

AN ARTIFICIAL LARVAL DIET FOR REARING OF *ANASTREPHA STRIATA* (DIPTERA: TEPHRITIDAE)

EMILIO HERNÁNDEZ¹, J. PEDRO RIVERA¹, DINA OROZCO-DAVILA¹, MIGUEL SALVADOR² AND JORGE TOLEDO³

¹Programa Moscafrut-Desarrollo de Métodos (SAGARPA-IICA), Central Poniente No. 14-Altos, Tapachula, Chiapas, 30700, Mexico

²Centro de Biociencias, Universidad Autonoma de Chiapas, Tapachula, Chiapas, 30700, Mexico

³Departamento de Entomología Tropical, El Colegio de la Frontera Sur, Apartado Postal 36, Tapachula, Chiapas, 30700, Mexico

ABSTRACT

An artificial larval diet for *Anastrepha striata* (Schiner) was developed and the changes in the rearing and quality parameters through 6 generations during the adaptation were characterized. In the first experiment we tested diet formulations that had already been developed for the mass-rearing of *Anastrepha ludens* (Loew), *A. obliqua* (Macquart), *A. serpentina* (Wiedemann) and *Ceratitis capitata* (Wiedemann) by sowing *A. striata* eggs (20-40% hatched) in each diet. In those tested diets, the maximum larval recovery percentage was 4.82%. In the second experiment, in the AOII modified diet of *A. obliqua*, we substituted the protein source, torula yeast by Nutrifly™, torula yeast-casein and hydrolyzed protein. A formulated diet contained 4.83% Nutrifly™, 15% corn cob fractions, 8.0% corn flour, 8.33% sugar, 0.23% sodium benzoate, 0.11% nipagin, 0.13% citric acid, and 63.37% water allowed higher larval survival compared to diets with different protein sources. In the third experiment, we evaluated adaptation of the larvae to Nutrifly diet. Over 6 generations, the larval and pupal weights and pupation percentage decreased from parental to first generation and increased after the third generation, recovering the initial value. Larval recovery and adult emergence increased from parental generation to the next generations; and was maintained during the next 5 generations. Larval recovery only a light decreased in the third generation. The laboratory colonization of *A. striata* reared on this artificial diet required at least 5 generations for the larvae to adapt to the artificial diet and increase pupal weight and adult emergence.

Key Words: American guava fruit fly, colonization, sterile insect technique, mass rearing

RESUMEN

Se desarrolló una dieta para *Anastrepha striata* (Schiner), caracterizando los cambios ocurridos en los parámetros de cría y calidad durante seis generaciones. En el primer experimento se utilizaron huevos que registraron entre 20 y 40% de eclosión al momento de sembrarse sobre las dietas utilizadas en las crías de *Anastrepha ludens* (Loew), *A. obliqua* (Macquart), *A. serpentina* (Wiedemann) y *Ceratitis capitata* (Wiedemann). En estas dietas, la mayor recuperación larvaria fue de 4.8%. En el segundo experimento, en la dieta AOII modificada de *A. obliqua* fue sustituida la fuente de proteína, y la levadura torula por Nutrifly™, torula-caseína y proteína hidrolizada. En la dieta formulada con 4.83% de Nutrifly™, 15% de polvo de olote, 8.0% de harina de maíz, 8.33% de azúcar, 0.23% de benzoato de sodio, 0.11% de nipagin, 0.13% de ácido cítrico y 63.37% de agua, se obtuvo la mayor recuperación de larvas en comparación con las otras dietas formuladas con diferentes fuentes de proteína. En el tercer experimento, se caracterizó la adaptación de las larvas a la dieta Nutrifly™. Durante seis generaciones, el peso de larva y de pupa disminuyó de la generación parental a la primera generación, y ambos parámetros se incrementaron a partir de la tercera generación, hasta registrar un peso similar al observado en la generación progenitora. La recuperación larvaria y la emergencia de adultos se incrementó de la generación parental a las siguientes generaciones y mantuvo dicha tendencia durante las cinco generaciones, y solamente la recuperación larvaria registró una ligera disminución durante la tercera generación. Se discuten los resultados que la adaptación de *Anastrepha striata* a una dieta larvaria artificial requiere de al menos cinco generaciones.

Translation provided by the authors.

The American guava fruit fly, *Anastrepha striata* (Schiner), is the third most economically important fruit fly species in Mexico (Aluja 1994). It is distributed from southern United States

(Texas) to Brazil (Weems, Jr. 1982; Hernández-Ortiz & Aluja 1993; Jiron & Hedström 1988). In Mexico, it has been recorded in the tropical regions of both coastal plains in the Balsas depression. Also, it is present in the highland locations of the states of Aguascalientes and southern Zacatecas over an area of about ~20,000 hectares (Hernández-Ortiz 1992), where guava orchards suffer serious infestation by fruit flies. In these growing areas, the farmers are interested in the application of the Sterile Insect Technique (SIT) based on the success of this control method of *Anastrepha ludens* (Loew) and *A. obliqua* (Macquart) (Rull-Gabayet et al. 1996; Reyes et al. 2000).

The SIT requires the capability of rearing large numbers of sterile flies of the target pest. However, knowledge of *A. striata* is limited to some aspects of its physiology (Ramírez-Cruz et al. 1996), behavior (Aluja et al. 1993), insecticide susceptibility (González et al. 1997), natural hosts (Aluja et al. 1987; Boscan de Martínez et al. 1980), and population fluctuations (Celedonio-Hurtado et al. 1995; Carballo 1998). There is only one previous study regarding artificial rearing of this species and that includes description of an artificial substrate for oviposition and egg collection (Hernández et al. 2004).

