, Texas, USA, as well as technology developed by the Methods Development Department of the Mexico-Medfly Program.

The Biofactory was built with three production modules, two for fruit fly mass rearing and a third for parasitoids (*Diachasmimorpha longicuadata*). Each module has the capacity for producing 150 million individuals and at present is used for *Anastrepha ludens* and *A. obliqua* production. Besides these species, in the future it is intended to mass rear, in each module, *A. striata* and *A. serpentina,* species which are of the most economic importance in Mexico and included within the eradication strategies of the Campaign in the different Mexican states.

Goals of the Campaign are based in the success reached in mass production of *Anastrepha* spp. Applying the Sterile Insect Technique (SIT) combined with integrated control strategies in each specific area of the country.

This paper describes the methods used at present for mass rearing. *A. ludens*, taking into account that these methods are those which have worked through a series of previous evaluations.

2. HANDLING THE COLONY

The colony is one of the main pillars of insect mass rearing, since it depends totally on the insect adjustment capacity for reproduction in an artificial environment. The values or parameters used to assess the efficiency of the colony are egg fecundity and fertility.

The colony is kept confined in Mission type aluminum cages with the following dimensions: 2.15 × 1.80 × 0.30 m, covered by a mesh made of glass fiber. The oviposition panel is on the front and center of the cage and consists of linen fabric to simulate the host fruit surface where flies lay eggs. The fabric is covered with a silicon thin layer to obtain a soft surface to make easy egg collection. It is also within a chamber with aluminum walls and door made of acrylic which isolate eggs from the external environment to avoid egg desiccation.

Eggs are collected once a day in the morning (it starts at 7:00 a.m.) with the use of spray nozzle which sprays water on the panel, and eggs slip to the inner part of the cage where they are collected in a container; later, they are disinfected with chlorine at 200 ppm for 10 minutes; then they are placed in plastic decanters with a determined egg volume and sent to the incubation area.

After eggs are collected, the oviposition panels are disinfected with chlorine at 2,800 ppm to allow a new oviposition period in an environment free of microorganisms. Once oviposition starts and the excess moisture has decreased in the oviposition fabric, a very thin spray of gel fusselerone is applied. This gel is in a pressurized container and applied with a fan pattern nozzle. This procedure is repeated in the afternoon to assure that eggs remain in a hydrated medium and will not lose viability to dehydration.

An acceptable production is of 2×10^6 eggs per cage per day with 90% fertility. These parameters, considered as a minimum, are reached with a colony completely adjusted to be handled in artificial conditions, whereas there is a temperature of $27 \pm 1^\circ$ C and a relative humidity of 50-60%, which have been determined as ideal, as well as a photoperiod of 14:10 hours (light:darkness).

The use of these methods has evolved according to the problems faced. First of all the oviposition panel, which was made of plastic material called *curine* which, besides its high cost, needed to be perforated, since it has a plastic cover to ease the introduction of the ovipositor. Thus, linen is an alternative which works more efficiently and is practical to use.

Humidity injection is used to avoid egg dehydration within the oviposition chambers using ultrasonic dehumidifiers, which proved to be effective when handling fewer cages in the colony. The problem was when the colony increased to obtain a greater production and the oviposition chambers did not retain 100% of the humidity injected. For this reason, the relative humidity percentage in the environment increased up to 80%, causing low fly longevity, general contamination and loss of productivity. The use of the hydrating gel in a low concentration has solved such problems, and has assured an egg production with the desired quality.

Fly density inside the cages is another factor related to egg production efficiency and its quality. At present, a density of 150,000 flies per cage produce from 1 to 2 million eggs per cage, per day, in fly's productive life. Flies reach production peaks from the fifth to the tenth day. The tendency is to decrease fly populations per cage to a level of 110,000 individuals, which may produce similar amounts to those produced by 150,000 flies per cage, of acceptable quality, with better handling conditions and environment with less stress (Figure 1).

