



Rearing two fruit flies pests on artificial diet with variable pH

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Abstract

Fruit flies (Diptera: Tephritidae) are considered the main fruit pests worldwide. In Brazil, two species are predominant: the South American fruit fly, *Anastrepha fraterculus* and the Mediterranean fruit fly, *Ceratitis capitata*. In this study, we evaluated the effect of artificial diets with variable pH in their larval development and adult performance. The experiments were carried out in the laboratory at 25 ± 2 °C, $70 \pm 10\%$ RH and 12:12h (L:D) photoperiod. Semisolid diets with pH values of 6.0, 5.0, 4.0, 3.0, 2.0, 1.5, and 1.0, adjusted by adding hydrochloric acid were tested. Results indicated that the diet with pH 6.0 did not support larval development of both species of fruit fly. Diets with greater acidic pH values did not allow egg, larvae or pupae development and adult reproduction of *A. fraterculus*. For *C. capitata*, the pH of artificial diet exerts greater influence compared to *A. fraterculus* on the duration and viability of the larval stage, number of pupae, sex ratio and longevity of males.

Keywords: *Anastrepha fraterculus*, *Ceratitis capitata*, biological parameters, acidific agent.

Criação de duas moscas-das-frutas praga em dieta artificial com variação de pH

Resumo

As moscas-das-frutas (Diptera: Tephritidae) são consideradas as principais pragas da fruticultura mundial. No Brasil, duas espécies são predominantes: a mosca-das-frutas Sul-americana, *Anastrepha fraterculus* e a mosca-do-Mediterrâneo, *Ceratitis capitata*. Neste estudo avaliamos o efeito de dietas artificiais com pH variável no seu desenvolvimento larval e performance de adultos. Os experimentos foram realizados em laboratório a 25 ± 2 °C, $70 \pm 10\%$ UR e fotoperíodo de 12:12 horas. Foram testadas dietas semi-sólidas com valores de pH de 6,0; 5,0; 4,0; 3,0; 2,0; 1,5 e 1,0, ajustados pela adição de ácido clorídrico. Os resultados indicaram que a dieta com pH 6,0 não suportou o desenvolvimento larval de ambas as espécies de mosca-das-frutas. As dietas com pH ácido não permitiram o desenvolvimento de ovos, larvas ou pupas e a reprodução de adultos de *A. fraterculus*. Para *C. capitata* o pH da dieta artificial exerceu maior influência do que para *A. fraterculus* nos parâmetros de duração e viabilidade do estágio larval, número de pupas, razão sexual e longevidade de machos.

Palavras-chave: *Anastrepha fraterculus*, *Ceratitis capitata*, parâmetros biológicos, agente acidificante.

1. Introduction

Fruit flies (Diptera: Tephritidae) are considered the main fruit pests worldwide (Ruiz et al., 2014). In Brazil, two species are predominant: the South American fruit fly, *Anastrepha fraterculus* (Wiedemann, 1830), infesting 114 species and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824), with 93 reported hosts (Zucchi, 2008, 2012).

Host selection by Tephritidae is determined for several factors including the chemical properties of the fruit (Papachristos and Papadopoulos, 2009). The concentration of hydrogen ions (H^+), or pH, is vital to ensure biological process that affect physiology, survival and symbiosis

(DiMario and Mahowald, 1986; Koval and Suppes, 1990). In general, insects regulate pH to support acidic environments and intestinal pH changes, caused by the type of food infested (Harrison, 2001).

For fruit flies, the host fruit pH is considered a limiting factor, as reported in guava fruits (*Psidium guajava* L.) for *A. fraterculus* (Oliveira et al., 2014) and citrus (*Citrus* spp.) for *C. capitata* (Papachristos et al., 2008). However, despite its importance, few studies have discussed the influence of pH in artificial diet on the biology of Tephritidae (Vargas et al., 1984; Chan Junior and Jang, 1995; Hu et al., 1999; Vera et al., 2014).

Artificial diets for rearing and maintaining insects in the laboratory have applications in basic and applied research (Parra, 2009). The knowledge of the target species biology is crucial for the success of mass rearing (Cladera et al., 2014). Any change in the diet quality during immature development can have a significant effect on their biological characteristics (Chapman, 2013; Nestel et al., 2016).

The pH influences the palatability and stability of the diet, the activity of preservatives, the solubility of nutrients and probably many other not yet determined factors (Cohen, 2004). In addition, the pH currently used for rearing from fruit flies (4.5), may result in microbial growth and early deterioration of the diet (Vera et al., 2014). Therefore, we evaluated artificial diets with different pH values for egg, larvae and pupae development of *A. fraterculus* and *C. capitata*.

2. Material and Methods

The study was conducted at the Entomology Laboratory of Brazilian Agricultural Research Corporation (EMBRAPA) (Pelotas, Rio Grande do Sul, Brazil), in a rooms kept at 25 ± 2 °C, $70 \pm 10\%$ RH and 12:12h (L:D) photoperiod. The adults of *A. fraterculus* and *C. capitata* used in the experiment were obtained at the Food Irradiation and Radioentomology Laboratory of CENA/USP (Piracicaba, São Paulo, Brazil).

