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ECOSYSTEMS

Functional response and preference of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) in *Ceratitis capitata* and *Anastrepha fraterculus* (Diptera: Tephritidae)

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Abstract: *Diachasmimorpha longicaudata* is the most used braconid in biological control programs for Tephritidae fruit flies worldwide. The aim of this work was to assess the functional response and preference of this parasitoid to larvae of *Ceratitis capitata* and *Anastrepha fraterculus*, in different densities of hosts. The functional response of females of *D. longicaudata* was assessed, independently, in two hosts (third instar larvae of *C. capitata* or *A. fraterculus*), in seven densities 1, 3, 5, 10, 25, 35 or 55 larvae of fruit flies per one female of parasitoid exposed in unit of artificial parasitism, for three hours, in at least 20 repetitions. The species showed a Type III functional response regardless of the density of host larvae, in both species, indicating that they are feasible hosts for multiplication of the parasitoid, under the conditions tested. The number of individuals parasitized and the percentage of female emergence were superior in *A. fraterculus*, when compared to *C. capitata*. Parasitism in field and progeny of female parasitoids can be incremented using larvae of *A. fraterculus* in the rearing of *D. longicaudata*.

Key words: Artificial rearing, behavior, fruit fly, parasitoid.

INTRODUCTION

Diachasmimorpha longicaudata (Ashmead) (Hymenoptera: Braconidae) has been considered the most important biological agent in Tephritidae fruit fly control programs in Latin America (González et al. 2007). It is a coinobiont endoparasitoid that oviposits on the last larva instar of tephritidae and completes its development in the host pupa stage (Van Nieuwenhove et al. 2012). After its introduction in American countries, *D. longicaudata* has been recorded parasitizing *Anastrepha* spp., *Ceratis capitata* (Wiedemann) and *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) (Ovruski et al. 2000). Its efficiency depends on several factors, among which, the host density (Vargas et al. 1993), size (Sivinski 1991), intra-specific competition (Montoya et al. 2012) and super parasitism (González et al. 2007, Altafini et al. 2013).

The efficacy of a natural enemy in regulating a pest population has been associated to its functional response (Fujii et al. 1986) and its knowledge is decisive for the success of releases of natural enemies in the field (Neil & Specht 1990). The functional response describes the relation between the numbers of hosts attacked as function of their density (Holling 1965). According to the author, three types of responses can be acknowledged: type I (linear), when parasitism increases linearly with increasing hosts density; type II (curvilinear), when the number of parasitized hosts increases with increasing hosts density, though in a

descending rate, until reaching a plateau from which it stabilizes, and type III (sigmoid), described as an initial increase and subsequent decrease in the proportion of parasitized hosts with growing of hosts density.

While assessing the functional response of *D. longicaudata* to larvae of *Anastrepha suspensa* (Loew), Montoya et al. (2000) observed, for isolated females, a type III curve, presenting direct dependence on density, considering the proportion of larvae attacked. However, the authors observed that for females in groups, data of attack to larvae fit in the type II curve model. Parasitizing larvae of *C. capitata* Harbi et al. (2018) also described type II functional response, with variations related to temperature and laboratory conditions or semi-field, testing 1:5, 1:20 or 1:40 female proportion per larva.

In addition to responses related to hosts density, factors like their specific preference and nutritional quality can determine higher or lower rates of parasitism, essential information for mass rearing and use of parasitoids as biological control agents (Silva et al. 2007). *D. longicaudata* preference for larvae of *A. fraterculus* or *C. capitata* was assessed by Ovruski et al. (2011), who recorded significant difference in the choice among hosts when they were separately offered to the braconid females. However, the authors verified that in double choice tests, *D. longicaudata* showed strong preference for *A. fraterculus*, presenting a higher number of the parasitoid females emerged from this species of fruit fly. The preference of this parasitoid for larvae of *A. fraterculus* against those of *C. capitata* was also recorded by Ovruski et al. (2011), Altafini et al. (2013) and Meirelles et al. (2013). The performance of a parasitoid can also be associated to the size of the host (Nicol & Mackauer 1999, Chau & Mackauer 2001). Females of *D. longicaudata* preferred larger larvae for the development of their offspring (Cancino et al. 2002, López et al. 2009). However, in any of these works the influence of the original host of the parasitoid females was assessed, a factor that can influence parasitism rate (Papaj & Lewis 1993) and be determinant to the parasitoid efficiency, in case of release in field.

Thus, this work aimed to determining the type of functional response of *D. longicaudata* under different densities of larvae of Tephritidae *A. fraterculus* and *C. capitata*, as well as the preference for parasitizing these larvae, considering the original host of the parasitoid.

