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Does *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) have a preferential instar to parasitize Tephritidae (Diptera)?

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ABSTRACT. *Diachasmimorpha longicaudata* (Ashmead, 1905) is a koinobiont parasitoid of Tephritidae larvae, the third instar larvae of which is considered preferential, but it is able to parasitize other larval stages and compete with native parasitoids. This study investigated the preference and parasitism capacity of *D. longicaudata* in larvae of different instar of *Anastrepha fraterculus* (Wiedemann, 1830) (AF) and *Ceratitis capitata* (Wiedemann, 1824) (CC). The experiments were carried out under laboratory conditions, one instar being offered at a time in parasitism units, with the following choices among the hosts: 25 AF larvae and 25 CC larvae (first, second and third instar were evaluated). The other test was a multiple-choice in relation to the instar, for larvae of the same host species, with three parasitism units being offered, with 15 larvae of each instar. The mean number of formed pupae, emerged parasitoids, parasitized pupae, unviable pupae and sex ratio were evaluated. In the first bioassay, the mean number of emerged parasitoids and parasitized pupae in the AF host were significantly higher in treatments with first and second instar larvae. For CC there was no difference between the instars tested. In the second bioassay, the mean value of emerged parasitoids and parasitized pupae, was higher in second and third instar larvae for CC, and for AF was in second instar larvae. The sex ratio was biased for males in all treatments in both bioassays. The results show that *D. longicaudata* can parasitize and be successful in all available larval instars, being able to compete with parasitoids of any instar.

KEYWORDS. *Anastrepha fraterculus*, *Ceratitis capitata*, exotic parasitoid, tephritids.

RESUMO. ***Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) tem um ínstar preferencial para parasitar Tephritidae (Diptera)?** *Diachasmimorpha longicaudata* (Ashmead, 1905) é um parasitoide coinobionte de larvas de Tephritidae sendo que o terceiro ínstar larval é tido como o preferencial, mas pode parasitar outros estágios larvais e competir com os parasitoides nativos. Este estudo investigou a preferência e capacidade de parasitismo de *D. longicaudata* em larvas de diferentes ínstares de *Anastrepha fraterculus* (Wiedemann, 1830) (AF) e *Ceratitis capitata* (Wiedemann, 1824) (CC). Os experimentos foram realizados em condições laboratoriais, sendo oferecido um ínstar por vez em unidades de parasitismo, havendo escolha entre os hospedeiros: 25 larvas de AF e 25 larvas de CC (foram avaliadas larvas de primeiro, segundo e terceiro ínstar). O outro teste foi de múltipla escolha em relação ao ínstar, para larvas da mesma espécie hospedeira, sendo oferecidas três unidades de parasitismo, com 15 larvas de cada ínstar. Avaliou-se o número médio de pupários formados, parasitoides emergidos, pupários parasitados, pupas inviáveis e razão sexual. No primeiro bioensaio o número médio de parasitoides emergidos e pupários parasitados no hospedeiro AF foram significativamente superiores nos tratamentos com larvas de primeiro e segundo ínstar. Para CC não houve diferença entre os ínstares testados. No segundo bioensaio, o valor médio de parasitoides emergidos e de pupas parasitadas foi maior nas larvas de segundo e terceiro ínstar para CC, e para AF nas larvas de segundo *ínstar*. A razão sexual foi desviada para machos em todos os tratamentos, nos dois bioensaios. Os resultados demostram que *D. longicaudata* pode parasitar e ter sucesso em qualquer ínstar larval disponível, podendo competir com parasitoides de qualquer ínstar.

PALAVRAS-CHAVE. *Anastrepha fraterculus*, *Ceratitis capitata*, tefritídeos, parasitoide exótico.

