MASS REARING METHODS FOR FRUIT FLY

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Abstract

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The most common rearing methods used for mass rearing of fruit flies, with emphasis on those of economic importance in Mexico such as *Anastrepha ludens* (the Mexican fruit fly), *Anastrepha obliqua* (the mangoe and plum fruit fly) and the exotic fruit fly *Ceratitis capitata* (the Mediterranean fruit fly) are described here.

1. INTRODUCTION

The strategies for eradication of fruit fly utilizing the Sterile Insect Technique, have been adapted from the successful screwworm (*Cochylyomia hominivorax* Coquerel) eradication program, which has eliminated this pest from the southern United States, Mexico and a great part of Central America. This success, initiated in the 1960s, coincided with the elimination of the melon fly (*Bactrocera cucurbitae* (Coquillet)) from the island of Rot in 1962-1963 and the oriental fruit fly (*Bactrocera dorsalis* (Hendel)) from the island of Guam in 1963, (Fay, 1989).

Seven polyphagous species of Tephritidae have been identified for mass rearing or investigation: *Bactrocera tryoni* (Froggatt) (Queensland fruit fly), *Bactrocera dorsalis* (Hendel) (oriental fruit fly), *Bactrocera curcurbitae* (Coquillet) (melon fly), *Anastrepha suspensa* Loew (Caribbean fruit fly), *Anastrepha ludens* Loew (Mexican fruit fly), *Anastrepha obliqua* Macquart (West Indian fruit fly) and *Ceratitis capitata* (Wied) (Mediterranean fruit fly). All of these species are of tropical and subtropical origin and all are polyphagous and multivoltine, characteristics that have favored adaption to artificial rearing, in comparison to *Bactrocera oleae* (Gmelin) (olive fruit fly) or *Rhagoletis spp.*, which are monophagous or more host specific, which have made artificial rearing artificial more difficult for the moment, (Fay, 1989).

In Mexico we first began *Anastrepha ludens* rearing in the 1950s, when we obtained, in experimental form, small quantities of this species (SARH, 1995). The Sterile Insect Technique became very relevant in Mexico in 1977, when Mediterranean fruit fly invaded the south of the territory. Among the actions taken for integrated pest control was the release of millions of sterile flies, which were produced since 1979 at the rearing and sterilization facility at Metapa de Dominguez, Chiapas, Mexico, (Schwarz, 1985).

Mexico initiated an ambitious phytosanitary project with the National Fruit Fly Campaign against Mexican fruit flies, based on the success obtained from the eradication of Mediterranean fruit fly from south Mexico. Seeing the necessity of controlling the economically important *Anastrepha* complex, a fruit fly and parasitoid plant was built at Metapa de Dominguez, Chiapas, Mexico, which, along with the medfly production plant at the same place, forms the greatest bioindustrial complex in the world (SARH, 1995).

The present paper describes the most common rearing methods used for mass rearing production of fruit flies, with emphasis on those of economic importance in Mexico.

2. MAINTENANCE AND HANDLING OF THE COLONY (ADULTS)

2.1. Confinement of adults in cages

Adult fruit flies are confined in diverse cage designs, whose capacity depends on the amount of insect to be produced. These can be metal or board sheets with plastic screen ,which will allow correct ventilation and prevent insects from escaping (Vargas, 1989). Density of adults per cage can vary depending on the species and is the critical factor for survival and fertility. Density effects should be studied in order to determine the size of the cage and the population level confined (Fay, 1989). In the case of *Anastrepha ludens*, from Mission, Tex., we have a report of 110,000 flies per cage and 140,000 in Metapa, Chiapas, Mexico. In *Anastrepha obliqua* the density handled per cage is 50,000 flies. In both cases the cage is 1.5 m high X 1.4 m wide X 0.3 m deep, so these species use similar sizes. Among the smallest tephritid is the Mediterranean fruit fly (*C. capitata*), which confines densities of 300,000 adults in a cage 2.8 m high X 2.0 m wide X 0.2 m deep.