2.1. Fruit flies rearing

Adults of *A. fraterculus* were kept in plastic cages ($57 \times 39 \times 37$ cm). Water and solid diet, composed of refined sugar, wheat germ, and yeast at a ratio of 3:1:1 were offered (Nunes et al., 2013). Eggs were collected from screens placed on the cage sides and were transferred to Erlenmeyer-type glass containers (500 mL), where they remained for a 24 h aeration process. For the rearing of *C. capitata*, adults were kept in plastic cages ($48 \times 30 \times 30$ cm) containing water and same solid diet as described. The methodologies used for collecting eggs and for the aeration and inoculation processes were based on Gonçalves et al. (2013).

2.2. Diets preparation

The semisolid diets were prepared according to the methodology described by Salles (1992) and Nunes et al. (2013). For the diets preparation, the following components were homogenised in blender: refined sugar, lyophilized brewer's yeast Brewcell (Biorigin, Lençóis Paulista, SP), cread wheat germ (Walmon, São Paulo, SP) and distilled water. The components, methylparahydroxybenzoate Nipagin™ (Vetec, Química Fina Ltda., Duque de Caxias, RJ) (diluted to 10% in ethyl alcohol), sodium benzoate (Vetec) (dissolved in 20 mL of distilled water) and hydrochloric acid (Synth, Diadema, SP) were later added to the other in blender. Finally, the bacteriological agar (Alphatec, Barueri, SP) was dissolved in 200 mL of distilled water and brought to the fire, having been stirred constantly for a boil. The agar was then placed in blender along with other components and agitated for 2 min until homogenized. Before solidification, were distributed 100 mL of diet in plastic containers (400 mL) with perforated lids.

Using a pH meter (Phtek, model PHS 3B with Ruosull E-900 electrode) each diet was adjusted to the desired pH value by adding hydrochloric acid (HCl, concentrated at 37%) (Synth®) in preliminary tests. The experiment was conducted in a completely randomized design with 7 treatments (diet pH) and 10 replications (diet containers). The treatments contained diets with pH 6.0 (without addition of HCl), 5.0 (adding 3 mL of HCl), 4.0 (adding 6 mL of HCl), 3.0 (addition of 9 mL of HCl), 2.0 (adding 12 mL of HCl), 1.5 (adding 15 mL of HCl) and 1.0 (addition of 18 mL of HCl) (Table 1).

2.3. Biological parameters

After 24h of the diet preparation, 0.1 mL of eggs of *A. fraterculus* (~1.170 eggs) and *C. capitata* (~2.485 eggs), were inoculated separately, on filter paper, for larval development. The containers were packed in an air-conditioned room and near pupation, the 3rd instar larvae were separated from the diet by rinsing under running water, using a sieve. Larvae were transferred to containers with moistened thin vermiculite and after 10 d, the containers were inspected daily to separate the pupae, which were weighted with 24h using a precision analytical scale (Shimadzu of Brazil, AUY 220 model) and packed in new containers until emergence.

Table 1. Components used to prepare the artificial diet for the larval development of *Anastrepha fraterculus* and *Ceratitits capitata*.

Components ^a	Diet pH						
	6.0	5.0	4.0	3.0	2.0	1.5	1.0
Refined sugar (g)	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Lyophilized brewer's yeast (g)	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Crude wheat germ (g)	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Water (distilled) (mL)	800.0	800.0	800.0	800.0	800.0	800.0	800.0
Methylparahydroxybenzoate (mL)	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Sodium benzoate (g)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Hydrochloric acid (mL)	0.0	3.0	6.0	9.0	12.0	15.0	18.0
Bacteriological agar (g)	3.6	3.6	3.6	3.6	3.6	3.6	3.6

^aComponents for 1 L of artificial diet (Salles, 1992; Nunes et al., 2013).

We evaluated the duration and viability of larval and pupal stages, duration of egg stage and egg-adult period, number and weight of pupae and sex ratio. For each fruit fly species, 25 couples of each treatment were separated and packed in transparent plastic cups (500 mL, with 6-mm holes at the top), containing solid diet (sugar, wheat germ and yeast, at ratio 3:1:1) and hydrophilic cotton soaked in distilled water, available in acrylic containers (10 mL). To determine fertility, artificial substrates were added to containers for oviposition, comprised of bacteriological agar (14.0 g), distilled water (350 mL), blackberry juice (100 mL) and methylparahydroxybenzoate Nipagin™ (4 mL), as described by Salles (1992). Daily observations were carried out, registering the number of eggs and mortality. Pre-oviposition and oviposition periods and longevity of males and females were calculated.

