

Advances and Perspectives in the Mass Rearing of Fruit Fly Parasitoids in Mexico

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ABSTRACT: Biological control by augmentation is applied in Mexico as part of an integrated pest management program against native fruit flies of the genus *Anastrepha* Schiner. The exotic parasitoid *Diachasmimorpha longicaudata* has been the most important species used within this context. A program for the mass rearing of 50 million parasitized pupa per week has been established in southeast Mexico, and these are released into the field according to a yearly national plan based on industry requirements. In order to reduce costs and optimize procedures, important advances have been made in the technology for mass production, including an increase in the weight of host larvae (24 mg), changes in the management of host exposition, improvements in the management of environmental conditions, suitability in time and motions are the main areas addressed. Furthermore, a quality control program is routinely applied, and the key parameters under constant evaluation are: 1) weight and volume of host larvae, 2) host mortality after exposure, 3) weight and volume of pupae, and 4) percent parasitoid viability and percent emergence. Good performance in these parameters produces adults with adequate longevity and fecundity, high flight ability and good searching behavior. The introduced egg parasitoid *Fopius arisanus* and the native pupal parasitoid *Coptera haywardi* are being evaluated for use in the future as a complement to releases into the field of *D. longicaudata*. Manipulating host size and exposition time, the use of starting diet and suppressing host development by irradiation, have permitted the effective use of *Anastrepha* eggs as hosts for the rearing of *F. arisanus*. Further achievements in the mass rearing of *C. haywardi* (e.g., the suppression of unparasitized hosts after irradiation), could give us the opportunity to employ new options to reinforce the augmentative biological control of *Anastrepha* fruit flies in Mexico.

Key Words: *Diachasmimorpha longicaudata*, *Anastrepha ludens*, *Fopius arisanus*, *Coptera haywardi*, Quality Control.

INTRODUCTION

The Mexican Government launched the National Campaign against Fruit Flies in 1992 to create free and low-prevalence zones of native fruit flies of the genus *Anastrepha* Schiner of economic importance (Reyes *et al.* 2000). The technical plan was based on the integration of different technologies and strategies that have been applied using an area-wide approach. These include: the use of specific lures and baits to detect and monitor fruit fly populations, the application of cultural practices such as mechanical control, aerial or ground applications of selective toxic baits, the deployment of the Sterile Insect Technique (SIT) against *A. ludens* and *A. obliqua*, the establishment of quarantine procedures, and the release of the fruit fly parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) in specific zones and for certain periods (Montoya *et al.* 2007).

Since 1995, the *Moscafrut* facility located in Metapa de Dominguez, Chiapas, Mexico, has produced 50 million pupae parasitized by *D. longicaudata* per week (Moreno 2004). These parasitoids are released in the field according to a yearly national plan based on industry requirements.

Several studies have shown that, if used appropriately, augmentative releases of parasitoids may be a sound alternative for the suppression of fruit fly populations (Wong *et al.* 1991, 1992, Sivinski *et al.* 1996, Montoya *et al.* 2000a). Along with previous studies, Barclay (1987) and Knipling (1992) argue that integrating this form of biological control with the SIT may result in a synergism since two different stages of the fly population (i.e., immature and adult stages) would be under attack simultaneously. According to these authors, and under the circumstances described by Montoya and Cancino (2004) (in areas with organic fruit production, canyons and barracks, and marginal zones such as backyard orchards, etc.), the use of augmen-

tative biological control against fruit flies may have wide-spread benefits.

Data from field releases of *D. longicaudata* in different states of Mexico (i.e., Michoacán, Sinaloa, Nayarit, Aguascalientes [see Montoya *et al.* 2007]), have shown high rates in the percentage of parasitism reached in *Anastrepha* species and notable reductions in the Flies per Trap per Day (FTD) indices. These results are important evidence that mass releases of parasitoids, in conjunction with other suppression measures, can make the control of fruit fly populations easier inside commercial orchards.

Natural limitations are, however, presented by *D. longicaudata* when it is released in areas with a great diversity of host fruits, since large fruit may serve as an excellent refuge for the pest (Montoya *et al.* 2000a). In this scenario, the complementary quota of parasitism that can be provided by species such as *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae), an egg parasitoid of fruit flies, and *Coptera haywardi* (Oglobin) (Hymenoptera: Diapriidae), a pupal parasitoid of fruit flies, could enhance the possibilities and achievements for the augmentative biological control of these pests.