2.2. Oviposition site

The most common way of collecting *C. capitata* eggs is by using screens or cloths, where the flies lay eggs, which are then collected in water containers, or the use of opened bottles which are removed after the collection, as done in Hawai. In the case of Mexico, Guatemala, Chile and Argentina, *Ceratitis capitata* lay eggs through a screen covering the cages and these fall into the water collectors. In *Anastrepha* spp., the ovipositor is thicker and needs a harder substrate, which allows major mechanical stimulation. For this reason screens with different hole sizes, smaller than those for *C. capitata*, are used. A peculiar characteristic of the oviposition substrate for *A.ludens, A. suspensa* and *A. obliqua*, is that these are covered with silicon or cement screen (for *A. suspensa*). This is a favorable characteristic because, for these species, egg collection is done by water spraying, allowing the eggs to fall towards the back of the oviposition chamber and inside the container. The tape placed on the oviposition substrate avoids humidity to be introduced inside the cage which would cause contamination.

The oviposition sustrate or panel is placed on the lateral area of the cage and occupies one third of the lateral space, from which the oviposition chamber overviews. Here the panel is separated from direct contact from the environment by means of a door, normally acrylic, in order to view the interior of the cage without trouble.

In these species of *Anastrepha*, dehydration of the egg is carefully avoided, separating it from the oviposition chamber and covering it twice daily with fine gel (furcelleran) to maintain the egg moisture until collection the following day. A variant for *A. suspensa* is the use of sponges placed all along the ovipostition chamber door, this causes a damp environment within the chamber, maintaining egg hydration. This problem is solved in *C. capitata* from the moment the egg is laid and drops into the water container, from where is is then collected.

In order to induce artificial oviposition in rearing of flies for experimental levels, a dome simulating a host fruit is used. This dome is covered with cloth with a hot parafin screen, which becoming solid and forms a soft surface, simulating the host fruit. Here the fly lays its eggs, which are then collected with water inside the cavity formed within the dome.

2.3. Sexual maturation of adults

The period of oviposition for *Anastrepha* species fluctuates from 8 to 10 days, while for *C.capitata* it is from 2 - 3 days.

The copulation period for mature adults occurs from 1-2 days before the preoviposition period. This point is very important when considering plans for how long flies will remain in the cage for productivity. This goes hand in hand with the longevity of cage-reared adults, which varies according to the species, the density of adults per cage, atmospheric conditions and with food and water.

2.4. Fertility

The fertility of a species can vary depending on the results obtained from the medium used, such as artificial oviposition substrate In the typical case, exposing female flies to preferred hosts will greatly increase fertility. This is why the selection of the artificial oviposition substrate in mass rearing of insects should be based on the natural wild behavior of the insect. Copying the natural atmospheric condition for artificial rearing will also help the insect gradually adapt.

Schwarz et al. (1985) report approximately 160 eggs/female *C. capitata* during a period from 12-13 days under lab conditions. Mission, Texas, rearing of *A. ludens* reports 560 eggs/female in 20 days of productive life, and, in the same species at Metapa, Mexico, there is average production of 464 eggs/female in a productive period of 13 days. In the case of *A. obliqua* we have values of 108 eggs/female in a period of 13 days at Metapa, Mexico, and 300 eggs/female in Weslaco, Texas. With the latter, we utilize domes with parafin and in the other cases with *Anastrepha* we utilize cloth panels covered with silicon.

By the experiencies obtained, it is not recommended to maintain adults confined for more than three weeks, because during this period great mortality leads to low egg production. Egg quality also starts decreasing during this time.

2.5. Food and water for adults

The traditional food for tephritids is sugar, enzymatically hydrolyzed protein and water, which provides the necessary amount of protein for egg development and maturation. Other sources explored are honey and fruit parts.

For *Bactrocera oleae* (Tzanakakis, 1992) liquid and solid food have been evaluated, where the mixture 1/4/5 of enzymatically hydrolyzed yeast, sugar and water, respectively, provides high fertility in females. Liquid food is rapidly consumed and solid food is consumed more slowly, with positive results with liquid diet and higher amount of egg production. The disavantage of a liquid diet is that it needs to be replaced two times a week, due to its rapid dehydration. For solid diet the advantage is that it lasts throughout the egg production period, although the area is damp, tends to become sticky, and flies are caught.

Actually, solid food is used most, varying with the addition of casein, cholesterol, vitamins, which may affect fertility and fecundity. Water is obtained by means of cotton, napkins, sponges or damp filter paper in contact with water contained it PVC tubes or their

equivalents. Water is generally chloride at a minimum of 2 ppm or treated with ultraviolet light to reduce microbiological contamination risks in adults.