To evaluate fertility, 30 eggs of the second oviposition day of each female were removed from the artificial substrate using a surgical blade and a brush. Eggs were placed in Petri dishes on moist paper, with sponge cloth. The dishes were then wrapped with PVC film and kept in the growth chamber (25 ± 2 °C) until eggs were hatched and the number of larvae recorded.

2.4. Data analysis

Data were tested for normality by the Lilliefors test (Campos, 1983). In the event of normality, the data were submitted to the analysis of variance (ANOVA) and means compared using the Tukey test. When normality was not observed, the data were submitted to the Kruskal-Wallis analysis and the means compared using the Dunn test (Hollander and Wolf, 1973). For these analyses, the statistical software Bioestat 5.3 (Ayres et al., 2007) was

used considering the probability of 5%. Longevities of males and females were compared using the Log-rank test in the statistical software R and probability of 5% (Ihaka and Gentleman, 1996).

3. Results

Diets with pH 6.0, 1.5 and 1.0. did not allow larval hatching for both fruit fly species. As for the duration of the egg stage of *A. fraterculus*, a significant difference was observed among treatments (Table 2). For the duration of the larval and pupal stages of *A. fraterculus*, no significant difference was observed (Table 2). However, smaller viability of the larval stage was observed when larvae were kept on diet with pH 2.0, as well as lower viability of pupae, but differing from the diet with pH 3.0. A greater weight of the pupae of *A. fraterculus* was obtained on diet with pH 4.0, as well as the largest number of pupae; however, this last parameter showed no difference composed to diet with pH 5.0 (Table 2).

The duration of the egg stage of *C. capitata* was similar to that of *A. fraterculus*, with longer period on diet with pH 2.0, not differing from the diets with pH 3.0 and 4.0 (Table 3). For the larval stage, the diets with pH 2.0 and 3.0 provided an increased development of *C. capitata*. A longer duration of the pupal stage of *C. capitata* was observed on diet with pH 4.0, not differing from the diets with pH 5.0 and 2.0 (Table 3), as well as the longest egg-adult period, larger number of pupae and increased viability of larvae. The viability of pupae remained above 90% on diets with pH 5.0, 4.0 and 3.0.

A longer period of oviposition, fecundity and fertility for *A. fraterculus* was registered in females kept on a diet

Table 2. Biological parameters of immature stages of *Anastrepha fraterculus* kept on artificial diet with different pH values.

Biological parameter	Diet pH				CV ^a (%)
	5.0 [10]	4.0 [10]	3.0 [10]	2.0 [10]	
Egg stage duration (days) ^b	4.3 ± 0.83b (3.0-6.0)	5.1 ± 0.66b (4.0-6.0)	4.6 ± 0.83b (4.0-7.0)	6.6 ± 1.01a (5.0-9.0)	16.2
Larval stage duration (days)	7.9 ± 1.28ns (6.0-10.0)	8.1 ± 1.52ns (7.0-11.0)	8.6 ± 2.59ns (6.0-11.0)	6.5 ± 2.01ns (4.0-10.0)	24.0
Pupal stage duration (days)	9.7 ± 0.94ns (8.0-11.0)	10.3 ± 2.05ns (7.0-13.0)	9.9 ± 1.72ns (8.0-13.0)	10.8 ± 1.31ns (9.0-13.0)	14.8
Egg-adult period (days) ^b	21.3 ± 0.82b (20.0-23.0)	23.5 ± 1.77a (22.0-26.0)	22.9 ± 1.28ab (22.0-26.0)	24.0 ± 1.24a (23.0-26.0)	5.5
Pupae weight (mg) ^c	12.7 ± 0.49bc (12.10-13.90)	15.7 ± 1.02a (14.40-17.70)	13.5 ± 0.56b (12.30-14.20)	11.9 ± 0.71c (11.00-12.80)	5.1
Pupae number ^b	19.6 ± 3.33ab (14.0-24.0)	34.8 ± 3.79a (30.0-39.0)	17.8 ± 2.04bc (15.0-21.0)	8.2 ± 1.93c (5.0-11.0)	15.7
Larval viability (%) ^b	75.8 ± 5.23ab (68.00-84.00)	83.0 ± 4.50a (78.26-91.18)	81.9 ± 5.30a (74.07-88.24)	65.8 ± 3.66b (60.00-72.73)	6.0
Pupal viability (%) ^c	82.2 ± 6.53a (70.83-90.48)	86.9 ± 6.46a (78.38-97.37)	82.9 ± 13.51ab (61.11-100.0)	65.8 ± 20.10b (33.33-88.89)	15.6

^aCoefficient of variation. Means (±SE) followed by the same letter in the row do not differ using the ^bDunn and ^cTukey tests ($P < 0.05$), ns: not significant. Values in brackets indicate the number of repetitions and values in parentheses indicate the variation range.

with pH 4.0 (Table 4). The sex ratio showed no significant difference between treatments. Females of *A. fraterculus* kept during the larval stage on artificial diet with pH 2.0 presented the longest pre-oviposition period. Longer pre-oviposition of *C. capitata* were recorded in diets with pH 3.0 and 5.0, being that the diet with pH 5.0 did not differ from the other treatments (Table 5). The oviposition and fecundity period showed no significant difference between treatments. Greater fertility of *C. capitata* was recorded on diets with pH 5.0 and 4.0; however, a higher sex ratio was

found on diet with pH 2.0. For *A. fraterculus* the greater fertility was observed on diet with pH 4.0.