MASS REARING OF *DIACHASMIMORPHA LONGICAUDATA*

The parasitoid *Diachasmimorpha longicaudata* is mass reared on third instar (8-day-old) *A. ludens* larvae produced at the Moscafrut facility, and these are irradiated at 45 Gy to prevent the emergence of adult flies from any un-parasitized pupae when the parasitoids are released in the field (Cancino *et al.* 2002a). The irradiated larvae plus the retained diet-holding *A. ludens* larvae are placed in cassette-type containers covered with mesh and inserted in aluminum-frame mesh-covered cages (30 cm x 30 cm x 41 cm) (Cancino 2000). In these cages, larvae are ex-

posed to adult parasitoids at a rate of 2 larvae per parasitoid female. Adult parasitoids are fed with crystallized honeybees and kept in these cages for seven d. After two h of exposure, the host larvae are collected and placed in trays with vermiculite to allow pupation. Fourteen days later, the pupae are ready to be packed and sent to different destinations for field releases (Fig. 1). A quality control system to evaluate the mass rearing process was performed for each batch produced. The key parameters under constant evaluation were: 1) weight and volume of host larvae, 2) host mortality after exposure, 3) weight and volume of pupae, and 4) percent parasitoid viability and percent emergence (Planta Moscafrut/DGSV/SAGARPA 2000). The complete rearing process of *D. longicaudata* has been described by Cancino (2000).

IMPROVEMENTS IN THE QUALITY OF *D. LONGICAUDATA* MASS PRODUCTION

Over the past six years, significant advances in the mass rearing of *D. longicaudata* have been achieved, which has enabled the mass production of 50 million parasitoids per week while maintaining a high level of quality. The percent emergence (60% at a minimum) of adults is the main indicator of success, which has been achieved due to the following advances:

a) Weight of the Host Larvae: An average weight of 24 mg per larva is a crucial factor for achieving robust development of the parasitoid. A strict quality control of host weight results in batches with emergence above 60%.

b) Exposure Time: Various studies have reported that *D. longicaudata* is a solitary parasitoid with a high incidence of superparasitism (Lawrence 1988, Montoya *et al.* 2000b). The factors governing this behavior in mass rearing are unknown; nevertheless, the management of larval exposure time in mass

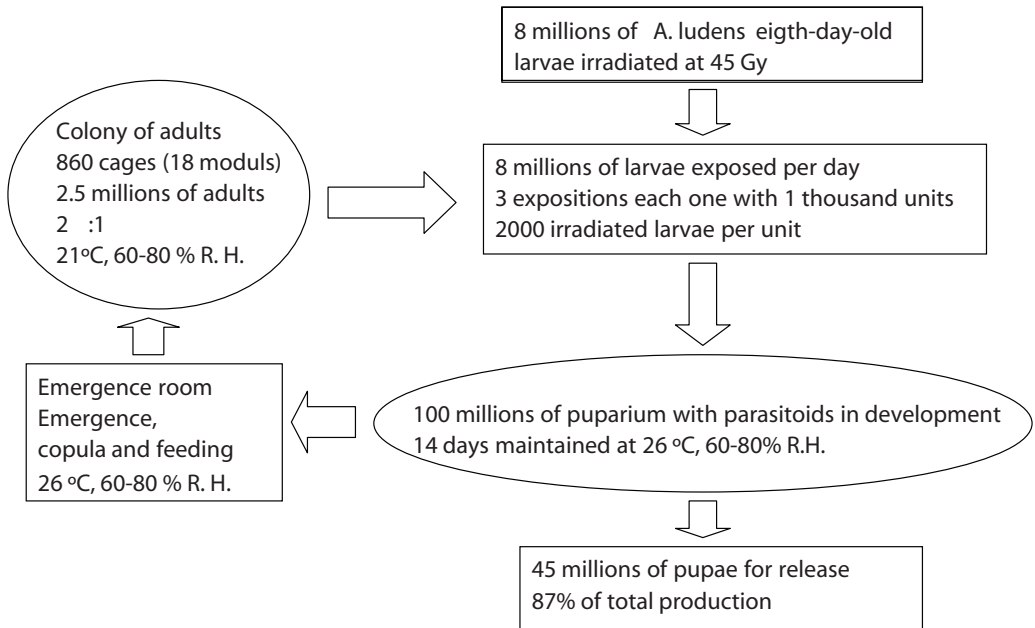


Fig. 1. Flow diagram of the main steps involved in the mass rearing of *D. longicaudata* at the Moscafrut Plant, Metapa de Domínguez, Chiapas, México.