MATERIALS AND METHODS

The work was performed in the Biology, Ecology and Biological Control of Insects Laboratory (BIOECOLAB), at Federal University of Rio Grande do Sul, Brazil, in climate-controlled room at 25 ± 2 °C, 65 ± 10 % RH and photoperiod of 14 h.

The rearing of *C. capitata and A. fraterculus* was based on methodology proposed by Terán (1977), with adaptations. Adults were kept in wood cages (45 x 30 x 30 cm), covered on the side with voile, and received distilled water and diet *ad libitum*. As substrate for *C. capitata* oviposition a 250 ml plastic orange tube was used (FAO/IAEA/USDA 2003). For *A. fraterculus*, the substrate was a plastic Petri dish with 15 cm of diameter, opening with 11 cm of diameter protected by voile covered with black cold silicone. This dish was placed on the upper face of the cage for breeding of *A. fraterculus*, containing water inside it. During incubation, eggs were kept in water and then placed on the artificial diet for larvae, made with raw carrot and corn flour.

The parasitoid rearing was based on Carvalho et al. (1998) methodology, using as hosts larvae of *C. capitata* and *A. fraterculus*. Third instar larvae were exposed to parasitoids

in parasitism units (40 to 50 larvae) for one hour, once a day.

Functional response bioassay

The functional response of females of *D. longicaudata* was evaluated, independently, in seven densities of two hosts (third instar larvae of *C. capitata* or *A. fraterculus*), defined after pilot bioassay. Densities were 1, 3, 5, 10, 25, 35 or 55 larvae of fruit flies per one female of parasitoid exposed in unit of artificial parasitism. The number of repetitions per density varied as function of host availability. For *C. capitata*, 46, 20, 35, 34, 35, 28 and 20 repetitions were made, respectively, for each density mentioned above, and for *A. fraterculus* 20 repetitions in all densities.

The larvae were exposed in parasitism units produced with two rectangular acrylic plates (10 x 8 cm), and one of them had an opening (4 x 6 cm) covered with voile, through which the parasitoid female had access to them, so that all larvae were equally exposed. Larvae of hosts were placed on the voile, covered with the breeding diet and at a thickness of 2mm, and, with the other plate, they were slightly pressed to limit their movement. The two plates were united by rubber band. Parasitism units were individually offered, hanging on the cage wall, in front of a light source, during three hours, for a paired female with oviposition experience, with four or five days old, randomly chosen, following methodology described by Montoya et al. (2000).

After three hours of exposure, the larvae were individualized and accommodated in glass tubes (8 cm x 2 cm ∅), identified with paper tags, containing sterilized sand previously sprayed with Nipagin® at 10%, diluted in alcohol 96°. The tubes were closed with plastic film and kept under the same environment conditions of the experiment until the emergence of parasitoids or flies.

The percentage of parasitism was recorded by [parasitoids emerged/(parasitoids + flies emerged)]. The number of emerged parasitoids added to the number of those found during the dissection of puparia, from which no insects emerged, defined the number of parasitized larvae (*Na*).

The natural mortality of fly larvae, due to handling and/or exposure condition, was obtained by keeping parasitism units with each of the seven densities, for three hours inside the experiment cages, in front of light source, without presence of the parasitoid female, which was considered as control. The larvae, after this period, were individualized in glass tubes until the emergence of flies. The average values of control mortality were compared to those recorded in the bioassays of exposure to *D. longicaudata*, through Kruskal-Wallis test, at 5% significance.

Estimate of parameters and numerical analysis of functional response

The number of hosts parasitized (*Na*) in the different densities of hosts (*No*), obtained through functional response assays was used to estimate searching efficiency (*E*), which is the probability of a given parasitoid to find any host in a given time (*T*), by the formula:

$$E = Na/No$$

The handling time (*Th*) of *D. longicaudata*, which covers the time of the host's location, its handling, oviposition, marking and time spent to generate new eggs, was estimated with Holling's discs equation (Holling 1959) by the non-linear least squares method (NLIN procedure, Marquardt method) through app SAS System (SAS Institute 2004). Based on *Th*, it was possible to estimate the total handling time (Th_{total}), which is the sum of handling times of each occasion; search time (*Ts*), which involves the

host localization mechanisms; attack rate (*a'*), which represents the proportion of parasitized individuals found, and; the maximum number of larvae that a female of *D. longicaudata* would be able to parasitize in a defined time (Na_{max}), by the formula:

$$Th_{total} = Th \times Na$$

$$Ts = T - (Th_{total})$$

$$a' = Na/(N \times Ts)$$

$$Na_{max} = T/Th$$

The type of functional response was estimated by adjusting the data observed to the model of sigmoid functional response (Hassel 1978) given by Holling's discs equation, after having their assumptions tested:

$$Na = No\ \{1-exp\ [-(bTNoP)\ /\ (\ 1+\ (cNo)\ +\ (bThNo^2)]\}$$

where:

Na = number of hosts parasitized

No = host density

T = total length of the experiment

P = number of parasitoids (1)

Th = handling time

b and *c* = constants (fixed values belonging to Holling's discs equation formula)

Differences in average values were submitted to Kruskal-Wallis test at 5% significance. The number of hosts parasitized (*Na*) was transformed by $\sqrt{x+1}$.

The quality of the adjustment to the functional response model was tested by pseudo-r^2 (SAS Institute 2004), calculated from the sum of squares (SQ) of the non-linear analysis, where: Pseudo-r^2 = 1 – (SQ /total corrected residue of SQ).

The percentage of parasitoid emergence and total mortality of fruit flies, among the densities tested, were compared through chi-squared test of heterogeneity, at 5% significance. Statistical analyses were made by Bioestat 5.0 (Ayres et al. 2007) and SAS System (SAS Institute 2004).

Assessment of preference of *Diachasmimorpha longicaudata* for host

The preference of females was evaluated by concomitantly offering ten third instar larvae of *A. fraterculus* and ten of *C. capitata* to an experienced female with five days old, in parasitism units, for three hours. The experience period consisted of offering both fruit fly larvae for one hour, 72 hours before the experiment. The females tested had two origins (from the standard laboratory rearing): emerged from larvae of *C. capitata* or from the first generation in larvae of *A. fraterculus*, 20 replicates were made for each parasitoid female origin.

After exposure, the larvae were accommodated in sterilized sand for pupation and kept until emergence. The number of parasitoids and/or flies emerged from each of the host species was recorded and compared by Wilcoxon test at 5% significance. The proportion of parasitized individuals in each host species, according to the origin, was compared by Fisher's exact test at 5% significance. Analyses were made in program Bioestat 5.0.

RESULTS AND DISCUSSION

Functional response of *Diachasmimorpha longicaudata*

The average number of larvae of *C. capitata* parasitized by a female of *D. longicaudata* was higher and similar when 10, 25, 35 and 55 larvae were exposed (H = 83.9439; df = 6; P < 0.0001) than those with 1, 3 or 5 larvae (Table I). The estimated percentage of parasitism increased from the lowest density (one larva), reaching a maximum of 23.3% in density of three, and

Table I. Mean number observed (gross values transformed by √x+1) and estimated of larvae of *Ceratitis capitata* parasitized by females of *Diachasmimorpha longicaudata* (*Na*), exposed to different host densities (*No*), percentage of parasitized larvae and estimated values of attack rate (*a'*), search efficiency (*E*), total handling time (Th_{total}) and search time (*Ts*), based on the random model of Holling's discs equation. Data refer to a total time of exposure of three hours. Values between brackets indicate the number of repetitions.

	Host densities (No)						
Mean ± SE	**1 (46)**	**3 (20)**	**5 (35)**	**10 (34)**	**25 (35)**	**35 (28)**	**55 (20)**
Na observed (gross values)	0.04±0.087c[1]	0.19±0.700 bc	0.19±0.943bc	2.03± 0.340ab	2.71±0.45ab	5.0±0.700a	4.85±0.850a
Na observed (transformed)	1.04±0.017 c	1.27±0.072bc	1.34±0.065bc	1.65±0.095ab	1.81±0.113ab	2.32±0.150a	2.29±0.177a
Na estimated	0.22	0.64	1.02	1.82	3.44	4.15	5.11
% parasitized larvae	8.7	23.3	18.9	20.3	10.9	14.3	8.2
a'	0.0005±2x10^{-4}b	0.0014±4x10^{-4}a	0.0011±2x10^{-4}a	0.001±2x10^{-4}a	0.0006±9x10^{-5}a	0.0008±9x10^{-5}ab	0.0005±5x10^{-5}ab
E	0.09±0.042b	0.23±0.064a	0.19±0.034a	0.20±0.029a	0.11±0.016a	0.14±0.016a	0.09±0.010a
Th_{total} (min)	1.83±0.886c	13.70±3.83bc	19.88±4.141bc	42.8±7.095ab	57.24±9.572ab	105.5±14.87a	102.29±18.121a
Ts (min)	178.17±0.886a	166.29±3.83ab	160.12±4.141ab	137.2 ±7.095bc	122.76±9.572bc	74.55±14.87c	77.71±18.121c

[1]**Distinct lowercase letters in lines indicate significant difference (Kruskal-Wallis, α=0.05).**

reduced in higher densities (Table I). While assessing parasitism of *D. longicaudata* in larvae of *C. capitata*, Harbi et al. (2018) recorded similar results, that is, higher parasitism percentage when host density was lower and when it was higher, this percentage was lower.