Diachasmimorpha longicaudata (Ashmead, 1905) is a solitary, koinobiont, endoparasitoid from the Indo-Australian region, where it parasitizes at least 14 species of *Bactrocera* Macquart, 1835 (Diptera: Tephritidae) (Wharton & Gilstrap, 1983). It is widely used as a biological control agent worldwide for parasitizing species of tephritids (Montoya *et al.*, 2000; Devescovi *et al.*, 2017). It can be easily reared in laboratory conditions and it has a low specificity for hosts, being able to parasitize *Ceratitis capitata* (Wiedemann, 1824) and several species of *Anastrepha* Schiner, 1868 (Diptera: Tephritidae) (Carvalho & Nascimento, 2002). It shows parasitism hability greater than 50% and can suppress up to 70% of the fruit fly populations innatural environment (Sivinski *et al.*, 1996; Montoya *et al.*, 2000).

Diachasmimorpha longicaudata usually parasitizes second and third instar of tephritids larvae (Sivinski *et al.*, 2001; Sime *et al.*, 2006), although there are records of preference for third instar and pupae (Carvalho, 2005b; Ovruski *et al.*, 2011; Montoya *et al.*, 2017). Due to these specificities, the research groups that advocate this species release to biocontrol fruit flies argue that this parasitoid would not compete for oviposition sites with other species, especially the native braconid *Doryctobracon areolatus* (Szépligeti, 1911) (Hymenoptera: Braconidae), which has

a preference for larvae in early stages of development (Matrangolo *et al.*, 1998; Carvalho *et al.*, 2000; Paranhos *et al.*, 2013). Nevertheless, Murillo *et al.* (2015) verified that *D. areolatus* can also parasitize larvae of up to the third instar, which brings the niches of these species even closer. *Diachasmimorpha longicaudata* was imported from the United States of America in 1994 and introduced in Brazil by *Embrapa Mandioca e Fruticultura Tropical*, with the aim of studying its behavior and effectiveness to control fruit fly, aiming the implementation of a biological control program, started in Northeast Brazil (Carvalho & Nascimento, 2002). However, evaluations carried out a few years after their release showed that there were alterations in the presence of native parasitoid species and suggested the existence of interspecific competition in oviposition sites (Carvalho, 2005a). On the other hand, Meirelles *et al.* (2016), after release *D. longicaudata* in Rio Grande do Sul field, did not detect a negative impact on native parasitoid populations. Despite parasitizing preferentially third instar larvae (Montoya *et al.*, 2018), we affirm that *D. longicaudata* is able of parasitizing and succeeding in any instar, differing from that generally described. The interaction between multiple species of parasitoids in the environment is not fully understood, and the release of *D. longicaudata* may be controversial. Thus, this work aimed to investigate the preference and parasitism capacity of *D. longicaudata* in larvae of native *Anastrepha fraterculus* (Wiedemann, 1830) and exotic *C. capitata* from different instars.

MATERIAL AND METHODS

Study site. The study was conducted at the Laboratory of Biology, Ecology and Biological Control of Insects (Bioecolab), at the *Universidade Federal do Rio Grande do Sul*, under controlled conditions of 26 ± 1 °C, 60 ± 10% RH, with 14 hours of photophase.

Host rearing. The adults of *A. fraterculus* and *C. capitata* were kept in wooden cages (45 x 30 x 30 cm), covered on the sides with voile fabric, receiving distilled water and a solid diet on an *ad libitum* basis, which consisted of crystal sugar, hydrolyzed protein, soybean extract (3:1:1) and vitamin complex (Lavitan – A-Z®), in the ratio of two macerated tablets per 250 g of diet (adapted from Jaldo *et al.*, 2001). As an oviposition substrate for *C. capitata*, a 250 ml yellow plastic tube with small perforations (FAO/IAEA/USDA, 2003) was used. For *A. fraterculus*, the substrate used was a blue tissue bag covered with silicone, as described in Meirelles *et al.* (2016). The eggs were collected daily and placed on polystyrene trays (23.5 x 18 x 1 cm), with an artificial diet based on organic carrot, beer yeast, corn flour, sugar, distilled water, sodium benzoate (Dinâmica®), nipagin (Synth®) and citric acid (Synth®) (modified from Terán, 1977). After seven days, these were placed inside larger plastic trays (51 x 30 x 9.5 cm), with sterile sand and covered by organza, where they remained for approximately seven days for the pupation. Subsequently, the sand was sifted and the pupae obtained were placed in plastic containers (6.6 x 6.6 x 6 cm) until emergence.

