



**Effect of Exposure Time and Ratio of Hosts to Female Parasitoids on Offspring Production of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) Reared on *Anastrepha fraterculus* (Diptera: Tephritidae) Larvae**

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Source: Florida Entomologist, 95(1) : 99-104

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.095.0116>

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## EFFECT OF EXPOSURE TIME AND RATIO OF HOSTS TO FEMALE PARASITOIDS ON OFFSPRING PRODUCTION OF *DIACHASMIMORPHA LONGICAUDATA* (HYMNEOPTERA: BRACONIDAE) REARED ON *ANASTREPHA FRATERCULUS* (DIPTERA: TEPHRITIDAE) LARVAE

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### ABSTRACT

*Diachasmimorpha longicaudata* (Ashmead) is a larval-prepupal endoparasitoid of tephritid fruit fly pests in tropical and subtropical countries. Because citrus-growing areas of northern Argentina are the most affected by *Anastrepha fraterculus* (Wiedemann), *D. longicaudata* presents as a potential candidate for augmentative releases against it. Within this context, parasitoid rearing must be focused on offspring yield optimization in order to sustain a successful mass-rearing process. Hence, the best ratio of *A. fraterculus* larva to female parasitoids and the best exposure time for achieving the greatest parasitoid yield with the highest proportion of female progeny were determined under laboratory conditions in the present study. The effect of superparasitism on the percentage of *D. longicaudata* emergence was also assessed. Third-instars of *A. fraterculus* were exposed for 30, 60, 90, and 120 min to 30 mated, 5-7 d-old parasitoid females at host/parasitoid ratios of 2:1, 4:1, 6:1, 8:1, and 10:1. Results showed that a low ratio of hosts exposed to parasitoids for a short time was essential to achieve parasitoid emergence rates exceeding 70%, and to achieve a ♀:♂ sex ratio of at least 61:39. Increasing both the ratio of host larvae to parasitoid further than 4:1 and the exposure time of *D. longicaudata* to hosts beyond 30 min did not significantly enhance overall parasitoid progeny yield. There was a significant negative correlation between the number of parasitoid 1st instars recorded per dissected host pupa and the percentage of parasitoid emergence. Conversely, a significant positive correlation was found between superparasitism and additional host mortality. Although a female-biased sex ratio resulted in all treatments, the greater parasitoid female progeny proportions were found in association with the higher levels of superparasitism.

**Key Words:** fruit flies, parasitoids, offspring production, superparasitism, biological control, Argentina

### RESUMEN

*Diachasmimorpha longicaudata* (Ashmead) es un endoparásitoide larvo-pupal de especies de moscas de la fruta plagas en países tropicales y subtropicales. Considerando que *Anastrepha fraterculus* (Wiedemann) es una seria plaga en las áreas cítricas del norte de Argentina, *D. longicaudata* surge como un potencial candidato para liberaciones aumentativas contra esta especie de tefrítido. En este contexto, la cría del parasitoide debe estar dirigida en la optimización de la producción de descendientes para mantener exitosamente el proceso de cría masiva. Por lo tanto, en el presente estudio se determinó la mejor proporción y tiempo de exposición de larvas de *A. fraterculus* para ser ofrecidas a una hembra del parasitoide bajo condiciones de laboratorio. El objetivo fue alcanzar la mayor producción de parasitoides con la más alta proporción de hembras en la progenie. Además, se evaluó el efecto del superparasitismo sobre el porcentaje de emergencia de *D. longicaudata*. Se expusieron larvas de *A. fraterculus* del tercer estadio durante 30, 60, 90, and 120 minutos a 30 hembras copuladas de *D. longicaudata* de 5-7 días de edad, en proporciones de 2, 4, 6, 8 y 10 larvas huéspedes por cada parasitoide. Los resultados indicaron que una baja proporción de huéspedes expuestos al parasitoide durante poco tiempo fue esencial para lograr porcentajes de emergencia del parasitoide superiores al 70% y para mantener una tasa sexual tendiente al 61% de hembras. El incremento de la proporción de larvas huéspedes por parasitoide más allá de 4:1 y el tiempo de exposición de *D. longicaudata* a huéspedes más de 30 minutos no aumentó significativamente la producción total de progenie del parasitoide. Se obtuvo una correlación negativa y significativa entre el número de larvas de *D. longicaudata* del 1er estadio y el porcentaje de emergencia de parasitoides. Por el contrario, se registró una correlación positiva y significativa entre el superparasitismo y la mortalidad adicional del huésped. Aunque en todos los tratamientos evaluados se obtuvo una tasa sexual con predominancia de hembras, la mayor producción de descendientes hembras del parasitoide estuvo asociada con los niveles más altos de superparasitismo.