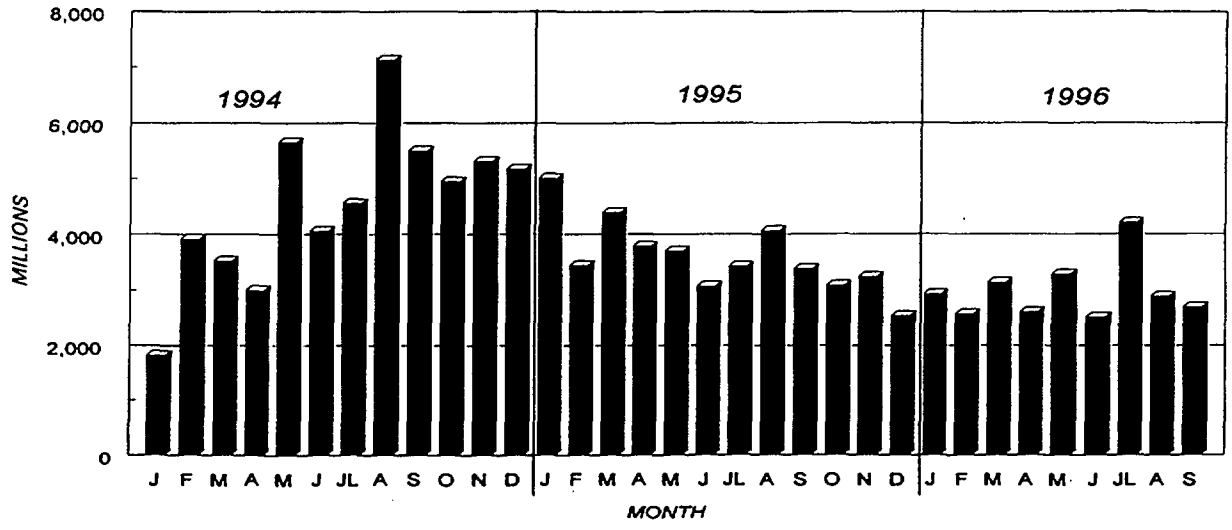

Figure 1. Egg production.

The handling of temperature is another factor that directly influences colony production. If the temperature is less than 26°C, sexual activity also diminishes (less matings) and, at the same time, the production index decreases. If the temperature is greater than 28°C, there is an increase in sexual activity and a greater egg production but longevity or productive life of the fly diminishes seriously. At present temperature inside the colony area has been kept between 26 and 28°C, and most of the time it remains between 27 and 28°C, a fact that has favoured the reduction of sexual maturity time from 10 days, which was considered normal for many years, to eight days, the value accepted at present. This helps greatly to make productive life of flies greater than 12 days, for a total of 22 days of confinement in the cage, with eggs since the first day of oviposition with 90% fertility.

3. EGG INCUBATION

After collection, disinfected eggs are placed in polycarbonated 8 liter plastic bottles to which 240 ml of eggs plus, 4,800 ml of water are added to get an egg:water ratio of 1:20, Water is previously purified by microbiological filters and Ozone action to diminish the possibility of contamination of the egg that may affect its viability. Egg incubation takes place after four days at an environmental temperature of 27°C and from 23 to 26°C inside the bottles. The incubation water is changed daily to avoid microbial population increases and, at the same time, at 48 hours of incubation, eggs are disinfected again with 5ppm of chlorine within the same bottle for 10 minutes. Water is eliminated by decantation and clean water is added at 2 ppm of chlorine to allow incubation to continue.

Eggs inside the bottle are kept oxygenated with pressurized air (20psi), which makes them remain in a continuous movement, avoids sedimentation and allows homogeneous incubation conditions.

Each ml of eggs contains approximately 21,000 eggs. Inside each bottle around five million eggs are incubated.