Parasitoids rearing. The rearing has started from the parasitized pupae of *A. fraterculus*, from *Embrapa Clima Temperado*, Pelotas, RS, Brazil. The adults were kept in wooden cages (19.5 x 16.5 x 25.5 cm), covered with organza material and fed with honey dissolved in water (7:3), offered in Petri dishes (5 x 5 x 1.5 cm) with cotton, water was provide by capillarity through a strip of Spontex Resist® fabric. Third instar *C. capitata* larvae were placed in parasitism units, which consisted of a circular plastic plate (4 cm in diameter), with a 0.3 cm border, formed by a small layer of silicone, wrapped with white organza fabric stuck with a rubber band. After one hour of exposure, the larvae were returned to the artificial diet in polystyrene trays (15.5 x 15.5 x 1 cm) and stored in plastic trays (41 x 28 x 7 cm) on a layer of sand sterilized until the pupae formation. After five days, the sand was sifted, and the pupae were packaged in the same manner as for fly breeding, waiting for parasitoids emergence that were reintroduced to the breeding in new cages.

Parasitism in different instars between two host species. The females preference was evaluated by concomitantly offering 25 larvae of *A. fraterculus* (AF) and 25 larvae of *C. capitata* (CC) to five couples of parasitoids (eight days old). First, second and third instar larvae of the two host species were evaluated. The larvae were offered daily for five days, completing 60 replicates and totaling 1,500 larvae evaluated by treatment. The couples were kept in wooden cages (15 x 15.5 x 20 cm), covered with organza, offered water and food. The larvae were offered in parasitism units, consisting of a circular plastic plate (2.7 cm in diameter), with a border of 0.2 cm, formed by a small layer of silicone and encased in white voile, trapped with an elastic band, disposed on pots with 3.8 cm in height as support. The units were exposed for eight hours, and the larvae were then returned to the artificial diet in polystyrene trays and placed in plastic containers (35 x 17.5 x 10 cm) on a layer of sand until pupa formation.

In order to evaluate larval mortality without action of parasitoids (control treatment) 25 larvae of *A. fraterculus* and *C. capitata* (total of 50 larvae per cage) were placed in parasitism units and these remained in cages for eight hours without parasitoids presence. Following that, the larvae were kept in the same manner as described for breeding.

Multiple-choice parasitism test with different larval instars of the same host. The preference of *D. longicaudata* females was evaluated in cages as described previously with three parasitism units containing 15 larvae of first, second and third instar (total of 45 larvae per replicate) of one host species – AF or CC – to five couples of parasitoids (eight days old). The larvae were offered daily for five days, totalizing 30 replicates and 1,350 larvae evaluated. The units remained exposed for eight hours, and the larvae were then conditioned as described previously.

To evaluate larval mortality, without action of the parasitoids (control treatment), 15 instar larvae each, totalizing 45 larvae per cage, or *A. fraterculus* or *C. capitata* were placed in parasitism units and kep in the cages for the

same time as described above, but without the presence of parasitoids.

For both bioassays, after five days, the sand was sifted and the pupae packed in plastic pots until the emergence of flies or parasitoids. The pupae of which there was no emergence were dissected for check the presence of parasitoids or flies. The mean numbers of formed pupae were recorded, as well as parasitized pupae (emerged parasitoids + pupae dissected with parasitoids), emerged parasitoids, unviable pupae [number of offered larvae - (number of flies emerged + emerged parasitoids)], sex ratio of parasitoids, and parasitism rate.

Statistical analysis. The mean values were analyzed for normality by the Shapiro-Wilk test and submitted to analysis of variance, the means being compared by ANOVA, followed by the Tukey test, with a significance level of 5%.

The sex ratio (Rs) was estimated using the formula: Rs = number of females / number of females + number of males. The Chi-square (χ^2) of heterogeneity was used to compare Rs between treatments. The parasitism index was calculated using the formula: IP = number of emerged parasitoids / number of pupae formed × 100. The tests were performed using the BioEstat 5.0 software (Ayres *et al.*, 2007).