Mass-rearing of fruit flies requires the development of an artificial diet that is effective, easy to prepare and manage, and inexpensive. For insects not previously reared on an artificial diet, one approach is to adopt or modify an existing diet of a closely related species (Cohen 2004; Parker 2005). Modified diets of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wied.) have proven useful in developing diets for the Mexican fruit fly, *A. ludens* (Stevens 1991), the South American fruit fly, *A. fraterculus* (Wiedemann) (Jaldo et al. 2001), and the West Indian fruit fly, *A. obliqua* (Artiaga-López et al. 2004). Diet formulations usually include a source of protein, bulking agents, acidifying agents, preservatives, and water. The bulking agents generally comprise 15-20% of the diet (w/w), and the more common agents used are sugar cane bagasse (Peg & Rhode 1970; Vargas et al. 1983), texturized soy (Schwarz et al. 1985), or corn cob fractions (Stevens 1991; Vargas et al. 1994; Artiaga-López et al. 2004). Yeast is typically a source of protein, but it also provides lipids, vitamins, mineral, carbohydrates, and micro-nutrients (Moreno et al. 1997). Sugar cane is the common carbohydrates source. Citric acid, methyl paraben, and sodium benzoate are the typically acidifiers and preservative agents (Stevens 1991; Jaldo et al. 2001; Artiaga-López et al. 2004).

When flies are introduced to the laboratory on an artificial diet for larval development, the establishment might require several generations. Once the insects are adapted, however, there is a

good development and many quality parameters such as pupal weight and adult performance are improved (Pinson et al. 2006; Liedo et al. 2007).

Using the knowledge obtained from another species as a starting point, we describe development of the first artificial larval diet for artificial rearing of *A. striata*. We monitored quality control parameters over 6 generations to describe how the larva of *A. striata* was adapted to the diet and displayed increased rearing performance.

MATERIALS AND METHODS

Biological Material

The eggs used in this study were obtained from a strain maintained during 10 generations on guava fruits (*Psidium guajava* L.) at the Colonization and Rearing Laboratory, Development Methods Department, Moscamed-Moscafrut mass rearing facility (SAGARPA-IICA) at Metapa de Domínguez, Chiapas, Mexico, according to the methods described by Hernández et al. (2004). This strain was started in 2004 with 8,000 larvae obtained from infested guava fruits collected near Tapachula, Chiapas, Mexico.

Previously Formulated Larval Diets

Larval diets investigated were based on known formulations used in mass-rearing of *A. ludens* (ALU diet) (Stevens 1991), *A. obliqua* (AOI diet) (Artiaga-López et al. 2004), *A. serpentina* (ASE diet) (Pinson et al. 1993), and *C. capitata* (CCA diet) (Schwarz et al. 1985). In addition, 2 formulations, AOII and AOIII, derived from the AOI diet were included. The compositions of the 6 diets are summarized in Table 1. The AOI was replicated 11 times; ALU, ASE and CCA were replicated 10 times, while AOII and AOIII were replicated 5 times to give a total of 51 experimental units. Plastic rearing trays (20 Length × 4 Width × 15 Height cm) contained 500 g of diet. For each replication the diet was prepared independently with enough ingredients for only 1 batch.

Eggs collected during the second oviposition day, were uniformly dispersed on pieces of black cloth on moistened filter paper in Petri dishes (150 mm diameter × 25 mm depth), and maintained in a Lindberg/Blue M, Stabil-Therm (Asheville NC, USA) incubator for 3 d at 28 ± 1°C. When 50% of the eggs had hatched in the Petri dishes, eggs were seeded onto the larval diet surface of each tray. Before placement on the diet, the eggs were disinfected with chlorine at 100 ppm, and 0.1 mL eggs (≈1,100 eggs) were suspended in 50 mL of 0.4% guar gum solution that had been homogenized, and the mixture poured on the surface of the diet. During the first 2 d, the trays with egg-sown diet were kept at 29 ± 1°C, and 90% R.H. to complete hatching. Subsequently, the trays with

TABLE 1. COMPOSITION (% BY WEIGHT) OF THE DIETS PREVIOUSLY DEVELOPED FOR OTHER SPECIES OF THE ANASTREPHA GENUS AND TESTED FOR THE DEVELOPMENT OF ANASTREPHA STRIATA LARVAE.

Ingredients	Diet Type					
	CCA	ALU	ASE	AOI	AOII	AOIII
Corn-cob fractions ¹	0.00	10.20	10.20	15.00	15.00	15.00
Corn flour ²	0.00	0.00	7.00	8.00	8.00	8.00
Carrot meal ³	0.00	7.00	0.00	0.00	0.00	0.00
Texturized soy ⁴	14.20	0.00	0.00	0.00	0.00	0.00
Wheat germ ⁵	14.20	0.00	0.00	0.00	0.00	0.00
Sugar ⁶	7.30	7.00	7.00	8.33	8.33	8.33
Yeast ⁷	6.70	5.00	9.00	5.83	4.83	6.83
Methylparaben ⁸	0.60	0.20	0.20	0.11	0.11	0.11
Sodium benzoate ⁹	0.00	0.01	0.20	0.23	0.23	0.23
Guar gum ¹⁰	0.00	0.00	0.00	0.10	0.10	0.10
Citric acid ¹¹	0.00	0.00	0.44	0.63	0.63	0.63
HCl ¹²	0.60	0.60	0.00	0.00	0.00	0.00
Water	57.00	69.99	69.99	61.77	62.77	60.77

