SEMI-ARTIFICIAL REARING OF THE LARVAE OF *ANASTREPHA OBLIQUA* (DIPTERA: TEPHRITIDAE) IN MANAUS, AMAZONAS-BRAZIL

LILIAN A. SALDANHA[1] AND NELITON M. SILVA[2]
[1]Department of Entomology, University of Nebraska-Lincoln
Lincoln, NE 68583-0816

[2]Universidade do Amazonas, Manaus, Amazonas, Brazil.

ABSTRACT

Larvae of the West Indian fruit fly, *Anastrepha obliqua* (Macquart), were successfully reared on a meridic diet of: agar, wheat flour, sucrose, nipagin, antibiotic, sodium benzoate, yeast, water and different amounts from 10 to 50 percent of powdered dehydrated araçá-boi (*Eugenia stipitata*) (Myrtaceae). For each evaluated diet, 100 larvae were reared. *Anastrepha obliqua* F_1 were successfully reared. The meridic diets with 20 to 40 percent of powdered dehydrated araçá-boi were observed to provide the optimum growth medium for larval development. Under these conditions, 38% of the larvae completed development at 26 ± 1°C, 88 ± 6% RH, and a photoperiod of 12:12 (L:D). The average life cycle was 43.3 days (eggs, 4 days, larvae, 26.3 days, pupae, 13 days). These results provide a basis for rearing *A. obliqua* in semi-artificial conditions. However more studies are needed to better understand pupal humidity requirements.

Key Words: West Indian fruit fly, larvae rearing, meridic diet, nutrition, diet selection, Insecta

RESUMEN

As moscas-das-frutas são conhecidas mundialmente como pragas pelos danos que causam a fruticultura. Para a realização de testes de métodos de controle é necessário o conhecimento da biologia da praga. Para tanto foi desenvolvido o presente trabalho de criação de larvas de *Anastrepha obliqua* em condições semi-artificiais utilizando dieta semi-artificial constituída de: dieta básica (ágar, amido, sucrose, lêvedo, benzoato de sódio, antibiótico, nipagin e água destilada) acrescida de quantidades crescente de fruto de araçá-boi (*Eugenia stipitata*) (Myrtacea) liofilizado, e polpa fresca nas percentagens que variaram de 10 a 50. As dietas após preparadas eram armazenadas em tubos de ensaio, nos quais colocava-se uma larva recém eclodida. O conjunto tubo-larva era mantido em temperatura ambiente (26ºC ± 1°C) umidade de 88 ± 6% e fotoperíodo de 12 horas, até atingir o período de pupa. Esta era então, após 24 horas, pesada e transferida para tubo de ensaio contendo areia peneirada autoclavada e levemente umidecida e observada até emergência do adulto. Foram criadas para cada dieta testada 100 larvas, num total de 1100 larvas. As dietas contendo 20, 30 e 40% de fruto liofilizado proporcionaram um desenvolvimento adequado das larvas, com tempo médio de desenvolvimento de 27.7 ± 3.68 dias; 24.7 ± 3.6 dias e 24.3 ± 4.5 dias, respectivamente. Pela primeira vez, para a espécie em estudo, obteve-se F_1 reprodutivamente viável, com tempo de desenvolvimento de 43.3 dias (ovos = 4; larvas = 26.3; pupas = 13 dias). A utilização de fruto de araçá-boi liofilizado revelou-se adequada para confecção de dieta de criação de larvas de *Anastrepha obliqua*. Entretanto maiores investigações sobre a umidade do substrato para pupa devem ser realizadas.

Throughout the world, various fruit flies are economically important pests. The females oviposit into the fruits and the larvae develop inside, feeding on pulp or seed. Many commercial fruits are affected making them inappropriate for commercialization and industrial purposes. The life cycle is completed in the soil, where the larvae pupate and the imagos emerge and start a new cycle (Morgante 1991).

According to Foote et al. (1993), the West Indian fruit fly *Anastrepha obliqua* (Macquart) occurs throughout the Greater and Lesser Antilles. *Anastrepha obliqua* is also found in more parts of world than any of the other *Anastrepha* species. It has been recorded in Central America (Belize, Costa Rica, Guatemala, Honduras, Mexico, Nicaragua, Panama), North America (USA), and South America (Argentina, Brazil, Colombia, Ecuador, Guyana, Peru, Suriname, Venezuela) (White and Elson-Harris 1992).

Thirty species of fruit flies occur in the Brazilian Amazon (Silva et al. 1996). *Anastrepha obliqua* is the most important economic fruit fly in the State of Amazon, damaging a variety of fruits (Silva 1993, Silva et al. 1996, Zucchi et al. 1996, Ronchi-Teles and Silva 1996). The amount of fruit production is increasing in this area. With the increase of host plants, there is a possibility that the number of *A. obliqua* will increase, as will their potential to damage the crops. Damage affects fruit exportation, increases the costs per acre to produce the crops and overall decreases fruit yield.