RESULTS

Multiple-choice parasitism in different instars between two host species

Anastrepha fraterculus. The mean number of parasitized pupae and emerged parasitoids was significantly higher ($F = 30.5686$; $df = 2$; $p < 0.0001$, $F = 35.4343$; $df = 2$; $p < 0.0001$, respectively) in larvae of first and second instars when compared to third instar larvae (Fig. 1) (Tab. I). The parasitism rate was 73.8, 74 and 34% in first, second and third instar larvae, respectively.

The mean value (± SE) of pupae formed in control treatment (without presence of parasitoids) was 21.0 ±

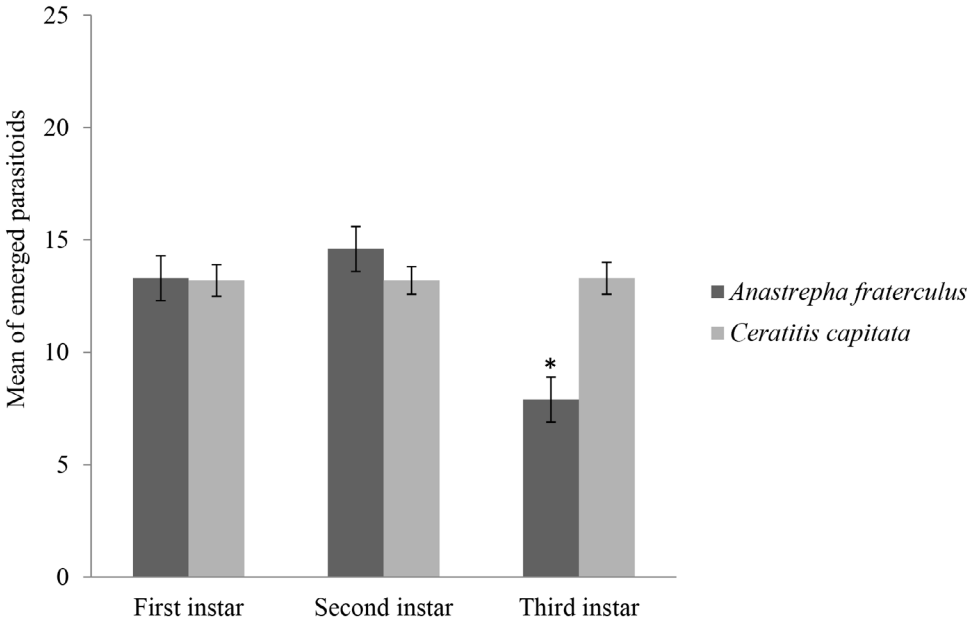

Fig. 1. Mean number of emerged parasitoids in hosts *Anastrepha fraterculus* (Wiedemann, 1830) and *Ceratitis capitata* (Wiedemann, 1824), exposed to parasitism by *Diachasmimorpha longicaudata* (Ashmead, 1905) on first, second and third-instar larvae. The bars correspond to the standard error. Bars with asterisk presented significant difference (ANOVA test, followed by the Tukey test, $p < 0.05$) of the other instars for the same host species.

Tab. I. Mean number (± SE) of formed pupae, parasitized pupae, unviable pupae and sex ratio of hosts *Anastrepha fraterculus* (Wiedemann, 1830) and *Ceratitis capitata* (Wiedemann, 1824), exposed to parasitism by *Diachasmimorpha longicaudata* (Ashmead, 1905) on first, second and third-instar larvae. Lowercase letters compare the different treatments with the same host species. Upper case letters compare the same treatments against the two host species. ANOVA test, followed by Tukey ($p < 0.05$). Sex ratio, tested by χ^2 for heterogeneity. (1) emerged parasitoids + pupae dissected with parasitoids; (2) number of larvae offered – (number of emerged flies + emerged parasitoids).