Regarding longevity of *A. fraterculus*, there was a significant difference for females ($P = 0.0013$) and males ($P = 0.0001$) (Table 4). Adults of *A. fraterculus*, when kept on a diet with pH 4.0, showed greater longevity. For females of *C. capitata*, there were no significant differences among treatments ($P = 0.2748$) (Table 5). The males of this species showed greater longevity when kept during the immature phase on the diets with pH 3.0, 4.0 and 2.0 ($P = 0.0046$).

Table 3. Biological parameters of immature stages of *Ceratitits capitata* kept on artificial diet with different pH values.

Biological parameter	Diet pH				CV ^a (%)
	5.0 [10]	4.0 [10]	3.0 [10]	2.0 [10]	
Egg stage duration (days) ^b	4.8 ± 0.99b (4.0-7.0)	5.7 ± 0.84ab (4.0-7.0)	6.1 ± 0.70a (5.0-8.0)	6.5 ± 0.85a (5.0-8.0)	14.9
Laval stage duration (days) ^b	7.3 ± 0.48ab (7.0-8.0)	7.7 ± 0.82a (7.0-9.0)	5.4 ± 0.84c (4.0-7.0)	6.1 ± 0.73bc (5.0-8.0)	11.2
Pupal stage duration (days) ^b	8.0 ± 0.47abc (7.0-9.0)	9.1 ± 0.87a (8.0-11.0)	7.2 ± 0.91c (6.0-8.0)	8.2 ± 0.63abc (7.0-9.0)	8.9
Egg-adult period (days) ^b	20.0 ± 0.47b (19.0-21.0)	22.1 ± 0.87a (21.0-24.0)	19.2 ± 0.91b (18.0-20.0)	21.2 ± 0.63ab (20.0-22.2)	3.5
Pupae weight (mg) ^b	13.3 ± 0.48a (12.30-13.90)	12.9 ± 0.32ab (12.50-13.50)	11.3 ± 0.70b (10.30-13.0)	10.0 ± 0.41b (9.20-10.80)	4.1
Pupae number ^c	52.0 ± 7.40b (44.0-67.0)	63.8 ± 6.10a (55.0-74.0)	40.3 ± 6.66c (33.0-53.0)	37.6 ± 7.38c (26.0-51.0)	15.0
Larval viability (%) ^c	82.1 ± 8.33b (70.97-97.10)	93.8 ± 2.78a (87.30-97.37)	64.1 ± 10.02c (53.23-81.97)	62.3 ± 12.32d (40.63-78.46)	12.1
Pupal viability (%) ^b	91.5 ± 5.99a (82.00-97.73)	98.7 ± 1.77a (95.16-100.00)	91.4 ± 2.19ab (87.88-94.12)	78.8 ± 5.84b (66.67-85.71)	4.5

^aCoefficient of variation. Means (±SE) followed by the same letter in the row do not differ using the ^bDunn and ^cTukey tests ($P < 0.05$). Values in brackets indicate the number of repetitions and values in parentheses indicate the variation range.

Table 4. Biological parameters of adults of *Anastrepha fraterculus* kept during the larval stage on artificial diet with different pH values.

Biological parameter	Diet pH				CV ^a (%)
	5.0 [23]	4.0 [25]	3.0 [18]	2.0 [21]	
Pre-oviposition period (days) ^b	13.9 ± 1.08b (13.0-16.0)	12.7 ± 1.64c (12.0-20.0)	16.2 ± 6.93bc (12.0-32.0)	16.9 ± 3.72a (15.0-31.0)	21.3
Oviposition period (days) ^b	14.6 ± 10.83b (1.0-41.0)	26.8 ± 11.45a (4.0-44.0)	12.0 ± 12.60b (1.0-51.0)	14.5 ± 10.93b (2.0-34.0)	7.4
Fecundity ^b	416.7 ± 31.02b (20.0-991.0)	1302.8 ± 60.82a (134.0-2181.0)	322.8 ± 36.33b (35.0-1323.0)	313.6 ± 26.47b (53.0-820.0)	7.9
Fertility (%) ^b	61.2 ± 5.68b (43.33-68.00)	73.1 ± 2.64a (70.00-76.67)	60.9 ± 4.13b (53.33-66.67)	57.7 ± 7.02b (43.3-66.67)	7.9
Sex ratio ^b	0.4 ± 0.06ns (0.40-0.61)	0.5 ± 0.01ns (0.46-0.52)	0.4 ± 0.09ns (0.36-0.63)	0.4 ± 0.18ns (0.20-0.71)	19.1
Survival (females) (days) ^c	31.2 ± 16.01b (13.0-83.0)	52.8 ± 22.66a (17.0-96.0)	34.0 ± 17.72b (11.0-80.0)	31.4 ± 14.12b (8.0-60.0)	0.5
Survival (males) (days) ^c	42.6 ± 25.71b (16.0-119.0)	80.4 ± 31.20a (19.0-126.0)	37.2 ± 15.52b (11.0-72.0)	30.2 ± 17.30b (10.0-80.0)	0.6