Zucoloto et al. (1979), Polloni (1981) and Jorge (1987) cultured *A. obliqua* in the laboratory on an artificial diet. Continuous rearing of *A. obliqua* has come up against two major obstacles. The first obstacle concerns the poor nutritional balance of the larval diet. The second obstacle is the unusual behavior of the emerged adults and their inability to reproduce.

This study was conducted to evaluate a variety of meridic diets for rearing larvae of *A. obliqua*. Meridic diet can be defined as a diet composed of defined chemicals but can contain crude components (Reinecke 1985). If found, the optimum medium can be used to study *A. obliqua* biology, including the basic life cycle and rearing requirements. This knowledge is important in developing management strategies for this pest.

Materials and Methods

Meridic diets were evaluated. Diets were prepared following standard laboratory procedures, however the diet was not autoclaved. Microbial development was inhibited by the addition of anti-fungal (nipagin) and antibiotic ingredients. The amounts of antibiotic and nipagin were determined by trial and error. The trials were based on the nutritional requirement ranges for insects determined by Parra (1979). The amounts are important to provide the correct balance of the microbial flora in the medium.

The basic composition of the diet was similar to the diet developed by Zucoloto et al. (1979) and evaluated by Jorge (1987). Adjustments were made to their original protocol in an attempt to reduce microbial contamination and to increase the nutritional value of the diet. The diets used in this experiment were prepared using the basic ingredients (Table 1) as well as additional ingredients, which varied with each trial. The varied ingredients were powdered dehydrated araçá-boi, varying in concentration from 10 to 50 percent, and araçá-boi pulp. Araçá-boi fruit was used, because it is the primary host of *A. obliqua*. The use of dehydrated fruit was proposed to improve larval development by reducing contamination caused by bacterial and fungal development, presented when fresh pulp was used, and also prevent first instar larvae drowning. Thus, eleven different diets were evaluated for rearing maggots (Table 2).

TABLE 1. INGREDIENTS FOR ARTIFICIAL DIET FOR *ANASTREPHA OBLIQUA*.

Basic diet	
Ingredients	Quantity
Agar	4.0 g
Yeast	9.0 g
Wheat flour	8.0 g
Sucrose	12.0 g
Sodium benzoate	0.05 g
Antibiotic	0.05 g
Nipagin	2.0 ml
Water	220 ml

The chemical ingredients were obtained from a local store. The powdered dehydrated araçá-boi was prepared at INPA-Food production laboratory by freezing a mixture of the fruit at each stage of maturity. The concentration of the nutrients in the fruit was determined following standard procedures of food analysis. Araçá-boi, dry matter has 8-10.75% protein, 5-6.5% fiber, 69.98-71.63% carbohydrate and contains the following micronutrients: phosphate, potassium, calcium, and magnesium. Furthermore, one hundred grams of fresh pulp contain 7.75mg of Vitamin A, 9.85mg of Vitamin B_1, and 7.68mg of Vitamin C (Pinedo et al. 1981, Cavalcante 1991).

The medium was prepared by mixing water, sucrose, wheat flour, and agar in a beaker. The mixture was heated for 5 minutes. The solution was placed in a vertical hood to cool for 3 minutes. The remaining ingredients were added, stirring constantly with a glass rod. The pH was checking using pH paper and adjusted to pH 3 using hy-

TABLE 2. PERFORMANCE OF *ANASTREPHA OBLIQUA* LARVAE IN DIFFERENT DIETS.

Diet number	Description	Performance[1]
1	Basic diet (Table 1)	Poor
2	Basic diet + 10% dehydrated fruit	Poor
3	Basic diet + 20% dehydrated fruit	Moderate
4	Basic diet + 30% dehydrated fruit	Moderate
5	Basic diet + 40% dehydrated fruit	Moderate
6	Basic diet + 50% dehydrated fruit	Low
7	Basic diet + 10% araçá-boi pulp	Poor
8	Basic diet + 20% araçá-boi pulp	Poor
9	Basic diet + 30% araçá-boi pulp	Poor
10	Basic diet + 40% araçá-boi pulp	Poor
11	Basic diet + 50% araçá-boi pulp	Poor

[1]The diet performance was evaluated based on the larval development.

drochloric acid. The mixture was poured into glass tubes (9.8 × 1.2 cm), stopped with a cotton ball and placed at a 30° angle to prevent the maggots from drowning. The tubes were stored at 4°C until use.