Variables evaluated	Instar					
	First		Second		Third	
	AF	CC	AF	CC	AF	CC
Formed pupae	17.9 ± 0.80 bA	18.2 ± 0.65 bA	19.6 ± 0.44 abA	18.5 ± 0.56 bA	21.4 ± 0.78 aA	21.7 ± 0.63 aA
Parasitized pupae (1)	13.3 ± 0.68 aA	13.2 ± 0.70 aA	14.6 ± 0.60 aA	13.3 ± 0.61 aA	7.9 ± 0.65 bB	13.4 ± 0.71 aA
Unviable pupae (2)	10.4 ± 0.72 bA	9.2 ± 0.66 aA	9.6 ± 0.62 bA	10.5 ± 0.58 aA	17.1 ± 0.71 aA	9.4 ± 0.70 aB
Sex ratio	0.24 bA	0.10 bB	0.31 aA	0.21 aB	0.27 bA	0.10 bB

0.79 (first instar), 18.6 ± 2.10 (second instar) and 23.2 ± 1.05 (third instar) and they were not significantly different ($p > 0.05$) from those that had the presence of parasitoids. The mean number of pupae, when parasitoids were present, was higher in the third instar only when compared to the first ($F = 6.1750$; $df = 2$; $p = 0.0030$) (Tab. I). In the presence of parasitoids, the mean number of unviable pupae was higher when the third instar larvae were exposed ($F = 35.7765$; $df = 2$; $p < 0.0001$), compared to the other two treatments (Tab. I). The mean (± SE) of unviable pupae in the control was 6.0 ± 1.05; 8.6 ± 2.09 and 4.8 ± 1.42, respectively, for the first, second and third instars, lower when compared to larvae of first and third instars that were exposed to parasitoids ($F = 5.6303$; $df = 1$, $p = 0.0194$, $F = 44.3177$, $df = 1$, $p < 0.0001$, respectively).

The sex ratio of the offspring was higher in larvae that were exposed to parasitism in the second instar ($\chi^2 = 20.6$; $df = 5$; $\alpha = 0.05$). In all treatments, there were a higher number of males (Tab. I).

Ceratitis capitata. The mean number of parasitized pupae and emerged parasitoids was not significantly different between treatments ($p > 0.05$) (Fig. 1) (Tab. I). The parasitism rate was 72.4% in first instar larvae and 71.3% and 71.1% in second and third instar larvae, respectively.

The mean value (± SE) of pupae formed in the control was 19.8 ± 2.01 in the first instar, 21.2 ± 0.55 in the second instar and 21.7 ± 0.63 in the third instar, was not significantly different among treatments ($p > 0.05$) from those that had the parasitoids presence. In the treatments with the parasitoids presence, the average pupae formed was higher in third instar larvae ($F = 10.0897$; $df = 2$; $p = 0.0002$) (Tab. I). In the treatments with the presence of parasitoids, there was no difference between the instars in the mean number of unviable pupae ($p > 0.05$) (Tab. I). In control, the mean numbers (± SE) were 7.4 ± 1.77; 7.4 ± 1.44 and 5.9 ± 1.95, respectively, for the first, second and third instars. There was no difference between treatments with parasitoids and their respective controls on larvae of the first and third instars ($p > 0.05$). The third instar had fewer unviable pupae in the control when compared to the treatment with parasitoids ($F = 4.1045$; $df = 1$; $p = 0.0440$).

The sex ratio of the offspring was higher in larvae exposed to parasitism in the second instar ($\chi^2 = 13.4$; $df = 5$; $\alpha = 0.05$). In all treatments, there were a higher number of males (Tab. I).

Host preference. When comparing the same instar between the two host species, the mean number of parasitized pupae ($F = 32.9505$; $df = 1$; $p < 0.0001$) (Tab. I) and emerged parasitoids ($F = 38.7731$; $df = 1$; $p < 0.0001$) was higher in *C. capitata* (CC) in the third instar (Fig. 1). Regarding the mean number of pupae formed, there was no difference between AF and CC in all treatments ($p > 0.05$). The mean number of unviable pupae was significantly higher only in the third instar of CC ($F = 59.1417$; $df = 1$; $p < 0.0001$). The sex ratio was always higher in the host AF (Tab. I), regardless of the instar in which the larvae were exposed ($\chi^2 = 22.7$; $df = 2$; $\alpha = 0.05$ – first instar larvae; $\chi^2 = 23.0$; $df = 2$; $\alpha = 0.05$ – second instar larvae, and $\chi^2 = 24.2$; $df = 2$; $\alpha = 0.05$ – third instar larvae).

