Performance of *Anastrepha obliqua* (Diptera, Tephritidae) larvae fed on artificial diets

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ABSTRACT. Fruit flies of the genus *Anastrepha* Schiner, 1868 are well-known for having economical importance since they damage commercially cultivated fruits. Nutritional demands during the immature and adult stages are different, so the larvae do not develop well using the same diet as the adults. Although the insect basic nutritional needs are well-known, there is also the challenge to elaborate rearing diets adequate to species with specific needs. The aim of this study was to determine the effect on the *Anastrepha obliqua* (Macquart, 1835) larvae performance of different kinds and amounts of carbohydrates in the diet. Larvae were individually reared until pupation in test tubes containing one of the artificial diets to be tested. The basic composition of the diets to be tested included 2.5 g agar, 3.25 g brewer's yeast and several different amounts of flour and sucrose. The suitability of the artificial diet for *A. obliqua* was tested evaluating the larvae and pupae survival (%) and the larvae, pupae and larvae-adults periods of development. The diet containing flour (2 g) and sucrose (2 g) and the diet containing only sucrose (5.5 g) have shown the best results regarding larval performance. All tested diets presented similar or superior results as compared to diets used in other studies. The importance of flour and its nutritional value for the larvae was discussed.

KEYWORDS. Fruit-flies, larvae, nutrition, artificial diets.

RESUMO. Desempenho das larvas de *Anastrepha obliqua* (Diptera, Tephritidae) alimentadas com dietas artificiais. Moscas das frutas do gênero *Anastrepha* Schiner, 1868 são conhecidas por sua importância econômica devido aos danos que elas causam nos frutos comerciais. As exigências nutricionais dos estágios imaturo e adulto são diferentes e as larvas não se desenvolvem bem utilizando a mesma dieta do adulto. Embora as necessidades nutricionais básicas dos insetos sejam bem conhecidas, existe ainda o problema de elaborar dietas de criação adequadas para espécies com necessidades específicas. O objetivo deste trabalho foi verificar o efeito de diferentes tipos e quantidades de carboidratos na dieta sobre a *performance* larval de *Anastrepha obliqua* (Macquart, 1835). Larvas foram criadas individualmente em tubos de ensaio contendo uma das dietas artificiais a serem testadas onde elas foram mantidas até a pupação. A composição básica das dietas testadas incluia 2,5 g de agar, 3,25 g de levedo de cerveja e quantidades variadas de sacarose e farinha de trigo. A adequação do meio artificial para *A. obliqua* foi testada pela avaliação da sobrevivência larval e pupal (%) e o tempo de desenvolvimento larval, pupal e de larva-adulto. A dieta contendo farinha de trigo (2 g) e sacarose (2 g) e a dieta somente com sacarose (5,5 g) foram as que apresentaram melhor *performance* larval. Todas as dietas testadas apresentaram resultados similares ou superiores às dietas utilizadas em outros trabalhos. A importância da presença da farinha de trigo e seu valor nutricional para as larvas são discutidos.

PALAVRAS-CHAVE. Moscas-das-frutas, larva, nutrição, dieta artificial.

Fruit flies of the genus *Anastrepha* Schiner, 1868 are well-known for having economical importance since they damage commercially cultivated fruits. In addition, they are biologically interesting organisms found in several habitats, showing quite diverse behaviors, and capable to explore different alimentary resources (Christenson & Foote, 1960).

The adults feed on a variety of items which include honeydew, juices and fruit tissues, nectar and bird feces (Christenson & Foote, 1960). During the larval stage, the diet is basically the host fruit, although they may feed on their own exoskeletons (Zucoloto, 1993), on other small animals and their exoskeletons (larvae, worms and other invertebrates) and on smaller co-specific larvae in the host fruit.