After four days of incubation eggs are ready to be seeded in the diet and the ratio of water:egg is modified to 10:1. Water used for seeding is mixed with gel (carragenine) to increase viscosity and water density. In this way eggs remain suspended until they are seeded in the diet, which is described as follows.

4. LARVAL DIET

The dream of any fruit fly breeder is to have a single food that may be recommended for several fruit fly species in any region of the world. Obviously, this is not yet possible due mainly to poor knowledge of nutritional requirements of these insects and because of conditions of the region where the work is carried out, which implies poor availability of ingredients, storage difficulties (shelf-life) and environmental problems among others.

Larval diet used at present is made with corn cob particles, corn flour (which works as a texturizer) to give body and volume to diet mixture; dried inactivated yeast (*Saccharomyces cervisiae*) as main source of aminoacids, the vitamin B complex and minerals; white sugar as a carbohydrate source; microbial inhibitors such as methyl-parabene (Nipagin) which has a greater action against fungus and yeast and, to a lesser degree, on gram-negative bacteria; sodium benzotate which acts to a greater degree on yeast and bacteria and to a lesser degree on fungus; citric acid is used to give acidity to larval food and acts in a direct way in microbial control since it creates an inadequate environment for growth of fungus and some bacteria; guar gum as stabilizer to avoid the sineresis effect or loss or non retention of moisture among diet particles; and finally water, which is the universal vehicle to disperse nutrients in the diet, makes up the target percentage in the formula (Table I).

Larval diet is prepared in belt mixers with a capacity of 3.5 tons each, following this mixing procedure:

Texturizers are placed in the mixer, as well as yeast and sugar, which are mixed dry for 10 minutes; then water, citric acid and diluted sodium benzotate are added. Methyl-paraben is left in hot water one day before to facilitate its dissociation since it is not 100% water soluble. The total mixture is homogenized for 30 minutes until it reaches the consistency and texture of the diet. The mixing process is monitored by the Quality Control Department to verify that diet humidity oscillates between 62 and 63% at a pH of 4.3 to 4.4. If it is not, the food will have to be adjusted to these parameters before it is sent to the diet reception area.

TABLE I. INGREDIENTS USED FOR LARVAL DIET

Ingredient	%
Corn cob particles	17.2
Corn flour	5.3
Sugar	9.2
Yeast	7.0
Guar gum	0.1
Nipagin (Metil-paraben)	0.2
Sodium benzoate	0.4
Citric acid	0.6
Water	60.0

All ingredients must fulfill specification requirements determined by the Quality Control Department for larval consumption, which are determined by evaluation and acceptance regulations. A rejected ingredient is not used in mass rearing since it would bring serious consequences and risk for production or deterioration of quality needed by the biological product.

Substantial changes were made in the larval diet formula this year basically to the use of quar gum which solved a problem of high larval mortality since it formed a water film in depressions of the food once placed in larval trays. Such depressions cause mortality of newly hatched larvae since they stayed trapped under the water and cannot breathe. Guar gum has the property of trapping water molecules and not releasing them; this fact avoided these troubles.

Preservatives have changed in concentration as microbiological problems have arisen which affected development and quality of the insect; most of the cases are derived from environmental control troubles.

5. LARVAL DIET RECEPTION

Prepared food is moved to the diet reception area where it is placed, weighted, and distributed in glass fiber trays previously disinfected. Seven kilograms of diet are placed in each tray, then it is distributed and the surface is flattened with a metallic plate to avoid the formation of crests on the diet since these get dry and eggs become dehydrated if are placed on such crests.

Trays are seeded with four ml of eggs suspended in the gel solution. Each trays contains approximately 84,000 eggs with 10 to 20% of hatched larvae to assure that the eggs have reached complete maturity.

The gel used for seeding helps to keep the egg moist until the moment of hatching, which has led to better egg viability.