AOI (Artiaga-López et al. 2004), CCA (Schwarz et al. 1985), ALU (Stevens 1991), ASE (Pinson et al. 1993), AOII (modified AOI), AOIII (modified AOI). ¹Corn cob fractions 100, Mt. Pulaski Products Inc., Chicago IL.; ²Maíz Industrializado del Sureste, S.A. de C.V., Arriaga, Chiapas; ³Carrot meal, ⁴Colpac, Navajoa, Sonora, México. ⁵Wheat germ, ⁶Ingenio Huixtla, Chiapas, Mexico; ⁷Lake States Div. Rhineland Paper Co. Rhineland Wis.; ⁸Mallinckrodt Speciality, Chemicals Co. St. Louis Miss.; ⁹Cia. Universal de Industrias, S.A. de C.V., México; ¹⁰Tic gums, Inc. Belcamp, Md.; ¹¹Anhidro acidulante FNEUM, Mexana, S.A. de C.V., Morelos, Mexico. ¹²Omnichem, Procesos Químicos Científicos, S.A. de C.V., Puebla, México.

diet and larvae were kept at $27 \pm 1^\circ\text{C}$, and 85-90% R.H, until larval development was completed. At the 10th day, when the first pupa was observed on the surface of the diet (larvae of *Anastrepha* spp. do not jump out of the diet), the larvae were separated by diluting the diet with water and pouring the mixture through a sieve (Mesh 14). Recovered larvae were mixed with fine vermiculite to eliminate excess humidity (Strong-lite fine, Sungro Horticulture, Seneca, IL, USA), and larvae were separated by sieving, counted, and weighed. To promote high pupation percentage in a short period of time and uniform distribution of the pupae, larvae were allowed to pupate without substrate (naked) at $21 \pm 1^\circ\text{C}$, and 70% R.H. on the surface of the tray. After 24 h, pupae were covered with vermiculite and maintained for 14 d at $25 \pm 1^\circ\text{C}$, and 80% R.H. to complete the maturation process. One day before adult emergence, the pupae were sieved to separate them from the vermiculite, counted, and weighed.

For each treatment, the following parameters were recorded: (1) larval recovery was defined as the estimated percentage of eggs seeded (on the assumption of 1,100 eggs per 0.1 mL) and developed into mature larvae, (2) Average weight (mg) per larva was estimated by weighting and counting the total number of individuals from each replication (Hernández et al. 2005).

Modification and Improving Larval Diets

The most economical diet (AOII) with the best larval recovery in the previous test was used in

further experiments by substituting torula yeast with another protein source. The treatments were as follows: (1) Nutrifly™ diet (commercial content: yeast, sugar, corn starch, rice starch and potato starch); (2) torula yeast and casein diet (3.62% casein, and 1.21% yeast) (TYC), and (3) hydrolyzed protein diet (HP) (MP Biomedical, Irvine, CA). Twelve replications were conducted for each treatment yielding a total of 36 experimental units (plastic trays described previously). Collection and incubation of eggs, addition of eggs to the diet, larval recovery, pupae separation, and adults were managed as described previously.

For each treatment, the following data were obtained: (1) larval recovery (%) and (2) larval weight (mg), as described in the previous section. We also recorded (3) pupation percentage at 24 h after larval recovery, (4) average weight per pupa (mg) before adult emergence, and (5) adult emergence (percentage based on relation of emerged adults/ pupae) (FAO/IAEA/USDA 2003; Hernández et al. 2005; Rivera et al. 2007).

Adaptation on Artificial Diet

In order to determine if *A. striata* when adapted to the artificial diet displayed increased rearing performance, the changes in rearing and quality parameters through the colonization process over 6 generations (parental, and 5 subsequent generations) were analyzed by using a Nutrifly™ diet for larval development and under the same environmental conditions. Each generation began with the collection of eggs from the 1000

newly emerged adults from each diet trays that were placed in 27-dm³ glass cages with one side covered a screen mesh to facilitate providing food to the flies. Adults were fed *ad libitum* with a mixture of sucrose and enzymatic yeast hydrolysate (3:1), and water was provided in plastic containers with a vertical strip of filter paper. The adult colonies were kept at 26 ± 1°C, 75% R.H. and a photoperiod of 14:10 (L:D) h. Light was provided by white 75-W fluorescent tubes placed 60 cm above the cages, and the light intensity was ~300 lux inside the cage. Ten days after emergence of the adults, mating were observed, and placement and examination of oviposition devices began the next day.

Eggs were collected with oviposition spheres prepared with furcelleran as described by Boller (1968). The spheres were placed inside the cages for 24 h, and the eggs were recovered by cutting the spheres into slices and dispersing them in water (22-24°C) by a bubbling system (aquarium air pump, airflow of 3 L/min, 4.5 p.s.i., 110V, 50/60 Hz) as was described by Hernández et al. (2009). The water was decanted, and the eggs from each daily collection were counted and placed on pieces of black cloth on a moistened filter paper in a Petri dish (150 by 25 mm). The eggs were incubated for 3 d at 28 ± 1°C, and egg hatch was estimated by counting the eggs and larvae from a sample observed under a stereoscopic microscope. When we observed between 20 and 40% of larval hatch, then were seeded them onto larval diet in order to start the evaluation of the next generation.