One of the main difficulties when studying nutrition and the feeding behavior of the fruit fly immatures is that the larvae mingle with the food, consequently some determinations as ingestion are difficult to carry out (Slansky & Scriber, 1985). In spite of their relative immobility, in the laboratory setting the fruit flies larvae are able to select the most appropriate food or part of it in order to develop (Zucoloto, 1988, 1993; Fernandes-da-Silva & Zucoloto, 1993; Canato & Zucoloto, 1993; Dukas *et al.*, 2001).

Regarding nutrition, adult and immature insects require carbohydrates, proteins, lipids, minerals and vitamins in order to develop (Dadd, 1985). The amount and quality of the ingested nutrients may affect the survival, the immature weight, the development period, the body chemical composition, the adult size and, in some cases, the production of egg's (Slansky & Scriber, 1985).

When fed on artificial diets containing exclusively proteins, the larvae in general show low performance as compared to those fed on diets containing proteins and carbohydrates. *Ceratitis capitata* (Wiedemann, 1824) (Canato & Zucoloto, 1998) and *Anastrepha obliqua* (Macquart, 1835) (Message & Zucoloto, 1980) do not survive on diets containing carbohydrates as the only

nutritional source. On the other hand, when fed on diets containing proteins, they may complete their development showing, however, low performance.

Carbohydrates and lipids in the tephritids diet constitute their main energy source (DADD, 1985). STOFFOLANO (1995) reasons that the great success of the insects is due to the great availability of carbohydrates, abundant in nature, as well as to the behaviors and mouth structures adapted to explore these resources.

A number of studies have investigated the quality and adequate carbohydrate concentration in the adult tephritids diet. The presence of carbohydrates in the adult diet is important to guarantee the *A. obliqua* adults' survival (FONTELLAS & ZUCOLOTO, 1999). Laboratory experiments with female adults of this species have shown that the amount of sucrose, in association with brewer's yeast, has influenced diet ingestion, longevity, egg production and diet selection (FONTELLAS-BRANDALHA & ZUCOLOTO, 2003). ZUCOLOTO (1992) has observed that the concentration and type of carbohydrate in the diet influenced the egg's production in *C. capitata*. LANDOLT & SIVINSKI (1992) have shown that sugar deprivation reduces the attraction potential of the *Anastrepha suspensa* (Loew, 1862) males.

In general, the studies with larvae aim to find adequate artificial diets to rear these organisms in the laboratory. ZUCOLOTO *et al*. (1979) investigated the quality of different protein sources for *C. capitata* larvae and found that 4.5 g brewer's yeast /150 ml diet promote better larval performance as compared to other sources (wheat germ, oat, powder milk and soy). The brewer's yeast possibly provides most of the macro and micronutrients essential for the development of these insects (MORENO *et al*., 1997).

The nutritional requirements of the *Anastrepha* sp. immatures are little known (ALUJA *et al*., 2000). MESSAGE & ZUCOLOTO (1980) verified that the adults' emergence did not occur when the larvae were fed with artificial diets lacking a protein source. These authors and ZUCOLOTO *et al*. (1979) have tested different diets for larvae not changing the wheat flour concentration.

In this context, the objective of this study was to determine the effect of diets with different types and amounts of carbohydrates on the *A. obliqua* larvae performance.

MATERIAL AND METHODS

In this study, newly hatched larvae were obtained mating wild adults in the laboratory. The adults were collected in *Spondias venulosa* (Anacardiaceae) fruits. The experiments were carried out at the Insects Nutrition and Feeding Behavior Laboratory, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto – Universidade de São Paulo. The flies were maintained in the laboratory and fed on agar diets containing 11 g sucrose, 6.5 g brewer's yeast and 100 ml water (FONTELLAS & ZUCOLOTO, 1999; CRESONI-PEREIRA & ZUCOLOTO, 2001). During the oviposition period, Parafilm coated artificial oviposition substrates were provided (FONTELLAS-BRANDALHA & ZUCOLOTO, 2004). The eggs found in these artificial substrates were daily removed and transferred to Petri dishes containing water. The newly hatched larvae were individually placed in test tubes containing the test diet (1.5 ml) where they remained until pupation. The pupae were then transferred to Petri dishes containing sterilized sand. They were identified according to the rearing diet and pupation date and maintained in the dishes until the adults' emergence. The experiments were conducted under controlled temperature (27 ± 1ºC) and humidity (70 ± 5%), 12:12 light/dark cycle with fluorescent light (400 lux).