A density of four ml of eggs per tray was considered suitable after evaluating densities of 3, 3.5, 4, 4.5 and 5ml. Which results in an acceptable larval transformation, but affected size and weight, which must vary between 25 and 27 milligrams.

Trays already seeded are piled in amounts of 30 trays on metallic platforms and moved to the larval starting room prior to this, the platforms were completely covered with a fabric called "pañalina"(kind of gauze used for diapers) to avoid introduction of *Drosophilla melanogaster* (vinegar fly).

6. LARVAL DEVELOPMENT

Larvae have three instars; for this reason, within the rearing process there are three different environments in three different and independent rooms, for nine days of development until they reach maturity.

TABLE II. TEMPERATURE AND RELATIVE HUMIDITY REQUIRED IN EACH DEVELOPMENTAL STAGE IN MASS REARING *A. ludens*

Room	Temperature (°C)	Relative humidity (%)
Colony	27 ± 1	55 ± 5
Incubation	27 ± 1	
Larval starting room	28 ± 1	85 ± 5
Larvae I	27 ± 1	75 ± 5
Larvae II	26 ± 1	75 ± 5
Pupation	21 ± 1	60 ± 5
Pupal maturation	24 ± 1	60 ± 5
Packing	16 ± 1	60 ± 5

6.1. Larval starting room

In this room environmental conditions are established with a temperature of 28 ± 1°C and a relative humidity of 85 ± 5% with the purpose of accelerating egg hatching to avoid dehydration and also to obtain homogeneous larval development.

Newly hatched larvae are kept under these conditions for three days and then moved to the Larvae I room.

6.2. Larvae I room

When larvae end the first instar they are placed in an environment with a temperature of 27 ± 5%. In this room, larvae complete the second instar; the fabric that covers the trays is taken off since this instar generates too much metabolic heat. First instar larvae remain on the diet surface feeding with nutrients dissolved in superficial water since their oral organs are not yet developed.

6.3. Larvae II room

At this place temperature and relative humidity temperature and relative humidity oscillate between 26 + 1°C and 75 ± 5%, respectively. Larvae are located from the middle depth of the diet to the bottom of the tray, looking for more humidity and nutrients available. At this stage the most metabolic heat is generated; temperature in the room diminishes since humidity gets lost gradually at the diet surface and as the third instar advances, the diet changes texture to a fine powder. If the loss of humidity in the diet is very extreme, on the seventh day of development a spray of water with benzoate is applied (1g/liter) on the food surface rehydrate it. When the ninth day of larval development is completed, larvae are a creamy color, the digestive system vanishes from sight and it is considered that it is mature, ready to be separated from the diet.

The remarkable changes in larval development have demanded the division of developmental instars in independent areas to provide ideal conditions for each larva. The result of this change is that the period of 10 days of larval development was shortened to nine days, getting in this way an optimization of the rooms for a greater production capacity (Figure 2).