In *A. fraterculus*, the average number of larvae parasitized by female of *D. longicaudata* was superior to that of *C. capitata* in densities 25, 35 and 55 (H = 83.7169; df = 6; P < 0.0001), and also larger than the densities of 1, 3, 5 and 10 for the same host species (Table II). The highest percentage of parasitism (55%) was observed in the density of three larvae per unit, similarly to that recorded *C. capitata* (Table II).

The percentage of parasitoids emerged from larvae of *C. capitata* varied in relation to densities (- χ^2_{calc} = 178.6304, - χ^2_{tab} = 12.592, df = 6 and P ≤ 0.05), from 6.5% to 21.6% and reduced with the increase in number of larva (1: 6.5%; 3: 21.6%; 5: 18.3%; 10: 17.8%; 25: 9.4%; 35: 12.1%; 55: 7.7%). The total mortality of *C. capitata*, adding death in the larval and pupal phase of the fly and added to the number of emerged parasitoids, was significantly higher as the density of larvae offered increased, from density 5 to 25 (- χ^2_{calc} = 168.99, - χ^2_{tab} = 12.592, df = 6 and P ≤ 0.05) (1: 47.2%; 3: 78.1%; 5: 56.2%; 10: 58.4 %; 25: 61.8%; 35: 51.8%; 55: 50.9%).

In *A. fraterculus* as host, the percentage of parasitoids emerged also differed between densities (- χ^2_{calc} = 101.4673, - χ^2_{tab} = 12.592, df = 6, P ≤ 0.05) being the highest value recorded in density three (1: 24.9%; 3: 36.6%; 5: 9.1%; 10: 14.6%; 25: 12.5%; 35: 27.3%; 55: 15.4%). *A. fraterculus* total mortality differs between densities (1: 80.2%; 3: 66.3%; 5: 56.4%; 10: 64.3%; 25: 53.1%; 35: 49.2%; 55: 46.1%) (- χ^2_{calc}=59.4562, - χ^2_{tab}=12.592, df=6, P ≤ 0.05).

By comparing parasitism of *D. longicaudata* between the two species of fruit fly, the average

Table II. Mean number observed (gross values transformed by √x+1) and estimated of larvae of *Anastrepha fraterculus* parasitized by females of *Diachasmimorpha longicaudata* (*Na*), exposed to different host densities (*No*), percentage of parasitized larvae and estimated values of attack rate (*a'*), search efficiency (*E*), total handling time (Th_{total}) and search time (*Ts*), based on the random model of Holling's discs equation. Data refer to a total time of exposure of three hours. Values between brackets indicate the number of repetitions.

	Host densities (No)						
Mean ± SE	**1 (20)**	**3 (20)**	**5 (20)**	**10 (20)**	**25 (20)**	**35 (20)**	**55 (20)**
Na observed (gross values)	0.099±0.25c[1]	0.232±1.65c	0.139±0.5c	0.296±1.85bc	0.513±3.85ab	0.954±11.05a	0.983±10.85a
Na observed (transformed)	1.10±0.027c	1.59±0.075c	1.18±0.055c	1.59±0.098bc	2.06±0.130ab	3.34±0.185a	3.28±0.233a
Na estimated	0.001	0.02	0.05	0.26	4.02	10.99	10.84
% parasitized larvae	25	55	10	18.5	15.4	31.54	19.72
a'	0.0024±9x10^{-4}b	0.0046±7x10^{-4}a	0.0007±3x10^{-4}b	0.0012±3x10^{-4}b	0.0009±2x10^{-4}b	0.0021±3x10^{-4}a	0.0012±2x10^{-4}ab
E	0.25±0.099b	0.55±0.077a	0.10±0.004b	0.18±0.045b	0.15±0.031b	0.32±0.041 a	0.20±0.027ab
Th_{total} (min)	19.10±7.59c	126.11±17.77c	38.21±16.17c	141.40±34.30ac	294.26±59.53ab	844.6±110.50b	929.27±113.94b
Ts (min)	160.90±7.59a	53.89±17.77a	141.79±16.17a	38.60±34.30a	---	---	---