The basic composition of the test diets was: 2.5 g agar, 3.25 g brewer's yeast, 0.5 ml propionic acid, 1.0 ml Nipagin in 20% alcohol solution, 75 ml distilled water, sucrose and/or wheat flour. The amounts of sucrose and flour are shown in table I.

The diets were prepared mixing all the components, with the exception of Nipagin and propionic acid, until homogenization. Afterwards, this mixture was placed in an Erlenmeyer (200 ml) and autoclaved (120ºC for 20 min). Nipagin and propionic acid were then added and the diets were distributed in test tubes.

The following parameters were observed: pupation (pupae/larvae ratio) and emergence (adult/larvae ratio) percentages, larvae and pupae stages duration and larvae-adults development period.

The data were analyzed by the Kruskall-Wallis test, followed by the Dunn's *post hoc* and two-way ANOVA tests. The tests were conducted using the SigmaStat program for Windows, version 2.03.

RESULTS AND DISCUSSION

Although diet A had the lowest carbohydrates concentration when compared to other tested diets, it had high yeast content (protein source), therefore a percentage of the individuals reared on this diet could pupate and reach the adult stage (Tab. I). The protein requirement is high during the larval stage (MESSAGE & ZUCOLOTO, 1980; CANATO & ZUCOLOTO, 1998). Studies with *C. capitata* have shown that the larvae can complete their development when fed on diets lacking carbohydrates, however not lacking proteins (CANATO & ZUCOLOTO, 1998).

Diets B and C were more adequate for the development of *A. obliqua* immatures (Tab. I). A small difference in some parameters as the pupation and emergence percentages, and the larval stage duration among diets may be due to the presence of wheat flour in diet B. Wheat flour contains 10 % proteins and 1 % lipids, thus increasing the amounts of these nutrients in the diets.

Another possible explanation for the different results found between diets B and C is that the larvae used the starch contained in the wheat flour. In nature, unripe fruit contains high starch concentrations (ROCHA *et al*., 2001), therefore this carbohydrate is present in the larval diet since hatching. Consequently, starch in diet B may have stimulated its ingestion.

Wheat flour may have facilitated feeding because it turned the diet into a more porous one. In the diets lacking flour (A and C) the larvae were observed scratching the substrate and trying with difficulty to

penetrate in the diet. Zucoloto *et al*. (1979) outlined the importance of the diet texture regarding the success of the larvae.

The larvae may present compensatory responses regarding the variations in availability and suitability of the food. These responses present themselves by pre-ingestion alterations (changes in behavior as regulation of ingestion) or post-ingestion alterations (physiological changes as alteration of efficiency in utilization, or development of physiological mechanisms to eliminate substances in excess in the organism) (Simpson & Simpson, 1990).

When compared to diets C and D, diet B presents the lowest total amount of carbohydrates (2.0 g sucrose and 2.0 g wheat flour). This lower amount of carbohydrates in diet B may have caused the extension in the larval stage duration in relation to diet C (Tab. I). This extension could have triggered higher ingestion of diet B by the larvae, so as to compensate the diet carbohydrates deficit, and may have resulted in a greater lipidic reserve, since in many insects the lipids are synthesized from proteins and carbohydrates (Zucoloto, 2000). The lipidic reserve was possibly used as a nutritional resource during the pupal stage, leading to a reduced pupation period. According to Selivon & Perondini (2000), during the larval development the insect accumulates and stores a great amount of reserve material, particularly lipids that will be used in metamorphosis and at the beginning of adult life. In diet D, containing the highest concentration of carbohydrates (5.5 g sucrose and 2.0 g flour), there was reduction in the larval and pupal viability (Tab. I).