^aCoefficient of variation. Means (±SE) followed by the same letter in the row do not differ using the ^bDunn and ^cLog-rank tests ($P < 0.05$), ns: not significant. Values in brackets indicate the number of repetitions (couples) used in the study on the biology of adults of *Anastrepha fraterculus* and values in parentheses indicate the variation range.

Table 5. Biological parameters of adults of *Ceratitis capitata* kept during the larval stage on artificial diet with different pH values.

Biological parameter	Diet pH				CV ^a (%)
	5.0 [18]	4.0 [25]	3.0 [25]	2.0 [19]	
Pre-oviposition period (days) ^b	12.2 ± 2.57ab (10.0-21.0)	11.1 ± 1.43b (10.0-14.0)	15.7 ± 6.16a (10.0-35.0)	13.7 ± 8.01b (9.0-38.0)	32.8
Oviposition period (days) ^b	11.9 ± 10.89ns (1.0-33.0)	17.7 ± 9.78ns (1.0-32.0)	15.2 ± 11.36ns (1.0-36.0)	13.0 ± 13.37ns (1.0-45.0)	81.0
Fecundity ^b	304.0 ± 29.23ns (9.0-1109.0)	429.0 ± 23.65ns (13.0-921.0)	311.4 ± 29.42ns (3.0-1067.0)	257.2 ± 28.22ns (10.0-910.0)	89.4
Fertility (%) ^b	73.3 ± 6.16a (63.33-86.67)	79.4 ± 4.55a (70.00-86.67)	64.5 ± 9.99b (50.0-95.0)	62.8 ± 7.04b (50.0-73.33)	10.2
Sex ratio ^b	0.5 ± 0.03ab (0.48-0.60)	0.5 ± 0.01b (0.50-0.51)	0.5 ± 0.04ab (0.50-0.65)	0.5 ± 0.07a (0.42-0.65)	7.3
Survival (females) (days) ^c	33.8 ± 24.06a (13.0-91.0)	47.1 ± 21.08a (22.0-93.0)	46.6 ± 23.49a (22.0-125.0)	44.0 ± 27.99a (8.0-101.0)	0.4
Survival (males) (days) ^c	25.0 ± 25.89b (14.0-93.0)	46.3 ± 15.98b (22.0-85.0)	43.6 ± 21.20b (19.0-112.0)	41.0 ± 31.31a (8.0-115.0)	0.5

^aCoefficient of variation. Means (±SE) followed by the same letter in the row do not differ using the ^bDunn and ^cLog-rank tests ($P < 0.05$), ns: not significant. Values in brackets indicate the number of repetitions (couples) used in the study on the biology of adults of *Ceratitis capitata* and values in parentheses indicate the variation range.

4. Discussion

On diet with pH 6.0, no larval hatching occurred for both species tested, possibly due to the rapid microbiological contamination of the diet. Vargas et al. (1984), found rapid deterioration in artificial diets with pH between 5.0 and 6.0. Vera et al. (2014) found contamination on diets for larval development with pH 4.5. On diets with pH 1.5 and 1.0, there was no contamination and no larval hatching either, which may be related to the infertility of eggs on the acid medium (Von Zuben, 1998).

For *A. fraterculus*, when the insects were kept on the diet with pH 2.0, delayed egg hatching occurred, causing longer egg-adult period. In general, immature forms of holometabolous insects complete their development in acidic medium, but in a longer period compared to those on alkaline or neutral media (Gullan and Cranston, 2012).

For both species of fruit fly, the smaller viability of the larval and pupae stage observed when larvae were kept on diet with pH 2.0, but differing from the diet with pH 3.0, is similar to results reported for *Bactrocera invadens* Drew, Tsuruta & White, 2005 (Diptera: Tephritidae) using liquid diet with pH 3.5 Ekesi et al. (2014). Vera et al. (2014) evaluated diets for *A. fraterculus* with addition of ascorbic, citric and lactic acid and found that, irrespective of the acid used, the pH of 3.5 appears to be too low for egg hatch, larval viability, egg-to-pupa recovery, and pupal weight.