2.6. Atmospheric conditions of the colony

Species of the tephritid family are mainly tropical or subtropical and need hot, damp conditions for optimum egg production. The range of temperature and humidity considered ideal fluctuates from 25-27°C and 50-80%, respectively, (Fay, 1989).

Another factor regulated in the colony is light, which in most of the cases is on continuously. This increases egg production compared with intervals of light/darkness. For *Anastrepha* spp. and *C. capitata*, light has been intensified from 3,000 to 10,000 lux (Fay, 1989). For *A. ludens*, *A. obliqua* and *C.capitata* in Metapa, Mexico, periods of light/darkness are utilized with excellent results on egg quality (percentage), maintaining adequate egg production.

It is important to note that during copulation, tephritids prefer crepuscular light, be it morning, afternoon or both. For these periods of light/darkness should be considered at least 12:12 hrs.

3. EGG HANDLING

3.1. Collection

For *A. ludens* and *A. obliqua*, eggs are collected daily from the oviposition panel with a fan spray which sprays water finely and eggs fall directly on the container. For *C. capitata* the egg is laid through a fine mesh and falls in a water collector, from where it will then be removed (Schwarz et al. 1985).

After collection, eggs are submitted to a disinfection treatment using, preferably, chloride at 250 ppm and sodium benzoate at 0.07%, for *A. ludens* in Metapa, Mex. For other species, such as *A. obliqua* and *C.capitata*, eggs should be washed only with distilled water and treated with ultraviolet light; no disinfectant should be added.

Fay (1989) reports the use of hydrochloric acid at 0.025% for 20-30 seconds for egg disinfection, washing them afterwards with clean water or using sodium benzoate at 0.03% to disinfect the egg's surface from microorganisms.

3.2. Incubation

Prior to collection, eggs continue in the incubation phase, under controlled temperature conditions, mainly fluctuating from 26-28°C. Under these conditions, *A. ludens* takes 4 days for incubation, *A. obliqua* 3 days, and *C. capitata* 2 days. The traditional incubation method is to use paper napkins or strips of filter paper, here the eggs, covered with hydrating gel, are added. These can be contained in petri dishes or other containers of greater dimensions hermetically sealed and with rigorous temperature control in incubation equipment. A variant is the use of water incubation, contained in bottles, in the ratio of 1:20 water:egg, oxygenated by air injection coming from specialized equipment, with air purifying filters.

This method is easier to implement when large quantities of eggs are handled and required handling space is less, with excellent results for *C. capitata, A. ludens, A. obliqua,* which present probability percentage above 90%.

3.3. Egg seeding

When egg eclosion reaches 5-25%, or more, in the water incubation system (up to 60%), eggs are ready to be seeded on larval diet.

Eggs will be seeded depending on the incubation systems. In the case of incubation on towels or strips of paper, the number of strips to be placed on the trays will be designated, depending on the density of egg/gram of diet. For *A. ludens* we handle 7.5, while for *A. obliqua* the report is of 7.75 and 14 for *C. capitata*. These are the values handled at Metapa de Dominguez, Mex., for mass rearing levels of the respective species.

4. ARTIFICIAL LARVAL DIET

4.1. Ingredients

One of the major efforts that has been invested in improving rearing of tephritids has been the larval diet. A diversity of ingredients has been used for rearing different species of tephritids, most of which have the same characteristics in common (Fay, 1989).

The major component is water, and its quantity depends on other components used, the physical characteristics and environmental conditions to which the diet is exposed. Water plays an important role in minimizing metabolic heat generated during the last larval stages, besides being the vehicle by which larvae obtain their nutrients.

The ingredients that occupy the second place in larval diet proportion, are texturized or volume agents, the most important of which are carrot powder, corn-cob powder, texturized soybean, wheat bran, wheat buds, wheat grits, cane bagasse, beet pulp (sugar beet), ground pasture, ground paper, corn flour, coffee husks, etc. All of these ingredients have in common a high fiber content (cellulose), which permits an optimum medium for larval development. The convenient amount of these ingredients depends on the species of tephritid to be reared, which oscillates between 5 and 26% of the total diet formula. Some of these ingredients provide nutrients to the larvae, including carrot powder, which is a source of vitamin A and carotenes, and grains from wheat by-products, which contain more or lesser protein and sterols grade. Hence, the use of these two ingredients is widely defended (Fay, 1989).