Twelve different batches of eggs were used to start each generation, each one with 500 g of diet with 0.1 mL of eggs collected from different cohorts. At each generation, records were kept on the following: (1) larval recovery, (2) larval weight, (3) pupation percentage at 24 h, (4) pupal weight, and (5) adult emergence, estimated as described earlier.

Data Analysis

The data for larvae recovered (%), pupation at 24 h (%), and adult emergence (%) were transformed to arc-sin by the function $\chi' = \sin^{-1} \sqrt{\chi}$; where χ corresponded to the original value as a

ratio (percent/100). When a Bartlett test (Zar 1999) for equal variances was not significant for arc-sin transformed data ($P \leq 0.05$), a one way analysis of variance was applied (Underwood 2005), and the separation of means was made by applying Tukey's test for larval recovery, pupation and adult emergence. If the Bartlett test was significant, a Kruskal-Wallis test was applied to compare treatments (larval and pupae weights) (SAS Institute 2003). The data for larval recovery (%), larval weight (mg), pupation percentage at 24 h, pupal weight, and adult emergence by generation, during the process to adaptation to artificial diet were subjected to a simple linear or quadratic regression analysis with JMP version 5.01 statistical software (SAS Institute 2003).

RESULTS

Previous Formulated Larval Diets

Larval recovery from the initial diet formulations tested ranged from 2.12 to 4.82%. The higher values were obtained in the AOI and AOII diets and the lowest in the ASE diet ($F = 3.2$; $df = 5, 45$; $P = 0.015$) (Table 2). Larval weight ranged from 15.35 to 16.41 mg, without significant difference among means of the 6 diet types (Table 2). Differences between treatments were not significant ($F = 0.4$; $df = 5, 45$; $P = 0.871$) (Table 2).

Improved Larval Diets

Larval recovery ranged from 7 to 38% with the improved diets. The comparisons indicated that the highest mean larval recovery (%) was from Nutrifly™ diet and the lowest larval recovery was recorded with the hydrolyzed protein diet ($F = 604.4$; $df = 2, 33$; $P < 0.001$) (Table 3). The Nutrifly™ and torula yeast-casein diets allowed higher larval weight than hydrolyzed protein diet ($H = 23.4$; $df = 2$; $N = 36$; $P < 0.001$) (Table 3).

The highest pupation value occurred with the Nutrifly™ diet, and the lowest on the hydrolyzed protein diet ($F = 54.7$; $df = 2, 33$; $P < 0.001$), with

TABLE 2. DEVELOPMENT OF *ANASTREPHA STRIATA* LARVAE ON ARTIFICIAL DIETS*.

Diet Type	Larval recovery (%)	Larval weight (mg)
AOII (modified AOI)	4.82 ± 0.32 a	16.31 ± 1.16 a
AOI (Artiaga-López et al. 2004)	4.77 ± 0.67 a	15.79 ± 0.63 a
AOIII (modified AOI)	4.73 ± 0.86 ab	15.35 ± 0.85 a
CCA (Schwarz et al. 1985)	3.21 ± 0.70 ab	16.21 ± 0.50 a
ALU (Stevens 1991)	2.51 ± 0.35 ab	16.41 ± 0.63 a
ASE (Pinson et al. 1993)	2.12 ± 0.56 b	15.56 ± 0.62 a

*Means within a column followed by the same letter do not differ significantly by Tukey HSD ($P < 0.05$) test.

TABLE 3. REARING AND QUALITY CONTROL PARAMETERS FOR *ANASTREPHA STRIATA* REARED ON 3 DIFFERENT DIETS UNDER LABORATORY CONDITIONS.

Parameter	¹ Nutrify™ Diet	² Torula Yeast - Casein (TYC diet)	³ Hydrolyzed Protein (HP diet)
Larval recovery (%)	38.50 ± 0.54 a	32.17 ± 0.82 b	7.17 ± 0.56 c
Larval weight (mg)	22.52 ± 0.29 a	22.65 ± 0.69 a	13.78 ± 0.66 b
Pupation (%) at 24 h	97.51 ± 0.58 a	93.33 ± 0.95 a	77.33 ± 2.24 b
Pupal weight (mg)	17.64 ± 0.23 a	16.26 ± 0.48 ab	15.21 ± 0.62 b
Emergence (%)	95.50 ± 0.83 a	91.95 ± 1.34 a	83.79 ± 2.82 b

Means within a row followed by the same letter do not differ significantly by Tukey HSD ($P < 0.05$) test.

¹Coltec (Comercializadora Agrotecnológica, Guatemala, C.A.).

²Casec® (Bristol-Myers Squibb de Mexico, Mexico, D. F.).

³ICN (MP Biomedicals, Irvine, CA).

no significant differences among the Nutrify™ diet and torula yeast-casein diet (Table 3).

The highest pupal weight was obtained with the Nutrify™ diet. The lowest average was observed in the hydrolyzed protein diet, while the torula yeast with casein diet presented an intermediate average ($H = 12.55$; $df = 2$; $N = 36$; $P = 0.002$) (Table 3).

Highest adult emergence was obtained with Nutrify™ whereas lowest emergence was observed in hydrolyzed protein diet adults ($F = 12.8$; $df = 2, 33$; $P < 0.001$). Average emergence obtained with Nutrify™ and torula yeast-casein diets were not significantly different (Table 3).