In the digestion process, the larvae of Tephritidae act in association with endosymbionts, which inhabit the intestinal lumen (Prokopy et al., 1993). These microorganisms present a mutualistic relationship essential for the proper growth and development of its host, ensuring its reproductive success (Dossi and Cônsoli, 2010). For fruit flies, *Enterobacter agglomerans* and *Klebsiella pneumoniae*

are the main bacteria associated with the digestive tract (Lauzon et al., 1998; 2009). According to Engel and Moran (2013), composition and metabolic capacity of intestinal microbiota depend on the conditions of the intestinal lumen of the insect, which may present extreme pH variations, once it is actively regulated according to the food ingested.

In relation to weight of the pupae of *A. fraterculus*, Nunes et al. (2013) found similar values (15.3 mg) using the same artificial diet with pH 4.0. The greater weight and larger number of pupae may be related to a favorable condition for larval development at this pH level, as well as for the bacteria associated to its intestinal flora. For the larval stage, diets with pH 2.0 and 3.0 provided an increased development of *C. capitata*. Papachristos et al. (2008) found that the longest period of larval development of *C. capitata* was found in lemon fruit (*C. limon*) and sour orange (*C. aurantium*), with pH values of 2.3 and 2.7, respectively.

The viability of pupae remained above 90% on diets with pH 5.0, 4.0 and 3.0, corroborating Chan Junior and Jang (1995). Vargas et al. (1984) also found lower pupae weight (7.8 mg) and viability of pupae (34.4%) of *C. capitata* on artificial diet with pH below 4.0, similar to the results obtained in this study.

Artificial diet with pH 2.0 caused the longest pre-oviposition period for *A. fraterculus*. In insects, egg production involves the synthesis of specific lipo-glycoproteins of females (vitelogenins), followed by their passage to the oocytes (Gullan and Cranston, 2012). DiMario and Mahowald (1986) found that ovarian tissue (follicular cells and oocytes) of *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae) kept *in vitro* presented low capacity of endocytosis of vitellogenin in environments with pH lower than 6.6. This fact could explain the longer

pre-oviposition period of *A. fraterculus*, given that the vitellogenesis process is critical for egg formation (Gullan and Cranston, 2012).

Greater fertility of *C. capitata* was recorded on diets with pH 5.0 and 4.0; and higher female sex ratio was found on diet with pH 2.0. This suggests that the diet with very acidic pH provided the largest number of females of *C. capitata*, although with low fertility. Vera et al. (2014) found similar results for *A. fraterculus*, with reduction in egg viability with decreasing diet pH. The diet with pH 3.5 resulted in lower egg hatch, corroborating our results. Regarding longevity, males of *C. capitata* showed greater longevity when kept during the immature phase on the diets with pH 3.0, 4.0 and 2.0. As a generalist species, *C. capitata* keeps genetic variation to allow expression of adaptive plasticity in a variety of environments compared to *A. fraterculus* (Forister et al., 2012).

In conclusions, artificial diets with pH below 2.0 do not provide suitable conditions for larval hatching of *A. fraterculus* and *C. capitata*. Diets with pH below 4.0 affect the development of *A. fraterculus*, promoting lower viability of larvae and delayed duration of the egg stage, egg-adult and pre-oviposition period. Diets with pH 4.0 and 5.0 provide greater fertility, weight and number of pupae of both fruit fly species and greater fecundity, oviposition period and longevity of males and females of *A. fraterculus*.

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References

AYRES, M., AYRES JÚNIOR, M., AYRES, D.L. and SANTOS, A.A.S., 2007. *BioEstat: aplicações estatísticas nas áreas das Ciências Bio-médicas*. Belém: ONG Mamirauá.

CAMPOS, H., 1983. *Estatística experimental não-paramétrica*. 2nd ed. Piracicaba: ESALQ. 349 p.

CHAN JUNIOR, H.T. and JANG, E.B., 1995. Diet pH effects on mass rearing of Mediterranean fruit fly (Diptera: Tephritidae). *Journal of Economic Entomology*, vol. 88, no. 3, pp. 569-573. <http://dx.doi.org/10.1093/jee/88.3.569>.

CHAPMAN, R.F., 2013. *The insects: structure and function*. 5th ed. New York: Cambridge University Press. 959 p.

CLADERA, J.L., VILARDI, J.C., JURI, M., PAULIN, L.E., GIARDINI, M.C., GÓMEZ CENDRA, P.V., SEGURA, D.F. and LANZA VECCHIA, S.B., 2014. Genetics and biology of *Anastrepha fraterculus*: research supporting the use of the sterile insect technique (SIT) to control this pest in Argentina. *BMC Genetics*, vol. 15, no. 2, suppl. 2, pp. 1-14. PMID:25471175.

COHEN, A.C., 2004. *Insect diets: science and technology*. 2nd ed. Boca Raton: CRC Press. 324 p.

DIMARIO, P.J. and MAHOWALD, A.P., 1986. The effects of pH and weak bases on the in vitro endocytosis of vitellogenin by

oocytes of *Drosophila melanogaster*. *Cell and Tissue Research*, vol. 246, no. 1, pp. 103-108. <http://dx.doi.org/10.1007/BF00219005>. PMID:3096571.