The larvae used in this experiment were the offspring of adults reared in laboratory conditions of 26 ± 1°C, 88 ± 6% RH and a photoperiod of 12:12(L:D). All of these adults were fed with two different solutions consisting of: 1) sugar cane hydrolysate (50ml) mixed with brown sugar (100g); 2) honey (10%). Two cotton balls on Petri dishes were placed in each cage. Each cotton ball was saturated with one of the above mentioned solutions.

Initially, artificial gel-forming compounds were placed in the cages to stimulate the females to lay eggs. However, these females did not use the structures provided, instead they laid eggs throughout the cage. The cages consisted of a wire skeleton covered with nylon fabric. Using a camel hair paint brush, the eggs were collected every morning and afternoon. The eggs were placed into Petri plates with 1% sodium benzoate solution until the larvae hatched. The larvae were washed in a new 1% sodium benzoate solution to reduce the microbial contamination on their bodies. One larva was placed in a glass tube with meridic diet. The tubes were closed with cotton and placed at 26 ± 1°C, 88 ± 6% RH and a photoperiod of 12:12 (L:D). The tubes were observed daily thereafter and pupation was recorded. Pupae were removed after 24 hours, their weights were recorded, and they were placed on moistened sand in glass tubes covered with cotton to prevent desiccation. They were incubated at 26 ± 1°C, 88 ± 6%RH and a photoperiod of 12:12(L:D). Adults that emerged were confined in cages, fed as described previously, and observed daily. Their reproductive capability was evaluated.

Results and Discussion

Only the diets with powdered dehydrated araçá-boi allowed the development of larvae to the adult stage (Table 2: Diets 3, 4, 5, and 6). The percentages of larvae that completed development and the developmental periods of West Indian fruit fly maggots with various amounts of powdered dehydrated araçá-boi are shown in Table 3.

The results of this experiment demonstrated that the amount of powdered dehydrated araçá-boi improved the rearing conditions and the reproductive capability of the emerged adults, because it increased the nutritional value of the diet. The use of powdered dehydrated araçá-boi is equally important because it can be used, not only during the fruit season, but also throughout the year. Additionally, the use of dehydrated fruit reduced microbial development and death of first instar larvae, when compared to the use of fresh pulp.

The percentage of pupation and the rate of growth in this experiment were consistent with previous results observed by Jorge (1987). However, emerged adults in this experiment showed reproductive capability when compared to the results given by Jorge (1987). Another result that differs from Jorge (1987) is the number of emerged adults. In this experiment more adults emerged than in Jorge's experiment. Using a protocol similar to the one developed by Zucoloto et al. (1979) we could not reproduce the 100% pupation that they observed. We could not compare emergence results because the authors did not report these results. Meanwhile, Moreno et al. (1997) found that diets containing casein is the best for pupal development. The authors measured the flight quality of the flies, while in this work reproductive ability was measured.

The different concentrations of powdered dehydrated araçá-boi in the diets influenced the numbers of larvae attaining maturity (pupation) and their rate of development. The percentages of larvae completing development varied from 0 to 38.

TABLE 3. COMPARISON OF MERIDIC DIET WITH DEHYDRATED ARAÇÁ-BOI AT 26°C, 88% RH AND A PHOTOPERIOD OF 12:12 (L:D) FOR REARING *ANASTREPHA OBLIQUA*.

Diet number	pH	N° Larvae	N° Pupae	N° Adults	Duration (Days) Larvae	Pupae	% Pupation	% Emergence
1	3	100	0	–	–	–	–	–
2	3	100	0	–	–	–	–	–
3	3	100	38	12	27.7	12.1	38	31.5
4	3	100	28	11	24.7	14.2	28	39.3
5	3	100	30	12	24.3	13.6	30	40.
6	3	100	24	4	25	12.8	24	16.7

Optimum development was obtained using powdered dehydrated araçá-boi at concentrations ranging from 20 to 40 percent (Table 3). Concentrations higher or lower than the optimal range yielded substantially fewer pupae or adults. The rate of growth (time to pupation) in this experiment (13.1 days, at 26 ± 1°C, 88 ± 6% RH, and a photoperiod of 12:12(L:D) was the same as observed by Jorge (1987) at 26.5°C (13.6 days). However, these results differ from those observed by Polloni (1981), who reported 18.2 days at 22.5°C, which was probably a result of lower temperature.

Instead of Petri dishes, we used glass tubes to hold the media for larval development, because we noted that larvae were drowning in the Petri dishes.

West Indian fruit fly larvae can be reared in semi-artificial conditions on meridic diets with powdered dehydrated araçá-boi from 20 to 40 percent. However, the methodology described here is only a beginning and further refinements are necessary. In particular, optimum conditions for pupae need additional investigation followed by further evaluation of more generations.

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