In *A. obliqua* studies, it was shown that the carbohydrate/protein ratio is important in the adults' performance, because they can select diets in order to ingest nutrient ratios more adequate to their development (Cresoni-Pereira & Zucoloto, 2001). In this study the carbohydrate/protein ratio appears to have influenced the adequate development of the larvae. When the amount of carbohydrates was considerably higher than the yeast (diet D), performance was negatively affected, resulting in the lowest percentage of adults' emergence (Tab. I). The interaction of wheat flour with the sucrose concentration in the diet also had effects in all the periods of development (Tab. II).

Saldanha & Silva (1999) tested semi-artificial diets in *A. obliqua* larvae. Such diets contained the same nutrients used in this study, but in different proportions, and contained the pulp of the fruits. Notwithstanding, most of the larvae did not reach the pupa stage. The highest emergence percentage obtained in the Saldanha & Silva (1999) study was 40%. It is not clear if this percentage refers to the initial number of larvae or to the number of pupae. It is difficult to discuss in nutritional terms the difference in the emergence percentage between this study (48.8%) and the Saldanha & Silva's (1999) experiments due to lack of information about the nutritional composition of the fruit pulps used by these authors.

The percentage of emergence found in this study was similar to that found by other authors that investigated the nutritional aspects of diets offered to *A. obliqua* larvae (Zucoloto *et al*., 1979; Message & Zucoloto, 1980; Moreno *et al*., 1997; Saldanha & Silva, 1999), in spite of the different proportions and/or combinations of nutrients which were used.

The presence of wheat flour in the artificial diet appears to be important for the development of *A. obliqua* larvae. Although the role of this component regarding the immatures has not been clearly defined (increased amount of available lipids, proteins and carbohydrates, presence of starch, adequate texture), its utilization in the artificial diets composition provided superior results to those obtained in previous studies. In this context, additional studies are necessary to investigate more adequate combinations and/or proportions of wheat flour, sucrose and yeast in the development of these larvae in the laboratory.

Table I. Performance of *Anastrepha obliqua* (Macquart, 1835) immatures fed on artificial diets containing brewer's yeast (3.25 g) and different quantities of sucrose in the presence or not of wheat flour (* values represent the mean±SE; values followed by different letters in the same column are significantly different (Dunn's test; $p<0.05$)).

	Diet	Components	Initial larvae number	% Pupation	% Emergence	Larval stage period (days)*	Pupation period (days)*	Larva-adult development period(days)*
	Sucrose	Wheat flour						
A	2 g	0 g	49	61	40.8	24.0±0.57[a]	17.6±0.44[a]	41.6±1.03[a]
B	2 g	2 g	43	65	48.8	24.4±0.74[a]	15.2±0.72[b]	38.8±0.93[a,b]
C	5.5 g	0 g	48	63	43.8	21.5±0.59[b]	15.90.19[b]	36.7±0.70[b]
D	5.5 g	2 g	44	50	31.8	25.5±0.62[a]	16.2±0.36[a,b]	41.3±0.84[a]

Table II. Calculated F and P values for larval stage, pupation and larva-adult development periods of *Anastrepha obliqua* (Macquart, 1835) larvae fed on artificial diets (*statisticaly significance (Two-way ANOVA; $p<0.05$)).

Dependent variable / Factor	Larval stage period	Pupation period	Larva-adult development period
Presence of wheat flour	F=11.793P<0.001*	F=4.607P=0.035*	F=1.070P=0.304
Sucrose concentration	F=0.979P=0.325	F=0.485P=0.489	F=1.821P=0.181
Factors interaction	F=8.006P=0.006*	F=7.540P=0.008*	F=16.666P<0.001*

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