Adaptation on Artificial Diet

There is a no significant fit to linear regression based on generational data for larval recovery (larval recovery = -0.11 generation + 37.35 , $r^2 = 0.03$) ($F = 0.16$; $df = 1, 70$; $P = 0.689$) (Fig. 1). Larval recovery ranged from 34 to 40% over 6 generations without significant differences among values ($F = 1.03$; $df = 5, 66$; $P = 0.405$). The lowest average was observed in the parental and third generation, while the highest corresponded to the first generation.

Larval weight was fitted to a quadratic equation, $y = 0.42x^2 - 0.04x + 18.87$, $r^2 = 0.78$ ($F = 5.89$; $df = 2, 69$; $P = 0.004$) (Fig. 2A), with mean values ranging from 18.1 to 21.4 mg through 5 generations. The highest average was observed in parental and fifth generation, which decreased in first and second generations, with medium averages in the third and fourth generations ($F = 3.03$; $df = 5, 66$; $P = 0.016$).

In the pupal weight, described by the equation $y = 0.19x^2 + 0.14x + 16.52$, $r^2 = 0.68$ ($F = 8.14$; $df = 2, 69$; $P < 0.001$) (Fig. 2B), the mean ranged from 16.8 to 18.6 mg through 5 generations. The highest average was observed in the parental and fifth generation, with significant decreases in the first and second generations; and medium averages in the third and fourth generation ($F = 5.12$; $df = 5, 66$; $P < 0.001$).

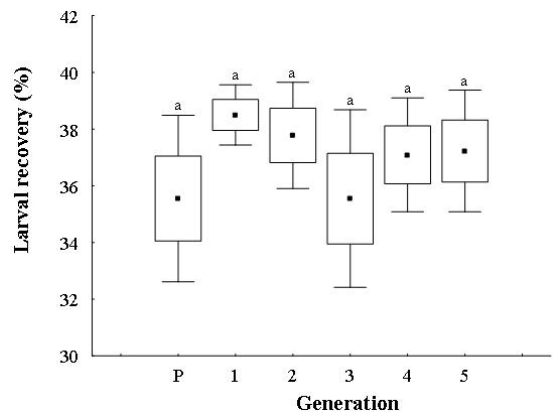


Fig. 1. Larval recovery of *Anastrepha striata* reared on Nutrify™ diet during colonization under laboratory conditions. [■ Mean, □ Mean ± S.E., ⊥ Mean ± (1.96) S.E.].

In pupation at 24 h, described by the equation $y = 1.84x^2 + 3.19x + 63.81$, $r^2 = 0.48$ ($F = 4.68$; $df = 2, 69$; $P = 0.012$) (Fig. 3), the mean ranged from 65.8 to 89.2% through 6 generations. In the parental and from third to fifth generation higher values were obtained than in the first and second generations ($F = 4.34$; $df = 5, 66$; $P < 0.001$).

The mean adult emergence over 6 generations ranged from 86.3 to 95.5% and was described by the equation $y = 1.46x + 86.29$, $r^2 = 0.77$ ($F = 22.87$; $df = 1, 70$; $P < 0.001$) (Fig. 4). The lowest average was observed in the parental generation while the highest corresponded to the fifth generation ($F = 6.21$; $df = 5, 66$; $P < 0.001$).

DISCUSSION

The developed for first time of an artificial larval diet for *A. striata* was based initially on the diets used for mass-rearing another species of fruit flies. The diet that yielded the highest number of larvae in the current study was the formulation developed for the mass-rearing of *A. obliqua* by

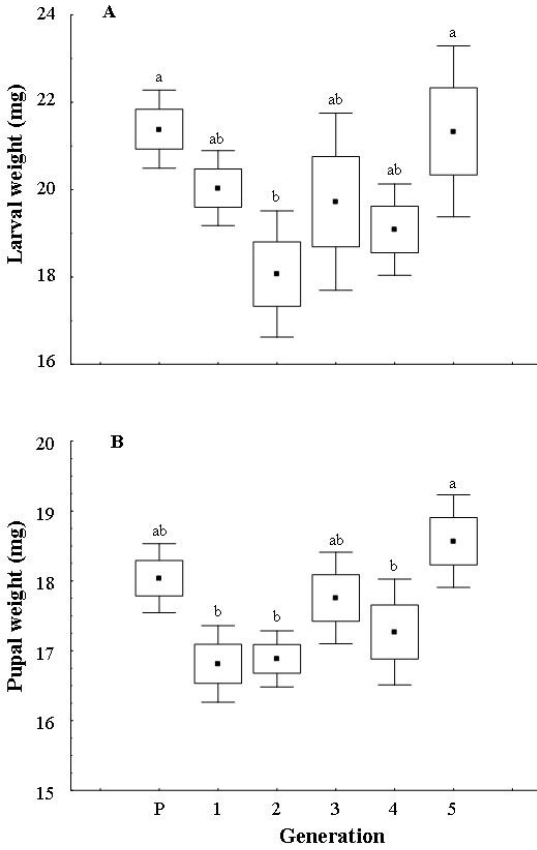


Fig. 2. Larval (A) and pupal (B) weight of *Anastrepha striata* reared on Nutrifly™ diet during colonization under laboratory conditions. [■ Mean, □ Mean±S.E., ⊥ Mean ± (1.96) S.E.].