rearing has permitted a notable improvement in the quality of the insects produced. At the beginning of mass production of this parasitoid, one of the main problems (high host mortality) was associated with high levels of superparasitism (Cancino *et al.* 2002b). Currently, three larval exposure periods per day are performed using different exposure times. The first exposure period (8:30 am) lasts an average of 1.5 h, the second exposure (11:00 pm) is done in 2 h, and the third (15:00) takes no longer than 3 h. This procedure results in adequate emergence percentages; furthermore, it favors a higher proportion of females above a 2♀:1♂ ratio, which is appropriate for the objectives of mass production (Gonzalez *et al.* 2007).

c) Control of Environmental Conditions:

Temperature and moisture are the main factors that affect the optimum development of mass-reared *D. longicaudata*. Wong and Ramandan (1992) reported that, compared with other temperatures, an average of $21 \pm 2^\circ\text{C}$ enhanced adult survival and increased

fecundity. Cancino (2002) reported that 21°C increased average female fecundity (76.21 offspring) when *A. ludens* was used as the host, which surpasses the 30.21 offspring, which are obtained at 26°C .

Temperature adjustment aimed at reducing variation has permitted the attainment of a seven-day average useful lifespan for parasitoids in production cages, with over 50% of the adult parasitoids remaining alive. Similarly, a temperature of 26°C in the emergence room leads to a shorter wait for adult emergence, thus reducing the need for space and keeping the conditions suitable for activities such as mating and feeding during the initial emergence period. When immature stages are developed at 26°C , however, the adults emerge in the required time, facilitating the coordination of shipments to various states of the country.

d) Suitability of Time and Motions: The suitable organization of technical personnel means that 22 people (who are distributed throughout the areas of colony maintenance,

larval exposure, immature stages maintenance, and packaging) can produce 50 million parasitized pupae with acceptable levels in the quality control parameters on a weekly basis.

DEVELOPMENT OF QUALITY CONTROL METHODS FOR THE MASS REARING OF *D. LONGICAUDATA*

The quality of mass-produced insects for augmentative releases in the field is a key factor in achieving adequate suppression of the target pest population (van Lenteren 1991, 2002, Messing *et al.* 1993, Bautista & Harris, 1997). The mass-reared insects should maintain their basic attributes (longevity, fe-

cundity, flight ability, search capacity) within the expected standards. For this reason, a rigorous system of quality control must be applied at two levels of the mass rearing process: 1) the quality of the host larvae and 2) the quality of the adult parasitoids obtained. For *D. longicaudata*, we consider that the most important attributes are: adult emergence, survival, fecundity, sex ratio, flight ability, and search capacity (Messing *et al.* 1993, Eben *et al.* 2000, Cancino *et al.* 2002b). In accordance with Cancino *et al.* (2006), we will briefly describe the principles and fundamentals concerning the main techniques, which these authors suggest for the quality control of *D. longicaudata* mass rearing. Table 1 shows the reference values used for the following quality control parameters:

Table 1. Values of reference for selected quality control parameters that are applied in the mass rearing of *D. longicaudata* (taken from Cancino *et al.* 2006).