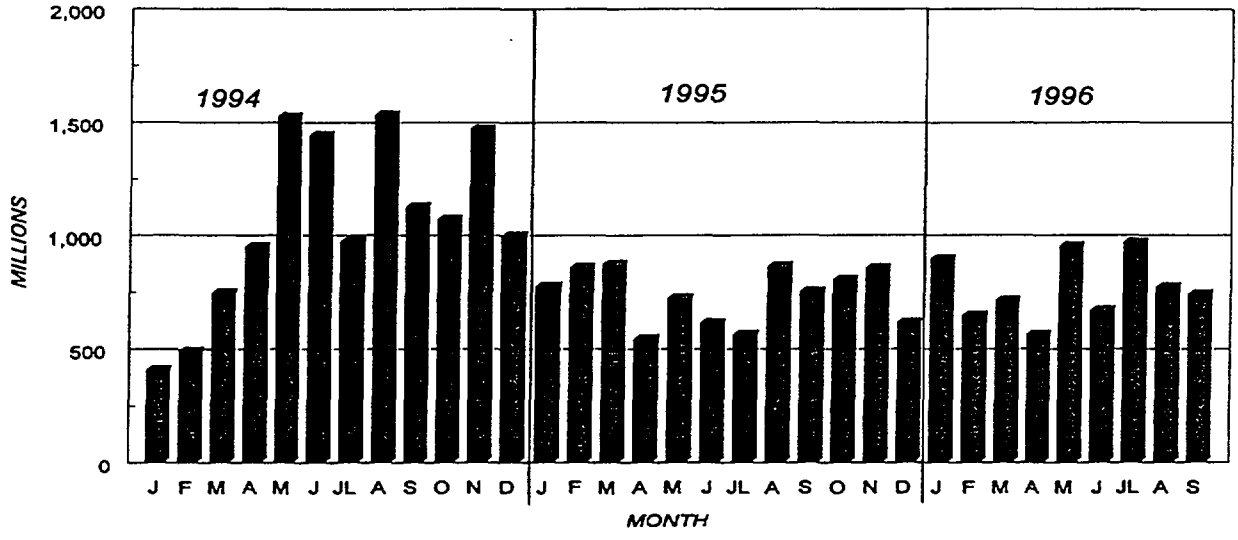

Figure 2. Larvae production.

7. LARVAL SEPARATION

When larvae are completely mature they are moved to the larval separation room, where the necessary equipment is available to separate then from the diet. The equipment consists of a reservoir made of glass fiber and a suction system which works by Venturi effect. The diet from 180 trays is emptied gradually into the reservoir. Trays are then cleaned in a washing machine where they are disinfected with chlorine and then returned to the diet reception area.

When the loading of the reservoir is completed, diet diluted in water is sucked through a plastic pipe in the bottom part of the reservoir; this pipe is connected to another plastic pipe which carries clean water through any intersection This produces a vacuum that sucks the mixture contained in the reservoir (Venturi effect). The mixture of clean water and diet is received in sieves that trap the larvae and release the diluted diet, which drops to a special pipe that carries to a collector tank out of the plant.

There is a dry method for larval-diet separation that uses tombolas. The diet is removed from the trays and emptied in the tombola to break compaction. Tombolas move circularly and the larvae also move within the tombola's sieve in an intermittent way. Larvae passes through the mesh when they are separated from the diet and fall into a container.

Separated larvae are placed in trays with a mesh bottom to drain the excess water, and later they are placed in aluminum trays, also with mesh in the bottom. To these trays, vermiculite is added as population substrate in amounts of one liter of larvae per two liters of vermiculite. Then trays are placed in shelves and moved to the pupation room.

8. PUPATION ROOM

This room is also called the dark room since it is kept in darkness because larvae are attracted by light and may come out of the trays. Temperature in this room is kept at 21 $\pm$ 1°C to induce larvae to be less active. Relative humidity is at 60 $\pm$ 5% to decrease the excess environmental humidity when humid larvae enter into this room; this fact delays pupation. Four dehumidifiers are used to keep humidity low, which assures the established levels.

Pupae are kept in this room for three days until they reach a minimum of 85% pupation. Later, the shelves are moved to the pupal maturation room.

9. PUPAE MATURATION AND PACKING

Pupal maturation ends at the eleventh day after finishing pupation, at a temperature of 24 $\pm$ 1°C and a relative humidity of 70 to 80%. Maturity is detected by eye coloration of immature stages, which marks the time to be irradiated (two days before emerging adults). At this time the puape are separated from vermiculite by means of a wind tunnel where vermiculite is deposited at the end of the tunnel; the bad quality pupae drop in the center and the good weight puape (minimum 17mg) are dropped at the beginning of the tunnel. In this way the biological material is selected to be released or sent to the colony.