Artiaga-López et al. (2004). The diets used for *A. ludens* (Stevens 1991), *A. serpentina* (Pinson et al. 1993), and *C. capitata* (Schwarz et al. 1985) permitted development of *A. striata* larvae although to a lesser degree. However, the low values regarding larval recovery indicate that it may be necessary to develop a diet with specific chemical, physical, and nutrient characteristics that permit the survival and growth of a greater number of larvae that would provide greater genetic variation in the population (Ochieng-Odero 1994).

Torula yeast appeared to benefit larval development, but allowed a greater quantity of recovered larvae and high larval weight suggesting that *A. striata* diets required casein. These results agree with observations made in *A. obliqua* in which larval weight increased when casein was added to the diet (Moreno et al. 1997; Rivera et al. 2007). For *A. ludens* and *A. serpentina*, torula yeast and corn flour provided the amino acids, vitamins, and other nutrients to promote larval growth (Rivera et al. 2007).

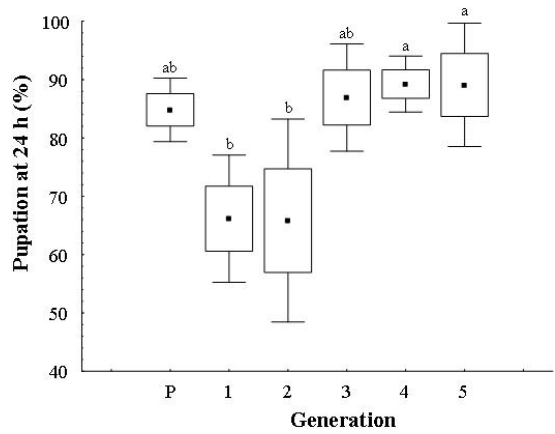


Fig. 3. Pupation (%) of *Anastrepha striata* reared on Nutrifly™ diet during colonization under laboratory conditions. [■ Mean, □ Mean ± S.E., ⊥ Mean ± (1.96) S.E.].

In this study we identified useful combinations of ingredients for rearing of *A. striata* on an artificial diet. This finding provides baseline information for development of low cost diets based on alternative substrates. Nevertheless, we found that larval and pupal weight and pupation at 24 h decreased from the parental generation maintained on guava fruits to the first generation maintained on artificial diet. With *C. capitata* and *A. obliqua* a precipitous decline in yield was observed during the first generation following a change from natural host to artificial mass-rearing methods (Leppla et al. 1983; Hernández et al. 2009). This trend could be explained by the fact that colony founders are forced through genetic bottlenecks to eliminate variability as well as phenotypes

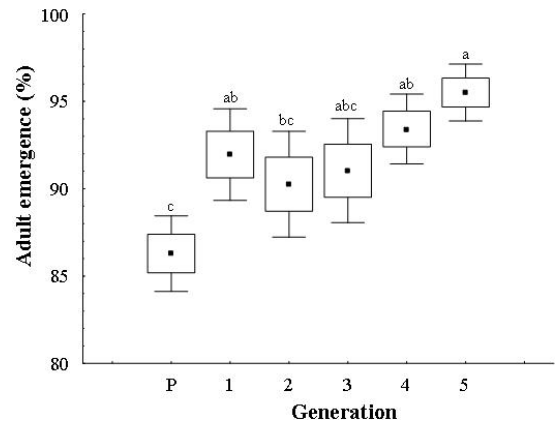


Fig. 4. Adult emergence (%) of *Anastrepha striata* reared on Nutrifly™ diet during colonization under laboratory conditions. [■ Mean, □ Mean ± S.E., ⊥ Mean ± (1.96) S.E.].

that prevent adaptation to the larval diet. Larval recovery and adult emergence of *A. striata* increased from the parental to the first generation. These results are similar with other previously reported in flies reared in artificial conditions (Manoukas 1983; Leppla 1989; Liedo et al. 2007; Hernández et al. 2009), which suggests that colonization for mass-rearing is a selection process in which insects are adapted to the rearing conditions and that attributes require several generations to improve. For example, the pupal recovery increased throughout the generations during the colonization of *Bactrocera invadens* Drew, Tsurutu and White (Ekesi et al. 2007), and the same result was observed for the pupal weight, demographic parameters, and mating attributes during the colonization of *C. capitata* (Liedo et al. 2007). Larval recovery and fecundity in *A. obliqua* showed the same trend through colonization (Hernández et al. 2009).

This study provides basic information regarding artificial diets for larval development of *A. striata*. However, additional studies into nutrient requirements are needed to optimize the rearing process to improve the production of quality insects at lower cost.

ACKNOWLEDGMENTS

The authors thank S. Ekesi and D. Nestel and 3 anonymous reviewers for helpful review of this manuscript. We are grateful to Y. Margoth García (Subdirección de Desarrollo de Metodos-Programa Moscafrut, SAGARPA-IICA), for technical help in the rearing procedures and Miguel Arenas for the computer assistance. This research was partially funded by the FAO/IAEA (Contract No. 13023/R0. 2005-2010) and the Campaña Nacional Contra Moscas de la Fruta, Programa Moscafrut (SAGARPA-SENASICA). We are grateful to Jorge Hendrichs, Andrew Jessup, and Carlos Cáceres of the IAEA staff.

