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

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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 desarrollo 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 nipagín, 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.
Anastrepha ludens (Loew) and A. striata (Stevens 1991), the Mediterranean fruit fly (medfly), Ceratitis capitata (Wied.) 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 Coloni- zation and Rearing Laboratory, Development Methods Department, Moscamed-Moscafruit mass rearing facility (SAGARPA-ICA) 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 × 15 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 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.

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diet and larvae were kept at 27 ± 1°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 ± 1°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 ± 1°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

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**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.**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>CCA</th>
<th>ALU</th>
<th>ASE</th>
<th>AOI</th>
<th>AOII</th>
<th>AOIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn-cob fractions¹</td>
<td>0.00</td>
<td>10.20</td>
<td>10.20</td>
<td>15.00</td>
<td>15.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Corn flour²</td>
<td>0.00</td>
<td>0.00</td>
<td>7.00</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
</tr>
<tr>
<td>Carrot meal³</td>
<td>0.00</td>
<td>7.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Texturized soy⁴</td>
<td>14.20</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Wheat germ⁵</td>
<td>14.20</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Sugar⁶</td>
<td>0.60</td>
<td>0.20</td>
<td>0.20</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>Yeast⁷</td>
<td>7.30</td>
<td>7.00</td>
<td>7.00</td>
<td>8.33</td>
<td>8.33</td>
<td>8.33</td>
</tr>
<tr>
<td>Methylparaben⁸</td>
<td>0.00</td>
<td>0.01</td>
<td>0.20</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Sodium benzoate⁹</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Guar gum¹⁰</td>
<td>0.00</td>
<td>0.00</td>
<td>0.44</td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Citric acid¹¹</td>
<td>0.60</td>
<td>0.60</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>HCl¹²</td>
<td>6.70</td>
<td>5.00</td>
<td>9.00</td>
<td>5.83</td>
<td>4.83</td>
<td>6.83</td>
</tr>
<tr>
<td>Water</td>
<td>57.00</td>
<td>69.99</td>
<td>69.99</td>
<td>61.77</td>
<td>62.77</td>
<td>60.77</td>
</tr>
</tbody>
</table>

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 we 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 \[ \chi = \sin^{-1} \sqrt{\frac{x}{100}} \]; where \( \chi \) 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*.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet Type</td>
<td>Larval recovery (%)</td>
<td>Larval weight (mg)</td>
</tr>
<tr>
<td>AOII (modified AOI)</td>
<td>4.82 ± 0.32 a</td>
<td>16.31 ± 1.16 a</td>
</tr>
<tr>
<td>AOI (Arriaga-López et al. 2004)</td>
<td>4.77 ± 0.67 a</td>
<td>15.79 ± 0.63 a</td>
</tr>
<tr>
<td>AOIII (modified AOI)</td>
<td>4.73 ± 0.86 ab</td>
<td>15.35 ± 0.85 a</td>
</tr>
<tr>
<td>CCA (Schwarz et al. 1985)</td>
<td>3.21 ± 0.70 ab</td>
<td>16.21 ± 0.50 a</td>
</tr>
<tr>
<td>ALU (Stevens 1991)</td>
<td>2.51 ± 0.35 ab</td>
<td>16.41 ± 0.63 a</td>
</tr>
<tr>
<td>ASE (Pinson et al. 1993)</td>
<td>2.12 ± 0.56 b</td>
<td>15.56 ± 0.62 a</td>
</tr>
</tbody>
</table>

*Means within a column followed by the same letter do not differ significantly by Tukey HSD (\( P < 0.05 \)) test.
no significant differences among the Nutrifly™ diet and torula yeast-casein diet (Table 3).

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

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

### Adaptation on Artificial Diet

There is no significant fit to linear regression based on generational data for larval recovery (larval recovery = -0.11 generation + 37.35, $r^2 = 0.03$) ($F = 0.16; \text{df} = 1, 70; P = 0.689$) (Fig. 1). Larval recovery ranged from 34 to 40% over 6 generations without significant differences among values ($F = 1.03; \text{df} = 5, 66; P = 0.405$). 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, $y = 0.42x^2 - 0.04 x + 18.87$, $r^2 = 0.78$ ($F = 5.89; \text{df} = 2, 69; P = 0.004$) (Fig. 2A), with mean values ranging from 18.1 to 21.4 mg through 5 generations. The highest average was observed in the parental and fifth generation, which decreased in first and second generations, with medium averages in the third and fourth generations ($F = 3.03; \text{df} = 5, 66; P = 0.016$).

In the pupal weight, described by the equation $y = 0.19x^2 + 0.14 x + 16.52$, $r^2 = 0.68$ ($F = 8.14; \text{df} = 2, 69; P < 0.001$) (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 ($F = 5.12; \text{df} = 5, 66; P < 0.001$).

In pupation at 24 h, described by the equation $y = 1.84x^2 + 3.19 x + 63.81$, $r^2 = 0.48$ ($F = 4.68; \text{df} = 2, 69; P = 0.012$) (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 ($F = 4.34; \text{df} = 5, 66; P < 0.001$).

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

### 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...
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).

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. 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.].

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

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, Tsuruta 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**

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