[1]**Distinct lowercase letters in lines indicate significant difference (Kruskal-Wallis, α=0.05).**

number of individuals parasitized (*Na*) was superior in *A. fraterculus*, in densities three (H = 8.4647), 35 (H = 13.2522) and 55 (H = 9.0215) (P ≤ 0.05). But *Na* was similar in both hosts, in densities one (H = 1.0978; P = 0.2947), five (H = 2.0845; P = 0.1488), 10 (H = 0.0845; P = 0.7713) and 25 (H = 1.2131; P = 0.2707). In higher densities, for both hosts, the mean number of parasitized larvae was higher, but this did not reflect a higher parasitism rate (Tables I and II). Two factors can explain these results, drastic reduction of eggs in ovaries of females exposed to high densities and limitation in handling time that hinders a parasitoid from attacking all hosts available (Hassell et al. 1977, Zanuncio et al. 2013).

Compared mortality between hosts (without considering parasitoid emergence), was higher in *C. capitata* than in *A. fraterculus* at densities of three larvae/unit (H = 5.3371; P = 0.0209), 35 (H = 17.1475; P ≤ 0.0001) and 55 (H = 10.0522; P = 0.0015), coinciding with densities where the number of parasitized larvae was superior for *A. fraterculus*. Mortality did not differ between species of fruit fly in densities one (H = 1.1335; P = 0.287), five (H = 1.715; P = 0.1903), 10 (H = 1.7219; P = 0.1805) and 25 (H = 2.5182; P = 0.1125).

During the parasitism activity, the puncture caused by females of *D. longicaudata* may have been harmful to larvae of *C. capitata*, smaller than those of *A. fraterculus*, leading then to death. This can aid in the control of pest species, however without generating a new parasitoid. Even for *A. fraterculus*, smaller individuals can be more affected by puncture. Van Nieuwenhove & Ovruski (2011) assigned the high mortality of larvae of *A. fraterculus* in the first and second instar to the wound caused by the insertion of *D. longicaudata* female ovipositor, since 90% of puparia not emerged dissected contained dead eggs or dead larvae of the braconid. For the population control of the pest species in field, this characteristic can be advantageous,

however, for purposes of the parasitoid multiplication through mass breeding, larvae of *A. fraterculus* after the third instar could present better results as hosts.

The parasitism data were adjusted to the random model, in both species of fruit fly, evidencing a type III functional response, confirmed by the high pseudo-r^2 (0.9768 and 0.9785, for *C. capitata* and *A. fraterculus*, respectively). Although sigmoid curves are not graphically observed, there is a sharp rise of parasitism rate across the three first densities, with inflexion of curves becoming more tenuous after density 10 (Figures 1 and 2). Moreover, the Th_{total} increased with the increase in hosts availability (Tables I and II), corroborating type III functional response. Through the non-linear least squares method it was possible to estimate the values for component *Th* (21.09 min in *C. capitata* and 76.43 min in *A. fraterculus*). The maximum number of hosts that can be parasitized in the time period considered (three hours) was estimated in 8.53 larvae of *C. capitata* and 2.35 larvae of *A. fraterculus*.

Although in review articles the type II response is more common in parasitoids (Fernández-Arhex & Corley 2003), Montoya et al. (2000) also obtained adjustment of type III functional response for *D. longicaudata*, having as host *Anastrepha ludens* (Loew). This same type of functional response was found for another *C. capitata* parasitoid, *Aganaspis daci* (Hymenoptera: Figitidae) in larvae provided in artificial diet or in fruit, under laboratory conditions, whereas in semi-field (greenhouse conditions), the response was type II (Pedro et al. 2017). According to Zanuncio et al. (2013), for *Campoletis flavicincta* Ashmead (Hymenoptera: Ichneumonidae) parasitizing larvae of *Spodoptera frugiperda* (J. F. Smith) the type III of response were found, but they argue that under natural field conditions, the sigmoid response could be more common since artificial lab conditions would be limiting.