Test/Parameter	Description of standards	
	(1)	(2)
Host weight: Humid sample	24.6 ± 2.0 mg /larva	291 ± 28.9 larvae/10 ml
Dry sample	23.5 ± 0.1 mg/larva	327 ± 33.3 larvae/10 ml
Percent of pupation (72 h)	93.5 ± 4.9 %	
Percent of mortality (72 h)	0.62 ± 0.1 %	
Pupal weight (14 d)	12.4 ± 1.5 mg	
Adult emergence	63.7 ± 6.3 %	
Sex-ratio (f/m)	2.4 ± 0.4	
Survival:		
With food and water (15 d)	Males 63.0 ± 23.8 %	Females 75.77 ± 20.8 %
Without food and water (7 d)	Males 0.33 ± 2.2 %	Females 0.25 ± 1.1 %
Fecundity	3.2 ± 1.8 sons/female/day	
Flight ability	80.1 ± 7.8 % flying adults	

(1) = Host weight: mg per individual larvae; (2) = number of larvae per sample of 10 ml; Sexual rate: f = female, m = male.

a) Host Weight: In the mass rearing of parasitoids, host quality is an indispensable requirement for assuring good results in the production of parasitoids. In the particular case of fruit fly parasitoids, good results in emergence and sex ratio are obtained in direct proportion to the age, size and weight of the host (Wong & Ramadan 1992, Cancino *et al.* 2002b). The size of the host has repercussions mainly on the percent emergence of the offspring and its sex ratio. It is generally thought that smaller hosts are selective for the development of male eggs, while larger hosts are preferred for female eggs (Godfray 1994).

b) Percent Pupation and Mortality at 72 h: Host quality can also be evaluated after its exposure to parasitoids using the parameters of host mortality and pupation at 72 h. *D. longicaudata* is considered to be a koinobiont parasitoid (Lawrence *et al.* 1978), allowing development of its host until pupation. The surveillance of the host and the formation of the pupa depend basically on host quality (Orozco *et al.* 1983), although these parameters are also affected by handling during and after exposure to parasitoids. Host mortality occurs when conditions have not been adequate, including such factors as contamination by microorganisms, humidity, and high host densities. An increase in host mortality is the main cause of low parasitoid production.

c) Pupal Weight: Host weight is considered to be a good indicator of pupal size (IAEA/FAO/USDA 1998, Cancino *et al.* 2002b). It is thus an important parameter in the quality of mass reared insects (Kobayashi & Ozaki 1980, Orozco *et al.* 1983, Churchill & Stanland *et al.* 1986, Cancino *et al.* 2002b). In *D. longicaudata*, this parameter is highly related with adult size, sex ratio and percent emergence (Cancino *et al.* 2002c).

d) Adult Emergence: This parameter may be the most indicative of quality in mass rearing. Most publications on the quality control of mass-reared parasitoids use this

parameter, along with flight ability and sex ratio, as the main reference (Calkins & Ashley 1987, IAEA/FAO/USDA 1998).

e) Survival and Fecundity: Both characteristics are very important in the quality control of mass-reared insects (Greenberg 1991, van Lenteren 2002, Cancino *et al.* 2002c, Orozco *et al.* 1983). Survival, a basic parameter in insect biology, is related to life expectancy, which must last long enough for the insects to find food, reach sexual maturity and mate. This parameter is of particular importance to the field performance of the parasitoid.

Fecundity is probably the most studied parameter in *D. longicaudata*. We have information concerning the average number of eggs produced per female per day (Lawrence *et al.* 1978, Ashley & Chambers 1979, Gonzalez *et al.* 2007), but due to different related variables (host, density, parasitoid-host relation, *etc.*) we need to study the best way of applying this parameter in a standardized way in the context of the quality control of mass-reared insects.

f) Flight Ability: This is one of the clearest and strongest quality control parameters in mass-reared insects (Schroeder *et al.* 1973). Traditionally, this parameter has been used as an indicator of dispersion capacity in the field, where a high level of flight ability is critical (Huettel 1976). Various studies have found that the confinement of insects to small cages with food and no adverse conditions may noticeably reduce their flight capacity (Fletcher & Economopoulos 1976, Cancino *et al.* 2002c). We compared a wild strain versus a lab strain of *D. longicaudata* and found that the percentage of flying parasitoids was higher in the wild strain.

g) Searching Behavior: This parameter is of crucial importance in the performance of parasitoids once released in the field. The fittest females develop the capacity to seek hosts more quickly (Lawrence 1981). In the mass rearing of *D. longicaudata*, it has been

observed that well-developed parasitoids are more efficient in the host-searching test. This test is carried out using a Y-tube into which infested and non-infested fruits are offered to female parasitoids. A simple wood-frame cage of 50 x 50 x 50 cm with walls of mesh netting may, however, also serve as a test site such that the types of fruit previously mentioned can be hung from the roof of the cage, allowing the free host to search for the female parasitoid.