Protein sources explored have been by excellency beer yeast (*Saccharomyces cereviseae*) and torula yeast (*Candida utilis*), which, beside containing from 45 to 50% protein, are rich in vitamin B (B complex), carbohydrates and minerals. Other sources of proteins utilized are casein, soy flour, cotton seed and wheat sprouts, utilized in a lesser degree as a complement for the yeasts. The principal function of proteins is for construction and regeneration of cells and organs (Fay, 1989).

Carbohydrates are commonly obtained from granulated sugar, which has a stimualatory effect on the insect. Other carbohydrate sources are texturized, such as soyflour, sugar cane bagasse, wheat by-products, rice bran and corn flour among others, besides yeast.

Carbohydrates contribute to the function and structure of insect tissue and can be found in the nucleus, cytoplasm and cellular membrane, as well as extracellular hemolymph as support for the tissue, (Fay, 1989). The principal input of fats (lipids) is constituted by dried inactive yeasts, as well as in some cases some textures such as soyflour, corn flour, wheat by-products (principally wheat sprouts). Lipids are essential components of cellular membranes and act as precursor of ecdysone hormone (principally sterols), essential constituents of epidermis, forming an impermeable screen which stops insect dehydration, and for prolonged flight (Dadd, 1973). In the case of vitamins, ascorbic acid (Vitamin C) is present in most of the diets for phytophagous species and is reported as constituent of tissues preventing infectious diseases.

Vitamin A is associated with the normal vision of the insects. The function of Vitamin E is to protect the cellular membranes, reducing toxic by-product of peroxidation of polyunsaturated fatty acids. Vitamin E is also associated with egg production and sperm probability (Sivrastava et al. 1977).

The B complex is reported as essential in the insect diet and is supplied by adding dried inactive yeasts. Insects also require substantial quantities of minerals such as potassium, phosphorous and magnesium, and small quantities of calcium, chloride phosphate and iron outlines, zinc, magnesium and copper, which act as enzymatic cofactors for reactions that occur in insect metabolism (Schwarz, 1989).

Most of the textures are agricultural by-products and have no rigorous quality control. Hence, by nature, they have a high microbial count (bacteria, fungi and yeasts). When used with other diet ingredients, microorganisms spontanously reproduce in this appropiate medium; for this reason, we commonly resort to the use of microbiological inhibators and acidulating agents to avoid food contamination and harm to the insect. Methyl paraben (Nipagin) is used for controling yeasts and fungi, and to a lesser extent for bacterias (especially gram-negative), in pH ranges of 4 to 8. Sodium benzoate is effective against yeasts and bacteria and, to a lesser extent, against fungi, at ranges of pH of 2.5 to 4.0, (Baudi, 1989).

Other microbiological inhibitors utilized are propionate and sorbate by-products. Cholidric and citric acids are normally used to obtain the desired pH, which are adequated acccording to optimum pH for antimicrobial action of microbiological inhibitors and optimum pH for larvae development (commonly between 3.5 and 5.5.), (Funke, 1983). Chart No. 1 presents different formulae for larval diets, for the mass rearing of different tephritid species.

4.2. Preparation of diet

Diet is prepared with special mixers designed according to the maximum amount of daily preparation and components of diet. If these are of particle or fiber size, its preferable to use a pallet mixer, since it is easier to homogenize and mix in less time. When the size of the particle is smaller, such as flour, stripped mixers are recommended, because of its configuration homogenizes small particles at maximum. Normally ingredients are mixed dry at the beginning (texturized, yeast, sugar, etc.), forming a homogenized mixture, and then water with previously diluted preservatives is added.