The difference of the present work against that of Harbi et al. (2018) who recorded type II functional response can be associated, among other variables, to the densities used in each study. The authors only used densities in the proportion of 1 (female): 5 (larvae), 1:20 or 1:40, while the present study assessed seven densities, two of them lower and one higher than that of the paper mentioned, which made

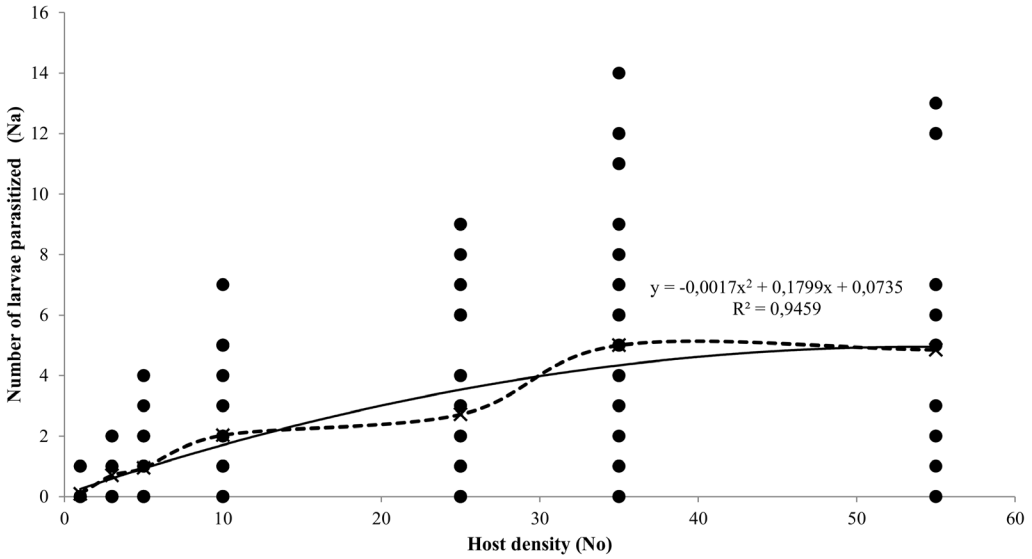

Figure 1. Functional response of *Diachasmimorpha longicaudata* in *Ceratitis capitata*: ●, number of larvae parasitized in each repetition; x, average number of larvae parasitized for each density. The continuous line refers to the adjustment of the polynomial model to data observed.

possible a more detailed observation of the response as function of density.

Th values estimated for the two hosts were higher than those of Th_{total} calculated based on data observed, for densities one, three and five in *C. capitata* and one and five in *A. fraterculus* (Tables I and II). Besides, in *A. fraterculus*, the maximum number of hosts that can be parasitized in the time period considered (2.35 larvae), was exceeded by data observed in densities 25, 35 and 55 (Table II), showing that *Th* may have been overestimated by the non-linear least squares method. While assessing the total handling time (Th_{total}) it was recorded, for the two species of fruit fly a trend to increase as the hosts density increased ($P \leq 0.05$), which is confirmed by the growing number of parasitized individuals (*Na*) observed with increase in densities (Tables I and II).

It was verified a reduction in the estimated search time (*Ts*) as hosts density increased (Tables I and II). Response that corroborates Hassell (1978) study, according to which species with sigmoid or curvilinear functional response present *Ts* reduction as hosts density increases. The estimated search efficiency (*E*) and the instant attack rate (*a'*) remained constant with increase in density for *C. capitata* ($P \geq 0.05$) (Table I), while in *A. fraterculus* these parameters presented significantly higher values in densities 3, 35 and 55 ($P \leq 0.05$), also corresponding to the highest percentages of parasitized larvae (Table II).

Type III functional response, according to Hassell et al. (1977) and Jervis & Kidd (1996), applies, most commonly, to the behavior of predator vertebrates, with type II being the most frequent among invertebrates, different from what was observed in the present work. According to Fujii et al. (1986), in type III, predators can learn to concentrate on a prey when it is abundant, increasing the time spent in handling, as demonstrated for *D. longicaudata* in this study, in highest densities.

Extrapolation of parameters obtained under laboratory conditions to field has been considered with caution due to the artificiality in which experiments are conducted and to the confinement of tests to arenas with restricted dimensions, so that in general they don't reproduce field densities (Jervis & Kidd 1996). Other factors, besides population density, like average room temperature, can also affect the parasitoids response, as demonstrated by Khan