REFERENCES CITED

- ALUJA, M., GUILLÉN, J., DE LA ROSA, G., CABRERA, M., CELEDONIO, H., LIEDO, P., AND HENDRICHS, J. 1987. Natural host plant survey of the economically important fruit flies (Diptera: Tephritidae) of Chiapas, Mexico. *Florida Entomol.* 70: 329-338.
- ALUJA, M., JACOME, I., BIRKE, A., LOZADA, N., AND QUINTERO, G. 1993. Basic patterns of behaviour in wild *Anastrepha striata* (Diptera: Tephritidae) flies under field-cage conditions. *Ann. Entomol. Soc. America* 86: 776-793.
- ALUJA, M. 1994. Bionomics and management of *Anastrepha*. *Annu. Rev. Entomol.* 39: 155-178.
- ARTIAGA-LÓPEZ, T., HERNÁNDEZ, E., DOMÍNGUEZ-GORDILLO, J., MORENO, D. S., AND OROZCO-DÁVILA, D. 2004. Mass-Production of *Anastrepha obliqua* at the Moscafrut fruit fly facility, Mexico, pp. 389-392 *In* B. N. Barnes [ed.], *Proc. 6th Intl. Symp. on Fruit Flies of Economic Importance*, 6-10 May 2002, Heriotdale, Johannesburg, South Africa.
- BOLLER, E. 1968. An artificial oviposition device for the European cherry fruit fly, *Rhagoletis cerasi*. *J. Econ. Entomol.* 61: 850-852.
- BOSCAN DE MARTINEZ, N., DEVORDY, J. R., AND REQUENA, J. R. 1980. Estado actual de la distribución geográfica y hospederas de *Anastrepha* spp. (Diptera: Tephritidae) en Venezuela (1). *Agronomía Tropical*. 30: 55-63.
- CARBALLO, V. M. 1998. Abundancia estacional y daño de *Anastrepha striata* en genotipos de guayaba y cas. *Manejo Integrado de Plagas* (Costa Rica) 50: 66-72.
- CELEDONIO-HURTADO, H., ALUJA, M., AND LIEDO, P. 1995. Adult population fluctuations of *Anastrepha* species (Diptera: Tephritidae) in tropical orchard habitats of Chiapas, México. *Environ. Entomol.* 24: 861-869.
- COHEN, A. C. 2004. *Insect Diets*. Science and Technology. CRC Press, Boca Raton. 324 p.
- FAO/IAEA/USDA/ 2003. *Manual for Product Quality Control and Shipping Procedures for Sterile Mass-reared Tephritid Fruit Flies*. Version 5.0 International Atomic Energy Agency, Vienna, Austria. 85 p.
- GONZÁLEZ, G. E., DEIBIS, J., AND CÁSARES, M. R. 1997. Susceptibilidad de poblaciones adultas, machos y hembras de *Anastrepha striata* Schiner al insecticida malatión, usando técnicas de aplicaciones tóxicas y consumo de cebos tóxicos. *Bol. Entomol. Venezolana* (N. S.) 12: 51-57.
- HERNÁNDEZ, E., ARTIAGA, T., AND FLORES, S. 2004. Development of an artificial oviposition device for *Anastrepha striata* Schiner (Diptera: Tephritidae), pp. 393-398 *In* B. N. Barnes [ed.], *Proc. 6th Intl. Symp. on Fruit Flies of Economic Importance*, 6-10 May 2002, Heriotdale, Johannesburg, South Africa.
- HERNÁNDEZ, E., FLORES-BRECEDA, S., SOSA-ITURBE, M. DE LA L., AND EZQUIVEL, H. 2005. Tamaño de unidad muestral y número de repeticiones para la estimación de los parámetros de desarrollo de *Anastrepha obliqua* y *Anastrepha ludens* (Diptera: Tephritidae). *Folia Entomol. Mexicana* 44: 155-164.
- HERNÁNDEZ, E., TOLEDO, J., ARTIAGA-LÓPEZ, T., AND FLORES, S. 2009. Demographic changes in *Anastrepha obliqua* (Diptera: Tephritidae) throughout the laboratory colonization process. *J. Econ. Entomol.* 102: 542-551.
- HERNÁNDEZ-ORTIZ, V. 1992. El género *Anastrepha* Schiner en México (Diptera: Tephritidae). *Taxonomía, distribución y sus plantas huéspedes*. Instituto de Ecología, and Sociedad Mexicana de Entomología. Publ. 33. México 162 p.
- HERNÁNDEZ-ORTIZ, V., AND ALUJA, M. 1993. Listado de especies del género neotropical *Anastrepha* (Diptera: Tephritidae) con notas sobre su distribución y plantas hospederas. *Folia Entomol. Mexicana*. 88: 89-105.
- JALDO, H. E., GRAMAJO, M. C., AND WILLINK, E. 2001. Mass rearing of *Anastrepha fraterculus* (Diptera: Tephritidae): A preliminary strategy. *Florida Entomol.* 84: 716-718.
- JIRON, L. F., AND HEDSTRÖM, I. 1988. Occurrence of fruit flies of the genera *Anastrepha* and *Ceratitis* (Diptera: Tephritidae), and their host plant availability in Costa Rica. *Florida Entomol.* 71: 62-73.
- LEPPLA, N. C., HUETTEL, M. D., CHAMBERS, D. L., ASHLEY, T. R., MIYASHITA, D. H., WONG, T. T. Y., AND HARRIS, E. J. 1983. Strategies for colonization and maintenance of the Mediterranean fruit fly. *Entomol. Exp. Appl.* 33: 89-96.