DEVELOPMENT OF MASS-REARING METHODS FOR *COPTERA HAYWARDI*

Coptera haywardi (Oglobin) is a solitary endoparasitoid of fruit fly pupae belonging to the Tephritidae Family. Its high specificity gives it an advantage as a candidate for the biological control of flies of the genus *Anastrepha* spp. (Sivinski *et al.* 1988). Among other significant criteria in opting for its mass rearing is the fact that pupal parasitoids are relatively easy to rear (Dresner 1954). Together with its demonstrated specificity, it also shows a highly developed discriminative capacity for pupae under attack by *D. longicaudata*. This lends support to the idea of setting up an attack on the pupal biological stage of fruit fly populations using *C. haywardi*, which would complement the actions already under way using *D. longicaudata* larval attack in the field.

A pre-requisite to the mass rearing of this species is the preliminary establishment of a robust brood stock to develop new methodologies for efficiently obtaining great numbers of individuals of high quality. Therefore, an average of seven million *C. haywardi* pupae per week will be included in the control plans of the National Campaign over the next few years. These parasitoids will be destined for use in those regions and circumstances where an integral biological control program by augmentative releases is deemed expedi-

ent (cf. Montoya & Cancino 2004). A detailed description of the complete process of mass production of this species is provided in Cancino *et al.* (*in preparation*).

REARING OF *FOPIUS ARISANUS* ON *ANASTREPHA LUDENS* EGGS

Since the introduction of *F. arisanus* into Mexico, the development of a strain raised on fly eggs of the genus *Anastrepha* has been a primary objective. The initial results were reported by Lawrence *et al.* (2000), who showed the possibilities of developing this parasitoid on *Anastrepha suspensa* (Loew) eggs. The earliest evaluations performed by Zenil *et al.* (2004) reported a low parasitism in three species of *Anastrepha*. Nevertheless, this study permitted defining activities so as to establish a special strain of *F. arisanus* developed on *Anastrepha ludens* (Loew) eggs. A small colony that is currently being kept has a 20–25% adult emergence rate and a sex ratio close to 2♀:1♂. These advances have been implemented because of the following developments:

a) Selection of host age and exposure time: Diverse studies report that 4-day-old eggs offer the best conditions for oviposition and development of *F. arisanus* using *A. ludens* as the host. Moreover, an exposure time of 4 h was adequate for obtaining parasitism percentages of approximately 80% (Montoya *et al.* *submitted*).

b) Artificial exposure units: The establishment of the strain was carried out by using a papaya chunk (Harris & Okamoto, 1991). Because this procedure has several drawbacks, the establishment of an artificial oviposition unit was sought. According to previous evaluations, we decided to use a unit made of PVC tubing (Fig. 2). This set-up consists of two halves of 2" PVC tubing measuring 15 cm longitudinally, joined together. A piece of water-saturated filter paper was placed in the

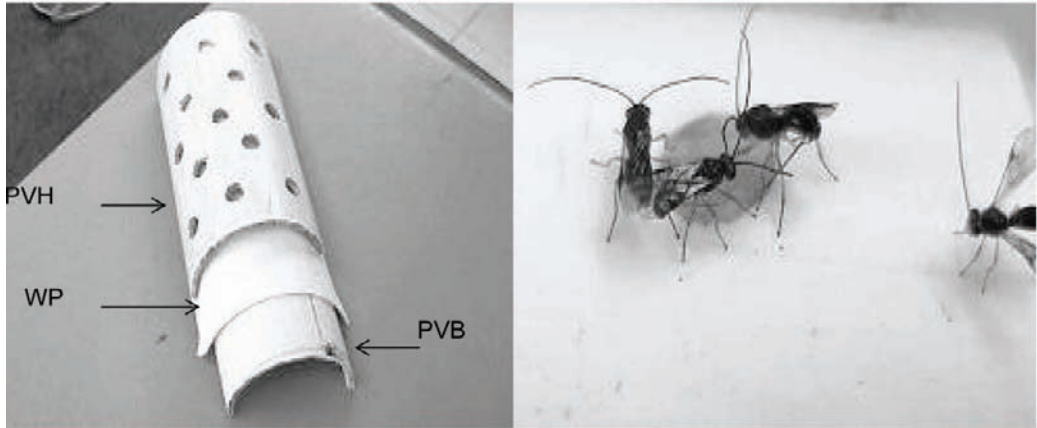


Fig. 2. Artificial oviposition unit for mass rearing of *F. arisanus* with *A. ludens* eggs. PVB= Base of PVC tube, WP= Wet paper filter, PVH= PVC tube with holes.