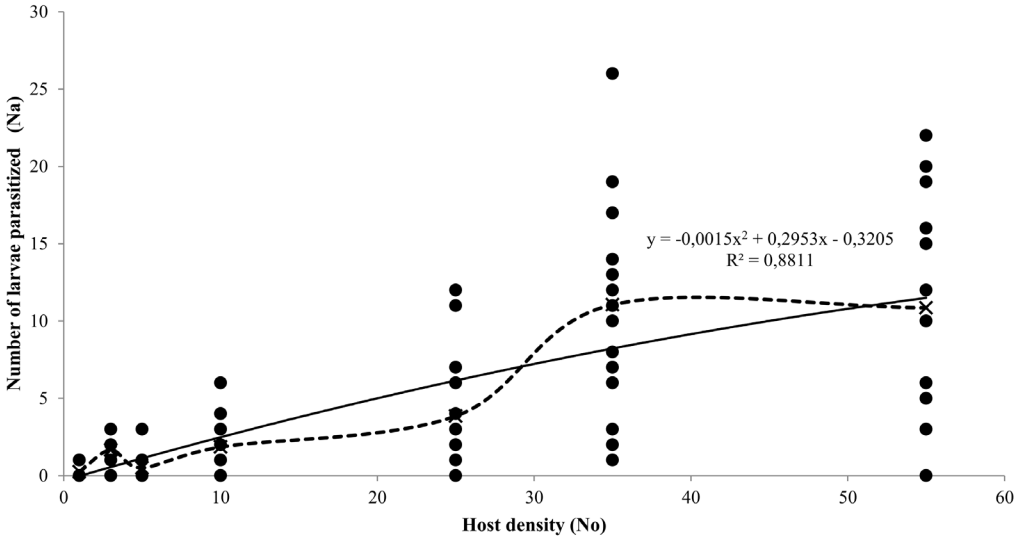

Figure 2. Functional response of *Diachasmimorpha longicaudata* in *Anastrepha fraterculus*: ●, number of larvae parasitized in each repetition; x, average number of larvae parasitized for each density. The continuous line refers to the adjustment of the polynomial model to data observed.

et al. (2016) while assessing other species of Braconidae, parasitizing Aphididae. The authors inferred that the parasitoid is more efficient at temperatures below 30 °C. It was observed by Harbi et al. (2018) that *D. longicaudata* is able to parasitize the Mediterranean fruit fly in temperatures ranging from 20–29 °C. In the present study this factor was not assessed, and the tested average temperature was kept around 25 °C. This is an aspect that should be evaluated, mainly considering different environmental conditions in different regions of the country where it is intended to use biological control by releasing this species.

The knowledge of the type of functional response presented by a natural enemy is important because it represents distinct effects on the stability of population interactions and so can affect the efficacy of a biological control program (Hassell 1978, Hassell & Waage 1984). Regardless of these limitations, the results herein presented, associated to other investigation on bioecology of species involved and, chiefly on the system population dynamics, will make feasible the obtention of a more realistic panorama of this interaction.

Evaluation of preference of *Diachasmimorpha longicaudata* for host

Diachasmimorpha longicaudata parasitism rate did not differ across host species when the parasitoid was reared in *C. capitata* (z = -0.169; P = 0.8658), indicating that there is no preference in the choice. However, the percentage of female emergence was superior in *A. fraterculus* (z = -2.1704; P = 0.03) (Table III).

When the parasitoid females were originated from *A. fraterculus*, they presented higher percentage of parasitism (z = -3.0584; P = 0.0022) and female emergence on this same host (z = -3.3316; P = 0.0009) (Table III). In this case, the host species where the female developed seems to influence the parasitism rate and this may occur due to learning, since it plays fundamental role in a variety of decisions made by different groups of insects (Papaj & Lewis 1993). Familiarity with the host can result in change of behavior, and the retention of the acquired information, in physiological terms, is referred to as memory (Matthews & Matthews 2010). It is known that females of *D. longicaudata* have innate preference for fruits of their host larvae and that preference can be altered by learning (Segura et al. 2016). Thus, it may be assumed that *D. longicaudata* females can recognize physiological characteristics appropriate to the hosts where they developed especially in mass rearing, where there are no host fruits.

Though being capable of parasitizing the two species of fruit fly, females of *D. longicaudata* reared in larvae of *A. fraterculus*

Table III. Average percentage (± SE) of parasitism and emergence of females of *Diachasmimorpha longicaudata* from different origins on *Ceratitis capitata* (n=20) and *Anastrepha fraterculus* (n = 20), in experiment of preference for host.