More than 5 exotic parasitoids were introduced into Argentina from Costa Rica and México in the 1960s for the biological control of both tephritid pest species *Anastrepha fraterculus* (Wiedemann) and *Ceratitis capitata* (Wiedemann) (Ovruski et al. 2000). One of them, *Diachasmimorpha longicaudata* (Ashmead) became firmly established on *A. fraterculus* in the citrus-growing areas of northeastern and northwestern Argentina (Oroño & Ovruski 2007; Schliserman et al. 2010). This opiine braconid is a koinobiont, solitary, larval-prepupal endoparasitoid of several tephritid genera that was originally collected in the Indo-Philippine region attacking *Bactrocera* spp. (Sivinski et al. 2006). At present, *D. longicaudata* is widely mass-reared for use in augmentative releases against economically important fruit fly species in tropical and subtropical countries, such as *Anastrepha* spp. (Sivinski et al. 1996; Montoya et al. 2000a), *Bactrocera dorsalis* (Hendel) (Purcell et al. 1994; Vargas et al. 2002), and *C. capitata* (Malavasi et al. 2007; Paranhos et al. 2008).

Because citrus-growing areas of northern Argentina are the most affected by *A. fraterculus*, which is one of the major fruit crop pests in Argentina (Guillén & Sánchez 2007), *D. longicaudata* presents as a potential candidate for augmentative releases against this tephritid pest (Ovruski et al. 2011) in combination with other environment-friendly methods by using an area-wide approach. In this framework, *D. longicaudata* rearing must be focused on parasitoid yield optimization in order to achieve and sustain a successful mass-rearing process (Cancino & Montoya 2004; Montoya et al. 2007).

To reach this goal, the different factors affecting the production of laboratory-reared parasitoids should be carefully assessed (Heimpel & Lundgren 2000). Among these factors, host density and exposure time to parasitoids may influence the reproductive efficiency of *D. longicaudata* in the mass rearing process (Ramadan et al. 1994; Montoya et al. 2000b; Cancino et al. 2002). In this regard, the aims of the current study were to determine the best ratio of *A. fraterculus* larvae to female parasitoids and the best exposure time in order to achieve the greatest parasitoid yield with the highest female-biased progeny ratio in the laboratory rearing of *D. longicaudata*. The present study also examined the effect of superparasitism on the percentage of emergence of *D. longicaudata* adults. This research paper is part of an assessment of *D. longicaudata* as a biocontrol agent of *A. fraterculus* in Argentina (Ovruski et al. 2011; Van Nieuwenhove & Ovruski 2011).

## MATERIALS AND METHODS

### Study Site and Insect Rearing

The study was conducted at the Biological Control Division of the Planta Piloto de Procesos

Industriales Microbiológicos y Biotecnología (PROIMI), located in San Miguel de Tucumán, Argentina. Laboratory conditions were  $25 \pm 1^\circ\text{C}$ ;  $75 \pm 5\%$  RH, with a photoperiod of 12:12 h L:D. The colony of *D. longicaudata* used in the assays was reared and maintained on late-third *A. fraterculus* instars at the PROIMI laboratory according to the rearing method described by Ovruski et al. (2011). The *A. fraterculus* rearing procedure was carried out as described by Vera et al. (2007).