Multiple-choice parasitism test with diferent larval instars of the same host

Anastrepha fraterculus. The mean number of parasitized pupae and emerged parasitoids was significantly higher ($F = 9.3968$; $df = 2$; $p = 0.0004$, $F = 9.3969$; $df = 2$; $p = 0.0004$, respectively) in second instar larvae (Tab. II). The parasitism rate was 66.9%, 86.9% and 60.3% in first, second and third instar larvae, respectively.

The mean value (± SE) of pupae formed in the control was 13.2 ± 0.86; 14.0 ± 0.31 and 14.8 ± 0.20 for first, second and third instar larvae, respectively. The control was not significantly different from the others treatments with parasitoids presence ($p > 0.05$). There was also no difference in the mean of puparia formed between the instars

Tab. II. Mean number (± SE) of formed pupae, parasitized pupae, emerged parasitoids, unviable pupae and sex ratio of hosts *Anastrepha fraterculus* (Wiedemann, 1830) and *Ceratitis capitata* (Wiedemann, 1824) exposed to parasitism by *Diachasmimorpha longicaudata* (Ashmead, 1905) on first, second and third-instar larvae. Lowercase letters compare the different treatments with the same host species. ANOVA test, followed by Tukey ($p < 0.05$). Sex ratio, tested by χ^2 for heterogeneity. (1) emerged parasitoids + pupae dissected with parasitoids; (2) number of larvae offered – (number of emerged flies + emerged parasitoids).

Variables evaluated	*Anastrepha fraterculus*		
	Instar		
	First	Second	Third
Formed pupae	11.4 ± 0.46 a	12.2 ± 0.57 a	12.1 ± 0.86 a
Parasitized pupae (1)	7.6 ± 0.64 b	10.6 ± 0.55 a	7.3 ± 0.59 b
Emerged pupae	7.6 ± 0.64 b	10.6 ± 0.55 a	7.3 ± 0.59 b
Unviable pupae (2)	5.2 ± 0.58 b	4.3 ± 0.54 b	7.7 ± 0.57 a
Sex ratio	0.27 b	0.32 b	0.70 a
	Ceratitis capitata		
Formed pupae	10.2 ± 0.50 b	13.9 ± 0.27 a	14.3 ± 0.15 a
Parasitized pupae (1)	8.0 ± 0.54 b	11.9 ± 0.43 a	11.4 ± 0.57 a
Emerged pupae	8.0 ± 0.54 b	11.9 ± 0.43 a	11.4 ± 0.57 a
Unviable pupae (2)	5.7 ± 0.50 a	2.9 ± 0.45 c	3.6 ± 0.57 b
Sex ratio	0.41 b	0.45 b	0.61 a

with the presence of parasitoids ($p > 0.05$) (Tab. II). In the treatments with parasitoids, the mean number of unviable pupae was higher in those exposed in the third instar ($F = 9.4386$; $df = 2$; $p = 0.0004$) (Tab. II). In the control, the mean value (± SE) were of 2.6 ± 0.67; 2.0 ± 0.83 and 0.6 ± 0.4 for the first, second and third instars, respectively, being lower than treatments with parasitoids only in the third instar ($F = 23.2425$; $df = 1$; $p = 0.0001$).

The sex ratio of offspring generated was higher in third instar larvae, with more females emerged ($\chi^2 = 47.9$; $df = 5$; $\alpha = 0.05$) (Tab. II).

Ceratitis capitata. The mean number of parasitized pupae and emerged parasitoids ($F = 16.6636$; $df = 2$; $p < 0.0001$; $F = 16.36637$; $df = 2$; $p < 0.0001$, respectively) was higher in the second and third instars (Tab. II). The parasitism rate was 78.2% for first instar larvae and 85.9% and 79.9% for second and third instar larvae, respectively.