Chart No. 1. COMPARATIVE CHART OF DIFFERENT LARVAL DIET FORMULAS FOR MASS REARING OF DIFFERENT TEPHRITIDS

Ingredients	C. capitata (Mexico)	C. capitata (Guatemala)	A. ludens (Mexico)	A. ludens (USA)	A.obliqua (Mexico)	A.obliqua (USA)	A.suspensa (USA)
Corn-cub powder	--------	-------	17.2	15.82	15.47	--------	26.44
Wheat bran	16.1	16.0	----------	---------	----------	---------	----------
Soyflour	16.1	----------	----------	---------	----------	---------	----------
Corn flour	----------	----------	5.3	---------	8.25	6.0	----------
Sugar cane Bagasse	----------	9.9	----------	---------	----------	---------	----------
Wheat bud	----------	----------	----------	8.43	----------	---------	3.12
Dried Yeast	8.6	9.9	7.0	6.32	6.01	4.52	4.15
Sugar	8.3	9.9	9.2	7.59	8.59	8.0	6.23
CholidricA.	----------	0.44	----------	0.95	0.56	0.5	0.73
Citric acid	0.7	----------	0.55	---------	----------	---------	----------
Sodium B.	0.25	0.29	0.40	0.10	0.24	0.1	0.11
Methyl Par.	0.65	----------	0.20	0.23	0.12	0.1	0.21
Guar gum	----------	----------	0.10	---------	0.1	---------	----------
Vitamins	----------	----------	----------	0.23	0.4	0.4	----------
Casein	----------	----------	----------	---------	----------	2.33	----------
Cholesterol	----------	----------	----------	---------	----------	0.05	----------
Beck salt	----------	----------	----------	---------	----------	0.08	----------
Agar	----------	----------	----------	---------	----------	0.3	----------
Water	49.3	54.0	60.05	60.45	60.20	77.62	59.01

Time of mixture depends on the nature of ingredients, thicker particles require less time for homogenization, while those with thin particles need a little more time. These values should be determined according to the final texture desired for the diet, taking care that the time of mixture is not excessive to avoid overheating of the diet, which could cause the quality of the nutrients to be lost.

5. LARVAL DEVELOPMENT

5.1. Containers

Larvae can be fed in a large variety of containers (trays, boxes, etc.). The model and configuration of the container will be determined by the amount of diet used and the volume it represents. The container should offer the facility for wide disipation of metabolic heat generated by the larvae, should be durable, easy to wash and if more space is required then it should be handy. Containers or trays could be aluminum, plastic or fiberglass, and are commonly rectangular.

5.2. Distribution of diet inside tray

Prepared diet is mesured by weight into the tray, and distributed homogeneously throughout the container, assuring a flat surface so that it is ready for egg seeding; eggs mst be distributed uniformly on the surface. The amount of diet per tray varies from 5 to 9 kg depending on the size of the tray.

5.3. Larval stages

Tephritid larvae pass through 3 larval stages within 5-9 days before completely maturing. In Metapa, Mexico, *A. ludens* completes its development in 9 days, *A. obliqua* in 8 days, and *C. capitata* in 7 days. *A. ludens* in Mission, Texas, USA, under different diet conditions, completes its development in 8 days, *A. suspensa* in Gainesville, Florida, USA, completes its development in 9-10 days.

In some cases, larval development requires two or three different rooms, in order to control temperature during each development stage. Temperatures for larval development range from 25°C to 28°C and relative humidity from 60-85%. Normally, to synchronize egg eclosion and homogenize the age of the larvae, its recomended that for the first stage the temperature should be 28-30°C, reduced to 27-28°C for the second stage and 25-27°C for the third. Table No. 2 presents various cases of atmospheric and handling conditions for different tephritid species.

5.4. Survival

Percentages of larvae-egg recovery are increased when a major adaptability of the insect in larval diet exists and when the production control process is standardized. The optimal level of larvae-egg recovery is estimated at 50%, however, some offspring, such as *C. capitata*, are reported at 60-70%.

Variation in larval production depends to a large extent on the quality of ingredients and fluctuations in atmospheric condition (out of parameters) or a combination of both factors. Under this situation, the rigorous control of ingredients is imperative, according to parameters determined as ideal for each species of insect.

5.5. Larval separation

The most common methods of separating larvae are: larvae jump (self-popping), sifting tumblers and damp separation. The method varies according to the species; hence, the popping method is utilized for *C. capitata, B dorsalis, B cucurbitae and A. suspensa.* With this method, larvae abandon the tray freely upon completing the third instar , jumping from the tray and dropping into water or another pupation substrate (vermiculite, sand, sawdust). With the tumbling method, diet is removed to break compaction and then dumped inside a mechanical sieve, which turns around intermitently, allowing larvae to be separated from the diet (Vargas, 1989). This method is used for *C. capitata* and *A. ludens* in Mexico and the United States.

The third method is damp separation, where the food is diluted in a water container, and injected with air pressure to maintain the water-diet-larva mixture in a homogenized form.

Larvae are then extracted from the container by means of PVC tubes connected to the base of the container, and joined by another Y tube connection, where air is generated (ventri effect) while injecting water at high pression, which pulls the water from the container and into manual sieves, where larvae are caught and diet is washed out. This method is utilized for *A. obliqua* and *A. ludens*, in circumstances where the diet is too damp and compacted to permit separations with the other methods.