	Origin of *females of D. longicaudata*							
	C. capitata				*A. fraterculus*			
Host species	Parasitism (%)		Emergence of females (%)		Parasitism (%)		Emergence of females (%)	
C. capitata	13.0 ± 3.70	Aa[1]	23.7 ± 8.65	Ba	16.0 ± 3.93	Ba	25.7 ± 8.93	Ba
A. fraterculus	14.0 ± 4.06	Ab	52.5 ± 11.16	Ab	40.0 ± 5.47	Aa	72.0 ± 7.20	Aa

[1]**Capital letters compare the same parameter among different host species, in columns. Lowercase letters compare the same parameter, in one same host species, among origins, in lines. Distinct letters indicate significant difference (Wilcoxon, α = 0.05).**

preferred larvae of this species for oviposition, when there was a choice. The preference for larvae of *A. fraterculus* was also observed by Ovruski et al. (2011), however, only in double choice tests. The host size, which would be associated to availability of resource for the parasitoids offspring development (Clausen 1939), seems to be the factor responsible for this preference, since Silva et al. (2007) observed that *D. longicaudata* does not discriminate odors of volatile substance emitted by *C. capitata* or *A. fraterculus*. On the other hand, it is known that females of parasitoids can use information both of their hosts and habitat (fly host fruits) to find larvae. Segura et al. (2016) demonstrated that females of *D. longicaudata* are guided, preferably, to the specific habitat of their hosts, in absence of direct signs, reducing the search areas. Messing et al. (1993), Cancino et al. (2002) and López et al. (2009) also demonstrated that females of *D. longicaudata* prefer larger larvae for the development of their offspring. Besides, the host size can directly influence that of the generated parasitoid (Godfray 1994) and larger parasitoids tend to present more chances of reproductive success, with higher fecundity and fertility (Jervis 2005).

The emergence of parasitoid females was higher in larvae of *A. frateculus* as host, regardless of the origin where these parasitoids were reared (z = -3.1792; P = 0.0015 and z = -2.0251; P = 0.0429) (Table III). Female emergence rate can be used as indicator of preference of one parasitoid for the host, and was observed in several studies as parameter for evaluation of preference in *D. longicaudata* (Eben et al. 2000, Mansfield & Mills 2004, Ovruski et al. 2011). In experiments made by Eben et al. (2000) with *D. longicaudata* reared in larger larvae, higher proportion of females in the offspring was recorded, corroborating the results found in the present work.

Lower emergence of *A. fraterculus* was observed in larvae exposed to females of parasitoids that were originally reared in this same species (z = -2.5956; P = 0.0094) (Table IV), evidencing preference and higher efficiency of the parasitoid to suppress the population of *A. fraterculus*, when reared in this same host. In choice test involving these two hosts, Ovruski et al. (2011) observed that females of *D. longicaudata* visit equally the larvae of the two species of fruit fly, but make higher number of proofs in those of *A. fraterculus*. In laboratory, Meirelles et al. (2013) demonstrated that females of *D. longicaudata* reared in *A. fraterculus* present higher net reproduction rate and larger individuals when compared to those from *C. capitata*.

Flies mortality did not differ between the two species, regardless of the parasitoids original species (*C. capitata,* z = -1.3491; P = 0.1773 and *A. fraterculus,* z = -1.609; P = 0.1089) (Table IV). The lack of difference in mortality between

Table IV. Average percentage (± SE) of emergence and mortality of *Ceratitis capitata* (n = 20) and *Anastrepha fraterculus* (n = 20) exposed to *Diachasmimorpha longicaudata* from different origins, in experiment of preference for host.

Origin of parasitoid	Emergence of		Mortality of	
	***C. capitata* (%)**	***A. fraterculus* (%)**	***C. capitata* (%)**	***A. fraterculus* (%)**
C. capitata	63.5 ± 6.25ns[1]	56.0 ± 4.88ns	23.5 ± 4.54ns	27.0 ± 3.25ns
A. fraterculus	59.5 ± 3.36a[2]	42.0 ± 5.55b	25.0 ± 3.66ns	18.5 ± 3.42ns

[1] **No significant difference (Wilcoxon α = 0.05).**
[2] **Distinct lowercase letters in lines indicate significant difference (Wilcoxon, α = 0.05).**

host species can be associated to the fact pointed out by Arthur (1981), according to whom the parasitoid females make proofs on larvae to assess their content and not always make oviposition, but affect the fly development.

This work data confirms that, both *C. capitata* and *A. fraterculus* are feasible hosts for the parasitoid multiplication under the tested conditions and that the efficiency of parasitism in field and the progeny of female parasitoids could be increased by using larvae of *A. fraterculus* in the rearing of *D. longicaudata*, particularly if the target control species is *A. fraterculus*.

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Each author contributed individually and significantly for the development of the study. DLA, LRR and SMJ conceived and planned the study. DLA carried out the experiments. DLA, LRR, CFSE and SMJ contributed to the interpretation of the results. CFSE and DLA took the lead in writing the manuscript. All authors provided critical feedback and reviewed the manuscript.