The mean values (± SE) of pupae formed in the control was 12.6 ± 0.74; 14.8 ± 0.20 and 14.6 ± 0.24 for first, second and third instar larvae, respectively. There was no difference between control and treatments parasitoids presence ($p > 0.05$). In the treatments with parasitoids, the second and third instars were the ones with the highest mean number of pupae formed ($F = 41.3569$; $df = 2$; $p < 0.0001$) (Tab. II). The mean number of unviable pupae in tests with parasitoids presence was higher in first instar larvae ($F = 8.2180$; $df = 2$; $p = 0.0008$) (Tab. II). The control had the mean values (± SE) of 2.4 ± 0.74; 0.2 ± 0.2 and 0.4 ± 0.24 for the first, second and third instars, respectively. All treatments that had the presence of parasitoids had a higher mean number of unviable pupae, when compared to their controls ($F = 6.7424$; $df = 1$; $p = 0.0134$ for first, $F = 5.6186$; $df = 1$; $p = 0.0224$ for second, and $F = 5.0216$; $df = 1$; $p = 0.0301$ for third instar).

The sex ratio of offspring generated was higher in larvae exposed in the third instar, with more females emerged ($\chi^2 = 64.4$; $df = 5$; $\alpha = 0.05$) (Tab. II).

DISCUSSION

The lack of difference in pupae number formed between treatments, even with the presence of parasitoids, is expected, considering that *D. longicaudata* is a koinobiont parasitoid (Ovruski *et al.*, 2000), that does not kill the larvae of its hosts immediately, allowing them to finish their development and pupate before causing death. This is known for Braconidae fruit fly parasitoids that emerge only at the pupal stage (Ovruski *et al.*, 2000; 2003). The higher mortality in some treatments, when compared to the control in this experiment, may be due to the stress caused to the larvae by parasitism, test punctures or even by superparasitism (Ovruski *et al.*, 2011; Harbi *et al.*, 2018). In our study, when only one instar was offered, *D. longicaudata* efficiently parasitized larvae of both the first and second instars of *A. fraterculus*, showing that their response may be conditioned to the environment, differing from other studies that registered their preference for the late larval stages (Ovruski *et al.*, 2011; Van Nieuwenhove & Ovruski, 2011; Montoya *et al.*, 2017). In addition, *D. longicaudata* showed no instar preference in *C. capitata* larvae when exposed only one at a time. On the other hand, when the three instars were offered concomitantly, the highest parasitism was in the second and third instar. In general, parasitoids usualy to have a preferential or single instar to parasitize, as seek to specialize in relation to the species they use as hosts and can be specialize in certain stages thereof (Mattiacci & Dike, 1995; Montoya *et al.*, 2018). In the case of *D. longicaudata*, there are records that it is able to parasitize the second and third instars (Sivinski *et al.*, 2001; Sime *et al.*, 2006). Additionally, this species has been shown a broad plasticity, adapting easily to environmental conditions (Carvalho & Nascimento, 2002).

When the three larval instars of *A. fraterculus* were exposed simultaneously, the second instar was preferred, differing from the studies that suggested the third as preferential (Ovruski *et al.*, 2011; Van Nieuwenhove & Ovruski, 2011; Montoya *et al.*, 2017). The interaction between *D. longicaudata* and *A. fraterculus* can be considered as a "new association", as they do not share an intense history of coevolution, a factor that may influence the parasitoid-host relationship (Hokkanen & Pimentel, 1989), and even change the parasitoid's preferences for the parasite. The fact that *A. fraterculus* larvae are larger than *C. capitata* (Meirelles *et al.*, 2013; Oliveira *et al.*, 2014; Sá *et al.*, 2018) or those of many *Bactrocera* species (Mau & Kessing, 1992; Thomas *et al.*, 2001; Singh *et al.*, 2010), their original hosts, may cause the *D. longicaudata* to parasitize also the first instars of the South American fruit fly, recognizing the youngest larvae as appropriate for their development, with sufficient nutritional quality and quantity to meet their needs, opposing previous studies (López *et al.*, 2009; Harvey *et al.*, 2012).