DOSSI, F.C.A. and CÔNSOLI, F.L., 2010. Desenvolvimento ovariano e influência da cópula na maturação dos ovários de *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). *Neotropical Entomology*, vol. 39, no. 3, pp. 414-419. <http://dx.doi.org/10.1590/S1519-566X2010000300015>. PMID:20676515.

EKESI, S., MOHAMED, S.A. and CHANG, C.L., 2014. A liquid larval diet for rearing *Bactrocera invadens* and *Ceratitisc fasciventris* (Diptera: Tephritidae). *International Journal of Tropical Insect Science*, vol. 34, no. S1, pp. S90-S98. <http://dx.doi.org/10.1017/S1742758414000113>.

ENGEL, P. and MORAN, N.A., 2013. The gut microbiota of insects: diversity in structure and function. *FEMS Microbiology Reviews*, vol. 37, no. 5, pp. 699-735. <http://dx.doi.org/10.1111/1574-6976.12025>. PMID:23692388.

FORISTER, M.L., DYER, L.A., SINGER, M.S., STIREMAN III, J.O. and LILL, J.T., 2012. Revisiting the evolution of ecological specialization, with emphasis on insect plant interactions. *Ecology*, vol. 93, no. 5, pp. 981-991. <http://dx.doi.org/10.1890/11-0650.1>. PMID:22764485.

GONÇALVES, R.S., NAVA, D.E., PEREIRA, H.C., LISBÔA, H., GRÜTZMACHER, A.D. and VALGAS, R.A., 2013. Biology and fertility life table of *Aganaspis pelleranoi* (Hymenoptera: Figitidae) in larvae of *Anastrepha fraterculus* and *Ceratitisc capitata* (Diptera: Tephritidae). *Annals of the Entomological Society of America*, vol. 106, no. 6, pp. 791-798. <http://dx.doi.org/10.1603/AN13044>.

GULLAN, P.J. and CRANSTON, P.S., 2012. *Os insetos: um resumo de entomologia*. 4th ed. São Paulo: Roca. 496 p.

HARRISON, J.F., 2001. Insect acid-base physiology. *Annual Review of Entomology*, vol. 46, no. 1, pp. 221-250. <http://dx.doi.org/10.1146/annurev.ento.46.1.221>. PMID:11112169.

HOLLANDER, M. and WOLF, D.A., 1973. *Nonparametric statistical methods*. New York: John Wiley & Sons. 503 p.

HU, X.P., PROKOPY, R. and AVERILL, A., 1999. Effects of pH on feeding responses in the apple maggot fly, *Rhagoletis pomonella* (Diptera: Tephritidae). *Journal of Insect Physiology*, vol. 45, no. 2, pp. 151-157. [http://dx.doi.org/10.1016/S0022-1910\(98\)00109-7](http://dx.doi.org/10.1016/S0022-1910(98)00109-7). PMID:12770383.

IHAKA, R. and GENTLEMAN, R., 1996. R: a language for data analysis and graphics. *Journal of Computational and Graphical Statistics*, vol. 5, no. 3, pp. 299-314.

KOVAL, T.M. and SUPPES, D.L., 1990. pH dependency of cell attachment and growth at both clonal and subculture densities of cultured lepidopteran cells. *In Vitro Cellular & Developmental Biology*, vol. 26, no. 7, pp. 665-670. <http://dx.doi.org/10.1007/BF02624422>. PMID:2384444.

LAUZON, C.R., MCCOMBS, S.D., POTTER, S.E. and PEABODY, N.C., 2009. Establishment and vertical passage of *Enterobacter* (Pantoea) *agglomerans* and *Klebsiella pneumoniae* through all life stages of the Mediterranean fruit fly (Diptera: Tephritidae). *Annals of the Entomological Society of America*, vol. 102, no. 1, pp. 85-95. <http://dx.doi.org/10.1603/008.102.0109>.

LAUZON, C.R., SJOGREN, R.E., WRIGHT, S.E. and PROKOPY, R.J., 1998. Attraction of *Rhagoletis pomonella* (Diptera: Tephritidae) flies to odor of bacteria: apparent confinement to specialized