6. PUPAE

6.1. Pupation methods

Once larvae are collected, they proceed to pupate on the pupation substrate, which could be vermiculate, sawdust, sand, inclusive wheat (wheat, rice).

The pupation sustrate simulates the ground where larvae normally pupate after leaving the fruit and is widely used for *Anastrepha* species and for *C. capitata*. A variant is naked pupation, used in Mexico for *C. capitata*, with excellent results (Shwarz et al. 1985), where extreme care should be taken to maintain high humidity to avoid loss of pupal weight.

6.2. Atmospheric conditions

In the tephritid species that are mass reared, a room for pupation is usually utilized where temperature is lower than the room assigned for pupal maturation. The objective is to counteract metabolic heat caused by larvae inside the container (tray). This room is also characteristically dark to minimize the number of larvae that pop out of the tray. Temperature for pupal development normally ranges between 24 and 20°C.

Another important factor is relative humidity, which should remain between 70 and 80% to avoid pupal weight loss. Generally, moisture (10-20 %) is added to the pupation substrate to help pupae retain weight.

Chart 2 presents values of temperature and relative humidity utilized for some tephritid species during the development phase for artificially reared pupae. Under these parameters, pupal deveopment is completed in 13 to 14 days for *A. ludens* and *A. obliqua* in Metapa, Mexico, Mission and Weslaco, Texas, USA, respectively; 12 days for *C. capitata* en Mexico, and 11-15 days for *A. suspensa* in Gainesville, Florida, USA.

6.3. Survival

Recovery of larvae to pupae varies between 80 and 90% in tephritid species, depending on adequate nutrition of the larvae and good control of atmospheric conditions. These factors are also closely related to the transformation percentages from pupae-adults, for which values of 75-95% are considered acceptable.

6.4. Separation and dyeing of pupae

To separate pupae from substrates (vermiculite, sawdust, etc.) a sieve is commonly used, which can be mechanical or manual (tumbler). This eliminates substrates through the screen and clean pupae are retained. This method, although the most common, is reported to

Chart No. 2. TEMPERATURES AND RELATIVE HUMIDITY FOR DEVELOPMENT STAGES OF FRUIT FLIES, FOR MASS REARING OF DIFFERENT SPECIES OF TEPHRITIDAE.

Area	C. capitata (Mexico) °C R.H.	A. Obliqua (Mexico) °C R.H.	A. ludens (Mexico) °C R.H	A. ludens (USA) °C R.H.	A. suspensa (USA) °C R.H.
Colony	27±1 70±5	27±1 75±5	26±1 55±5	24±1 65±5	26-27 65±5
Incubation	26±1 -----	26±1 ------	26±1 ------	25.5 ------	24 -----
Larvae I (1st. Stage)	30±1 90±5	28±1 90±5	28±1 85±5	28±1 80±5	27-28 80±5
Larvae II (2nd. Stage)	28±1 70±5	27±1 70±5	27±1 75±5	25.5±1 70±5	27-28 80±5
Larvae III (3rd. Stage)	28±1 70±5	26±1 70±5	26±1 75±5	25.5±1 70±5	21-22 90±5
Pupation	23±1 70±5	20±1 70±5	21±1 70±5	24±1 70±5	25.6-26.6 70±5
Pupae matu-ration	25±1 70±5	24-25 80±5	25±1 75±5	26±0.5 70±5	25.6-26.6 70±5
Packagin Room (Chill Room)	15-18 70±5	20±1 75±5	19±1 75±5	15.5 70±5	15.5 70±5

harm the wing muscles of some tephritid species (Osaki and Kobayashi, 1989), at level of up to 33% in *C. capitata, B. dorsalis*, and *B. curcurbitae.*

To solve this problem, in Metapa, Mexico, a wind tunnel is utilized, powered by a fan in a closed structure (tunnel form) covered in its interior with foam rubber to temper pupae while falling from the dosificator until they are in contact with air at moderate speed. Tbis air speed is sufficient to carry vermiculate out by difference in weight, and pupae fall on the container, free of substrates. This method is utilized for *A. ludens, A. obliqua* and *C. capitata*, with highly satisfactory results.

