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Original Article

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 ± 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, aciddific 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 predomindantes: 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 ± 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 ± 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 × 39 × 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 × 30 × 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 transfered 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 *Ceratitis 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

[a]Components 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

[a]Coefficient of variation. Means (±SE) followed by the same letter in the row do not differ using the [b]Dunn and [c]Tukey 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 *Ceratitis 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	5.7 ± 0.84ab	6.1 ± 0.70a	6.5 ± 0.85a	14.9
	(4.0-7.0)	(4.0-7.0)	(5.0-8.0)	(5.0-8.0)	
Laval stage duration (days)[b]	7.3 ± 0.48ab	7.7 ± 0.82a	5.4 ± 0.84c	6.1 ± 0.73bc	11.2
	(7.0-8.0)	(7.0-9.0)	(4.0-7.0)	(5.0-8.0)	
Pupal stage duration (days)[b]	8.0 ± 0.47abc	9.1 ± 0.87a	7.2 ± 0.91c	8.2 ± 0.63abc	8.9
	(7.0-9.0)	(8.0-11.0)	(6.0-8.0)	(7.0-9.0)	
Egg-adult period (days)[b]	20.0 ± 0.47b	22.1 ± 0.87a	19.2 ± 0.91b	21.2 ± 0.63ab	3.5
	(19.0-21.0)	(21.0-24.0)	(18.0-20.0)	(20.0-22.2)	
Pupae weight (mg)[b]	13.3 ± 0.48a	12.9 ± 0.32ab	11.3 ± 0.70b	10.0 ± 0.41b	4.1
	(12.30-13.90)	(12.50-13.50)	(10.30-13.0)	(9.20-10.80)	
Pupae number[c]	52.0 ± 7.40b	63.8 ± 6.10a	40.3 ± 6.66c	37.6 ± 7.38c	15.0
	(44.0-67.0)	(55.0-74.0)	(33.0-53.0)	(26.0-51.0)	
Larval viability (%)[c]	82.1 ± 8.33b	93.8 ± 2.78a	64.1 ± 10.02c	62.3 ± 12.32d	12.1
	(70.97-97.10)	(87.30-97.37)	(53.23-81.97)	(40.63-78.46)	
Pupal viability (%)[b]	91.5 ± 5.99a	98.7 ± 1.77a	91.4 ± 2.19ab	78.8 ± 5.84b	4.5
	(82.00-97.73)	(95.16-100.00)	(87.88-94.12)	(66.67-85.71)	

[a]Coefficient of variation. Means (±SE) followed by the same letter in the row do not differ using the [b]Dunn and [c]Tukey 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	12.7 ± 1.64c	16.2 ± 6.93bc	16.9 ± 3.72a	21.3
	(13.0-16.0)	(12.0-20.0)	(12.0-32.0)	(15.0-31.0)	
Oviposition period (days)[b]	14.6 ± 10.83b	26.8 ± 11.45a	12.0 ± 12.60b	14.5 ± 10.93b	7.4
	(1.0-41.0)	(4.0-44.0)	(1.0-51.0)	(2.0-34.0)	
Fecundity[b]	416.7 ± 31.02b	1302.8 ± 60.82a	322.8 ± 36.33b	313.6 ± 26.47b	7.9
	(20.0-991.0)	(134.0-2181.0)	(35.0-1323.0)	(53.0-820.0)	
Fertility (%)[b]	61.2 ± 5.68b	73.1 ± 2.64a	60.9 ± 4.13b	57.7 ± 7.02b	7.9
	(43.33-68.00)	(70.00-76.67)	(53.33-66.67)	(43.3-66.67)	
Sex ratio[b]	0.4 ± 0.06ns	0.5 ± 0.01ns	0.4 ± 0.09ns	0.4 ± 0.18ns	19.1
	(0.40-0.61)	(0.46-0.52)	(0.36-0.63)	(0.20-0.71)	
Survival (females) (days)[c]	31.2 ± 16.01b	52.8 ± 22.66a	34.0 ± 17.72b	31.4 ± 14.12b	0.5
	(13.0-83.0)	(17.0-96.0)	(11.0-80.0)	(8.0-60.0)	
Survival (males) (days)[c]	42.6 ± 25.71b	80.4 ± 31.20a	37.2 ± 15.52b	30.2 ± 17.30b	0.6
	(16.0-119.0)	(19.0-126.0)	(11.0-72.0)	(10.0-80.0)	

[a]Coefficient of variation. Means (±SE) followed by the same letter in the row do not differ using the [b]Dunn and [c]Log-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

[a]Coefficient of variation. Means (±SE) followed by the same letter in the row do not differ using the [b]Dunn and [c]Log-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 out 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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