Some years ago the separation of pupae from vermiculite was made inside a tombola with mesh 12 and 10 but it was detected that this caused mechanical damage to the pupae, decreasing their quality. The tunnel has the necessary characteristics for a better handling of the biological material since it uses a pedestal fan at low speed which pushes larvae that drop from a hopper in the top part of the tunnel to the bottom toward the inner part of it. Inner walls of the tunnel are covered with foam to lower the drop of the pupae.

Once pupae have been separated from vermiculite, they are colored with a fluorescent dye (Day Glo^{MR}). The process of painting pupae has the purpose of allowing a differentiation of the sterile flies released in the field from the wild (fertile) ones.

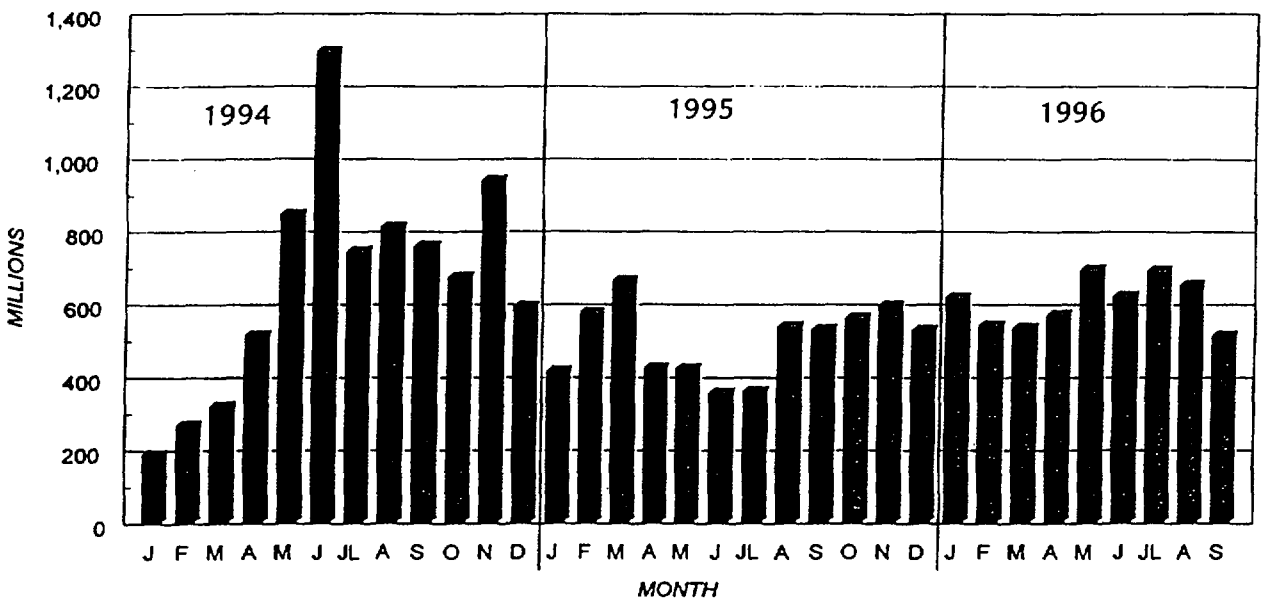

Figure 3. Pupae production.

Painted pupae are placed in sausage type bags to be packed in cardboard boxes in amounts of nine sausages per box. Each box contains 1.2 million pupae, approximately, ready to be irradiated.

Pupae remains one hour in hypoxia within the closed sausages to make ionizing radiation more effective. Each box has a radiosensitive film adhered to detect and certify that the material was adequately irradiated at eight Krads, when the film turns into a dark color.

Biological material is transported to the airport to be sent to the different states of the Mexican Republic, where pupae are packed in No. 20 paper bags and left in emergence rooms to be released in the field later (Figure 3).

10. ENVIRONMENTAL CONTROL OF THE PROCESS

The control of the environmental conditions is, undoubtedly, the most important factor of the mass rearing process. For this reason the lab was designed with double equipment in each room in a way that if one is out of order the other is ready to start working any time.

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