After separating the pupae, a certain percentage is returned for maintaining the colony and the rest is designated for release following irradiation. Pupae utilized for autocidal control are marked (dyed) with a phosphorescent dye (Day-glo), which allows diffirentiating sterile from wild adults when they are captured in trapping programs. The amount of the dye normally utilized is 1-2 grs. per liter of pupae. Dyeing of pupae is done in hermetically sealed plastic bottles or inside plastic bags. A designated volume of pupae corresponding to the amount of dye is placed inside, and these are slowly mixed with an oscillating movement until dyeing of the biological material is obtained.

7. PACKAGING AND IRRADIATION OF PUPAE

7.1. Selection of pupae

Irradiation of pupae normally occurs 2 days before adult emergence. The physiological age of a pupa is measured by determining the imago coloration (eye coloration),which varies

according to pupal age and is a simple parameter to use to determine an appropriate moment for irradiating pupae.

Color varies from a creamy white in young pupae to iridescent green in mature pupae of *A. ludens, A. obliqua* and *A. suspensa* and ochre red in *C. capitata.*

7.2. Hypoxia

In order for the action of irradiation to be efficient for inducing sterility of adult tephritids, pupae are exposed to a hypoxia process from 1-2 hours before irradiation and it is recommended that they be kept in this condition at a temperature of 15-17°C, so that under these circumstances, physiological activity (cellular division) of the insect is reduced to a minimum and direct harm to the cells of the reproduction organs can occur, which continue to divide.

7.3. Packaging

The hypoxia process normally occurs inthe packaging (Sausage bags or plastic pans) in which pupae are deposited to be transported to reception centers. If biological material is transported long distances, they should be transported in specially designed boxes for correct handling. In most cases, blue ice is utilized to maintain interior temperature in the box at 25-27°C.

7.4. Irradiation dose

Ability to induce high levels of sterility with minimum deterioration of insect quality is a basic requirement for any program utilizing the SIT (Hooper, 1989).

When reproductive cells are exposed to ionizing radiation, lethal dominant mutations result. In the Diptera, these mutations consist of loss of formation of chromosome fragments and/or formation of acentric and decentric chromosomes, which do not allow the adequate balance of chromosomes in the zygote. Thus, they die early in development (Hooper, 1989).

In Metapa, Mexico, the radiation dose for *C. capitata* is 14 krads (^{60}Co), to obtain 90% sterility, or inclusive higher. For *A. ludens* and *A. obliqua* 8 rads (^{60}Co) is utilized to obtain a similar sterility level.

In Mission, TX., USA, the same dose and same sources are used for *A. suspensa.* A radiation dose can vary depending on the presicion of the radiation source and age of pupae. As noted by Hooper (1989), sterility of 95-100% affects competivity of *C. capitata* males. The dose should be reviewed depending on the geographic location of the fly due to possible variation on genotypic characteristics.

8. HEALTH

As a preventive measurement in any installation for mass rearing, aseptic measures should be implemented to minimize contamination risks by microorganisms pathogenic for the insect.

Cleaning should be focused on material and equipment utilized for the different phases of insect development, as well as on the building. Use of sodium hypochlorite at 5-6% is recommended, and for cleaning activities for places with contamination risks, ammonium quaternary salts at 1% should be used.

9. FUNDAMENTAL ASPECTS FOR TEPHRITID MASS REARING

The greatest effort has focused on reducing operating costs on the most significant point, which is larval diet. Larval diet represents 40-50% of the investment for operation of the mass rearing plant. Alternative sources of ingredients of lesser price are a resource frequently used and can be combined with optimization of physical and chemical characteristics of the diet for better development of larvae.

Variation in insect quality is frequently caused by the quality of larval diet ingredients. These should be adjusted within the most strict parameters for physical-chemical, microbiological and nutrition, with the goal of obtaining the most standard ingredient.

The most important goals in application of SIT are to produce and release enough adults of optimum quality to cope with wild adults and be able to suppress or eradicate fly populations. To maintain a constant good quality and assure competitiveness of sterile adults in the field, the permanent procedures for rearing should be improved for better management in the different stages of insect development, adapting biology and behavior of the insect to the artificial rearing system.

Finally, the parameters for evaluating insect quality should be designed to predict the moment when it is necessary to replace the laboratory colony with wild flies, taking care to maintain the sexual aggressiveness of adults in order to achieve the principal goal of controlling and suppressing wild flies.

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