- LEPPLA, N. C. 1989. Laboratory Colonization of Fruit Flies, pp. 91-103 *In* A. S. Robinson, and G. Hooper [eds.]. Fruit Flies, Their Biology, Natural Enemies and Control. Vol. 3B. Elsevier: Amsterdam, The Netherlands.
- LIEDO, P., SALGADO, S., OROPEZA, A., AND TOLEDO, J. 2007. Improving mating performance of mass-reared sterile Mediterranean fruit flies (Diptera: Tephritidae) through changes in adult holding conditions: Demography and mating competitiveness. *Florida Entomol.* 90: 33-40.
- MANOUKAS, A. C. 1983. The adaptation process and the biological efficiency of *Dacus oleae*, pp. 91-95 *In* R. Cavaloro [ed.], Fruit Flies of Economic Importance. CEC/IOBC International Symposium, Athens, Greece, 16-19 November 1982. A. A. Balkema, Rotterdam.
- MORENO, D. S., ORTEGA-ZALETA, D. A., AND MANGAN, R. L. 1997. Development of artificial larval diets for the West Indian fruit fly (Diptera: Tephritidae). *J. Econ. Entomol.* 90: 427-434.
- OCHIENG-ODERO, J. P. R. 1994. Does adaptation occur in insect rearing systems, or is it a case of selection, acclimatization and domestication? *Insect Science Applic.* 15: 1-7.
- PARKER, A. G. 2005. Mass-Rearing for Sterile Insect Release, pp. 209-232 *In* V. A. Dyck, J. Hendrichs and A. S. Robinson [eds.], Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, IAEA, Springer. The Netherlands.
- PELEG, B. A., AND RHODE, R. H. 1970. New larval medium and improved pupal recovery method for the Mediterranean fruit fly in Costa Rica. *J. Econ. Entomol.* 63: 1319-1321.
- PINSON, E., CELEDONIO, H., AND ENKERLIN, W. 1993. Colonization and establishment of *Anastrepha serpentina* for mass-rearing: Preliminary results, pp. 281-284 *In* M. Aluja and P. Liedo [eds.]. Fruit flies: Biology and Management. Springer-Verlag. New York, USA.
- PINSON, E. P., TEJADA, L. O., TOLEDO, J., ENKERLIN, W., CELEDONIO-HURTADO, H., VALLE, J., PÉREZ, J. N., AND LIEDO, P. 2006. Caracterización de la adaptación de *Anastrepha serpentina* (Wied.) (Diptera: Tephritidae) a condiciones de cría masiva. *Folia Entomol. Mexicana* 45: 97-112.
- RAMÍREZ-CRUZ, A., HERNÁNDEZ-ORTIZ, V., AND MARTÍNEZ, M. I. 1996. Maduración ovárica de las moscas de la guayaba *Anastrepha striata* Schiner (Diptera: Tephritidae). *Acta Zool. Mexicana* 69: 105-116.
- REYES, F. J., SANTIAGO M., G., AND HERNÁNDEZ, M. P. 2000. The Mexican fruit fly eradication programme, pp. 377-380 *In* K. H. Tan [ed.], Area-Wide Control of Fruit Flies and Other Insect Pests. Penerbit Universiti Sains Malaysia. Penang, Malaysia.
- RIVERA, J. P., HERNÁNDEZ, E., TOLEDO, J., SALVADOR, M., AND SILVA, R. 2007. Dieta texturizada con agar para el desarrollo larvario de tres especies de moscas de la fruta (Diptera: Tephritidae). *Folia Entomol. Mexicana* 46: 37-52.
- RULL-GABAYET, J. A., REYES-FLORES, J., AND ENKERLIN-HOEFLICH, W. 1996. The Mexican national fruit fly eradication campaign: Largest fruit fly industrial complex in the world, pp. 561-563 *In* B. A. McPherson and G. J. Steck [eds.], Fruit Fly Pests. A World Assessment of Their Biology and Management. St. Lucie Press. Fla., USA.
- SAS INSTITUTE. 2003. JMP Statistical, Discovery Software, Version 5.0.1. SAS Institute Inc., Cary, North Carolina.
- SCHWARZ G. A., ZAMBADA, A., OROZCO, D., ZAVALA, J. L., AND CALKINS, C. O. 1985. Mass rearing of the Mediterranean fruit fly at Metapa, Mexico. *Florida Entomol.* 68: 467-477.
- STEVENS, L. 1991. Manual of Standard Operating Procedures (SOP) for the Mass-Rearing and Sterilization of the Mexican Fruit Fly, *Anastrepha Ludens* (Loew). USDA-APHIS, South Central Region, Mission Texas. 39 p.
- UNDERWOOD, A. J. 2005. Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press. Cambridge, UK. 504 p.
- VARGAS, R. I., CHANG, H., AND WILLIAMSON, D. L. 1983. Evaluation of a sugarcane bagasse larval diet for mass production of the Mediterranean fruit fly (Diptera: Tephritidae) in Hawaii. *J. Econ. Entomol.* 76: 1360-1362.
- VARGAS, R. I., MITCHELL, S., HSU, C., AND WALSH, W. A. 1994. Laboratory evaluation of diets of processed corncob, torula, yeast, and wheat germ on four developmental stages of Mediterranean fruit fly (Diptera: Tephritidae). *J. Econ. Entomol.* 87: 91-95.
- WEEMS, JR., H. V. 1982. *Anastrepha striata* Schiner (Diptera: Tephritidae). Entomology circular No. 245. December, 1982. Fla. Dept. Agric. and Consumer Serv. Division of Plant Industry. 2 p.
- ZAR, J. H. 1999. Biostatistical Analysis. Prentice-Hall, Inc. New Jersey. 663 p.