middle. The tube covering the concave section had 10 holes measuring 0.5 cm in diameter where the eggs were placed for exposure and kept moist by filter paper. Egg eclosion after exposure has traditionally been around 50 - 60% (Montoya *et al. submitted*).

c) The use of a starting diet: More than ten days are necessary for the complete development of *A. ludens* larvae from eggs parasitized by *F. arisanus*. To obtain good development of the parasitized larvae, two diets must be used. The first contains 10% more yeast than traditional diets, where the eggs are sown and kept for five days at high density (about 54,000 1st instar larvae per kg of diet). Later, the larvae are switched to a diet containing 10% more texturizer (ground corn cobs) from when the larvae finish their development until around day 12.

d) Suitability of conditions for mating: In general, *F. arisanus* is a parasitoid requiring special conditions for mating (Quimio & Walter 2000, Bautista *et al.* 2001). We designed a special place with a temperature of 24°C, 60-80% R.H., high lums intensity and an open space so air could freely circulate with a wind speed of 10m/s. These conditions induced 80% of the females to mate, thus promoting a suitable sex ratio and the stimulation of oviposition on the artificial units.

e) Separation of the parasitized material: To overcome the problem posed by the emergence of non-parasitized hosts in colony management, we irradiated the eggs at 30 Gy, which permitted the emergence of parasitoids but completely suppressed the development of flies from unparasitized eggs (Pérez 2005).

THE USE OF IRRADIATED HOSTS

The separation of non-parasitized flies, which developed together with parasitized hosts, in large-scale mass rearing of parasitoids is necessary. In the particular case of fruit fly parasitoids, the use of irradiated hosts has turned out to be a necessity. Sivinski and Smittle (1990) proposed using irradiated larvae for the mass rearing of *D. longicaudata*. Later, Cancino *et al.* (2002a) demonstrated how this could apply on a large scale. With this experience, host irradiation has permitted the incorporation of different species into mass rearing, including egg, larval and pupal parasitoids. The exclusive emergence of parasitoids as a result of the use of irradiated hosts gives a great advantage for the management of biological material, such as the process of mass rearing or field release activities.

PERSPECTIVES ON THE USE OF MASS-REARED PARASITIDS

The mass release of parasitoids in Mexico provides a fortuitous opportunity to analyze the success of biological control of fruit flies within a regional concept. In this context, mass rearing plays an important role, calling for consolidation and efficiency in the development of the biological control of fruit flies. The main topics of research for mass rearing can be grouped under three headings:

Production cost analysis: The study that used the most real data is the mass production of *D. longicaudata* using *A. ludens* as host, in accordance with the procedures documented in the Standard Operations Manual of the Moscafrut Plant (2002), which estimates the cost of producing one million pupae at \$400 million USD. Cost-benefit analysis requires further study and may include factors that afford a more accurate vision of the implementation of a biological control program by augmentation (Montoya & Cancino 2004).

The application of a Production Quality system: Despite all efforts, the effect of parasitoid quality on behavior in the field remains unknown. This implies that a

follow-up analysis of the data relevant to population suppression and a cause/effect feedback from the release of parasitoids are imperative. The main objective should be to obtain indices that would lead to improved parasitoid quality and use based on information obtained in the field.

Reduction in Production Costs: Production techniques in mass rearing must be constantly under analysis. In the case of the mass rearing of *D. longicaudata*, studies are being conducted to develop an automated rearing system to reduce costs and increase efficiency. For other species as well, the idea of applying artificial procedures, which do not impair the quality of the reared products,

are currently being investigated. This aspect, however, requires a good deal of study so as to determine the critical methods of rearing procedures and the influence on the quality of the final product.

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