- members of Enterobacteriaceae. *Environmental Entomology*, vol. 27, no. 4, pp. 853-857. <http://dx.doi.org/10.1093/ee/27.4.853>.
- NESTEL, D., PAPADOPOULOS, N.T., PASCACIO-VILLAFÁN, C., RIGHINI, N., ALTÚZAR-MOLINA, A.R. and ALUJA, M., 2016. Resource allocation and compensation during development in holometabolous insects. *Journal of Insect Physiology*, vol. 95, pp. 78-88. <http://dx.doi.org/10.1016/j.jinsphys.2016.09.010>. PMID:27650504.
- NUNES, A.M., COSTA, K.Z., FAGGIONI, K.M., COSTA, M., GONÇALVES, R.S., WALDER, J.M.M., GARCIA, M.S. and NAVA, D.E., 2013. Dietas artificiais para a criação de larvas e adultos da mosca-das-frutas sul-americana. *Pesquisa Agropecuária Brasileira*, vol. 48, no. 10, pp. 1309-1314. <http://dx.doi.org/10.1590/S0100-204X2013001000001>.
- OLIVEIRA, F.Q., BOIÇA JUNIOR, A.L., COSTA, M.L.Z., SANCHES, P.A., WALDER, J.M.M. and BATISTA, J.L., 2014. Preference and infestation level of *Anastrepha fraterculus* (Wiedemann, 1830) on fruits of some *Psidium guajava* L. cultivars and relation to their physicochemical parameters. *Phytoparasitica*, vol. 42, no. 4, pp. 475-483. <http://dx.doi.org/10.1007/s12600-014-0384-7>.
- PAPACHRISTOS, D.M., PAPADOPOULOS, N.T. and NANOS, G.D., 2008. Survival and development of immature stages of the Mediterranean fruit fly (Diptera: Tephritidae) in citrus fruit. *Journal of Economic Entomology*, vol. 101, no. 3, pp. 866-872. <http://dx.doi.org/10.1093/jee/101.3.866>. PMID:18613588.
- PAPACHRISTOS, D.P. and PAPADOPOULOS, N.K., 2009. Are citrus species favorable hosts for the Mediterranean fruit fly? A demographic perspective. *Entomologia Experimentalis et Applicata*, vol. 132, no. 1, pp. 1-12. <http://dx.doi.org/10.1111/j.1570-7458.2009.00861.x>.
- PARRA, J.R.P., 2009. A evolução das dietas artificiais e suas interações em ciência e tecnologia. In: A.R. PANIZZI and J.R.P. PARRA, eds. *Bioecologia e nutrição de insetos: base para o manejo integrado de pragas*. Brasília: Embrapa Recursos Genéticos e Biotecnologia, pp. 91-174.
- PROKOPY, R.J., COOLEY, S.S., GALARZA, L., BERGWELER, C. and LAUZON, C.R., 1993. Bird droppings as natural food compete with bait sprays against *Rhagoletis pomonella* flies (Diptera: Tephritidae). *Canadian Entomologist*, vol. 125, no. 3, pp. 413-422. <http://dx.doi.org/10.4039/Ent125413-3>.
- RUIZ, M.J., JUÁREZ, M.L., ALZOGARAY, R.A., ARRIGHI, F., ARROYO, L., GASTAMINZA, G., WILLINK, E., BARDÓN, A.V. and VERA, T., 2014. Toxic effect of citrus peel constituents on *Anastrepha fraterculus* Wiedemann and *Ceratitis capitata* Wiedemann immature stages. *Journal of Agricultural and Food Chemistry*, vol. 62, no. 41, pp. 10084-10091. <http://dx.doi.org/10.1021/jf503063b>. PMID:25237738.
- SALLES, L.A.B., 1992. Metodologia de criação de *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae) em dieta artificial em laboratório. *Annals of the Entomological Society of Brazil*, vol. 21, pp. 479-486.
- VARGAS, R.I., WILLIAMSON, D.L., CHANG, H. and KOMURA, M., 1984. Effects of larval diet pH on worker comfort and insect quality during mass production of Mediterranean fruit fly (Diptera: Tephritidae) in Hawaii. *Journal of Environmental Sciences*, vol. 19, no. 5, pp. 621-630. <http://dx.doi.org/10.1080/10934528409375181>.
- VERA, M.T., OVIEDO, A., ABRAHAM, S., RUIZ, J., MENDOZA, M., CHANG, C.L. and WILLINK, E., 2014. Development of a larval diet for the South American fruit fly *Anastrepha fraterculus* (Diptera: Tephritidae). *International Journal of Tropical Insect Science*, vol. 34, no. S1, pp. S73-S81. <http://dx.doi.org/10.1017/S1742758414000150>.
- VON ZUBEN, C.J., 1998. Comportamento de oviposaduras individuais, percentagem de eclosão e peso larval mínimo para pupação em populações de *Chrysomya megacephala* (F.). *Annals of the Entomological Society of Brazil*, vol. 27, no. 4, pp. 525-533. <http://dx.doi.org/10.1590/S0301-80591998000400004>.
- ZUCCHI, R.A., 2008 [viewed 15 October 2016]. *Fruit flies in Brazil: Anastrepha species their host plants and parasitoids* [online]. Piracicaba. Available from: <http://www.lea.esalq.usp.br/anastrepha/>
- ZUCCHI, R.A., 2012 [viewed 20 October 2016]. *Fruit flies in Brazil: hosts and parasitoids of the Mediterranean fruit fly* [online]. Piracicaba. Available from: <http://www.lea.esalq.usp.br/ceratitis/>