In the environment, hosts can be found at different stages and densities inside the fruits, which may reflect parasitoid choices (Núñez-Campero *et al.*, 2016). Thus, there is no ensure that *D. longicaudata* will not compete for the same oviposition niche of the native parasitoids. For parasitoids, a single host comprises its entire source of larval food and can have great influence on the adult's fitness. In general, larger hosts have more qualitative resources to supply parasitoid fitness (Mattiacci & Dicke, 1995; Ovruski *et al.*, 2011; Harvey *et al.*, 2012). This apparently did not influence in *D. longicaudata* choice in our study, being effective even in first and second instar larvae. In this case, possibly even smaller larvae can guarantee the quantity and nutritional quality for *D. longicaudata* development, as their hosts were originally species of *Bractocera* (Wharton & Gilstrap, 1983), smaller than those tested in this study (Singh & Ramamurthy, 2010).

The sex ratio of *D. longicaudata* offspring grown in both *A. fraterculus* and *C. capitata* was biased for males, indicating that host or environmental conditions may not have been propper for the parasitoid (Godfray, 1994). When different instars of the same host species were offered simultaneously, a larger number of females emerged in second and third instar larvae, respectively. The data found in our study corroborate the records that Tephritidae parasitoids

that parasitize larvae in later stages tend to produce a larger number of females (Eben *et al.*, 2000; Ovruski *et al.*, 2011; Van Nieuwenhove & Ovruski, 2011). On the other hand, Montoya *et al.* (2011, 2012) argue that larval size influences superparasitism, which, in turn, influences the sexual ratio of *D. longicaudata*. When moderate superparasitism occurs (2-6 scars per pupa), there is a trend of female emergence, with no detrimental effects on the demographic parameter to offspring, including longevity and fecundity (González *et al.*, 2007; Montoya *et al.*, 2011; 2012). It is possible that this occurred in our study on the second bioassay, although we did not record the number of scars left on the larvae, given that it could help to evaluate superparasitism and corroborate this hypothesis.

When the hosts *A. fraterculus* and *C. capitata* were exposed simultaneously, we observed that in *A. fraterculus* there was a higher proportion of females. In relation to the emergence of parasitoids and mortality, however, both had similar means, except for third instar larvae of *A. fraterculus*, with a higher mean number of unviable pupae and lower number of emerged parasitoids. Although *C. capitata* has been used for a long time in rearing of *D. longicaudata* in several places of the world, *A. fraterculus* has already been used, showing a good performance as a host (Messing *et al.*, 1993; Van Nieuwenhove & Ovruski, 2011, Meirelles *et al.*, 2016; Harbi *et al.*, 2018), and our study confirms this data. This aspect is important in mass rearing since studies such as those by Segura *et al.* (2007) and Tognon *et al.* (2013) have demonstrated that parasitoids that are reared in a given host are easier to recognize through chemical tracks, obtained by memory or learning, which would provide greater efficiency in the control of the target pest (Mattiacci & Dicke, 1995; Eben *et al.*, 2000).

Our study demonstrates the plasticity of *D. longicaudata* at the moment of host selection, and that it can be considered a good competitor. It is important that *D. longicaudata* coexist with other parasitoids, not leading their populations to decline. Therefore, before releasing exotic wasps species, it is important to know how they respond (behavior) in the field. Other factors such as biotic and abiotic conditions (Sivinski *et al.*, 2000), chemical tracks of plants (Eitam *et al.*, 2003; Silva *et al.*, 2007; Segura *et al.*, 2016) and patch isolation (Eitam *et al.*, 2004) may also interfere in search and parasitism. Considering that not all environments have abiotic and biotic barriers, which may help in the niches division, and that *D. longicaudata* is a competitive species, easily parasitizing any instar, its introduction into new environments should be well evaluated, so as not to cause suppression of other species and a subsequent imbalance in the environment.

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