### Experimental Procedure

To determine the optimal proportion of *A. fraterculus* larvae per *D. longicaudata* female and optimal amount of time to expose host larvae to parasitoids for rearing, 5 host/parasitoid ratios were individually analyzed: 2:1, 4:1, 6:1, 8:1, and 10:1. Thirty mated, 5-7 d-old *D. longicaudata* females that had no prior oviposition experience were used for each host/parasitoid ratio treatment. Therefore, the host densities were 60, 120, 180, 240, and 300 laboratory-reared, 9-11 d-old *A. fraterculus* larvae per artificial oviposition device. The larval age to expose hosts to parasitoids was chosen because the maximum yield of *D. longicaudata* offspring can be achieved using 9-12 d-old *A. fraterculus* larvae as hosts (Van Nieuwenhove & Ovruski 2011). The oviposition unit was an organdy screen-covered Petri dish (8 cm diam, 0.8 cm deep) similar to the widely used device for rearing opiine fruit fly larval parasitoids in the laboratory (Wong & Ramadan 1992,; Duan & Messing 2000). Each oviposition unit was filled with fresh larval diet (hydrolyzed brewer yeast + wheat germ + sugar + agar + water). Hosts at each density were exposed to parasitoid females in a separate cube-shaped Plexiglas cage (20 cm) for 30, 60, 90, and 120 min. These host exposure times were established on the basis of the time used for *D. longicaudata* mass rearing on *Anastrepha ludens* (Loew) larvae at the Moscafrut Metapa (México) facility, in which uses a 2 h exposure (Cancino & Montoya 2004). After exposure to parasitoids, larvae were transferred into plastic trays (15 × 11 × 1.5 cm) filled with fresh larval diet. The trays were then placed in a plastic container (33 × 23 × 11 cm) with a 2 cm-vermiculite layer on the bottom to allow pupation, and covered with organdy on the top. After 2 d, dead larvae were removed from trays and counted. Also, pupae were removed from the pupation medium and a sample of 10 pupae per treatment was dissected to record the number of 1st instar parasitoids per host (Montoya et al. 2000b). Host pupae dissection was performed to determine the effect of superparasitism on the percentage of emergence of *D. longicaudata* adults. The remaining pupae were held within plastic cups (8 cm diam, 5 cm high) with fresh vermiculite until the emergence of both flies and parasitoids. Then, the number and sex of emerged parasitoids were recorded. The pupae that did not yield parasitoids or flies after 40 d were con-

sidered as non-eclosed pupae. A control test, i.e., unexposed host larvae to parasitoids, was included per treatment to estimate natural host mortality. Thus, the number of dead larvae, emerged flies, and non-eclosed pupae were also recorded for control tests. Nevertheless, to be sure of the high quality of *A. fraterculus* larvae used in treatments, all host larvae sets with percentages of control flies emergence <85% were discarded from study. Treatments and control tests were replicated 12 times.

Data Analysis

The percentage of emerged parasitoids was calculated as the total number of emerged progeny divided by the number of recovered pupae. Sex ratio of parasitoid offspring was calculated as the percentage of emerged females. Host mortality was determined as the sum of dead host larvae plus non-eclosed pupae divided by the total number of larvae exposed in the oviposition device × 100. The mortality in the control was used to correct host mortality by subtracting control mortality from the treatment (Montoya et al. 2000b).

Data on parasitoid emergence, sex ratio of parasitoid offspring, additional host mortality, and number of *D. longicaudata* 1st instar larvae per host pupa recorded from each host/parasitoid ratio and host exposure time were compared by means of two-way multivariate analyses of vari-

ance (MANOVA) ( $P < 0.05$ ). Mean comparisons were analyzed by Tukey's honestly significant difference (HSD) test at  $P = 0.05$ . The relationship between either parasitoid emergence or additional host mortality, and the number of *D. longicaudata* 1st instar larvae per host was analyzed by Pearson's correlation test ( $P < 0.05$ ). To normalize distribution of the percentage and count data, arcsine  $\sqrt{\%}$  and  $L_n(x+1)$  transformations were applied, respectively. However, untransformed means ( $\pm$  SD) were presented in the text.

RESULTS

The ratio of host larvae exposed to *D. longicaudata* females greatly influenced the percentage of emerged parasitoids, the sex ratio of parasitoid offspring, the additional host mortality, and the number of *D. longicaudata* 1st instar larvae per host pupa (Wilks'  $\lambda = 0.043061$ ,  $F_{(12, 663.684)} = 74.64$ ,  $P < 0.0001$ ). Similarly, host exposure time to parasitoids significantly affected all the response variables mentioned above (Wilks'  $\lambda = 0.316994$ ,  $F_{(12, 574.420)} = 26.03$ ,  $P < 0.0001$ ). The interaction between both categorical factors (host/parasitoid ratio vs. host exposure time) was also significant (Wilks'  $\lambda = 0.733481$ ,  $F_{(48, 837.945)} = 1.46$ ,  $P = 0.0239$ ). Parasitoid emergence increased considerably at 4:1, 6:1 and 8:1 host/parasitoid ratios over the shorter host larvae exposure times (Table 1). Pro-

TABLE 1. MEAN ( $\pm$  SD) PERCENTAGE OF *D. LONGICAUDATA* EMERGENCE, SEX RATIO OF PARASITOID OFFSPRING (PERCENT FEMALES), PERCENTAGE OF ADDITIONAL *A. FRATERCULUS* MORTALITY, AND SUPERPARASITISM RECORDED FROM *A. FRATERCULUS* AT DIFFERENT HOST-PARASITOID RATIOS AND HOST EXPOSURE TIMES TO PARASITOID FEMALES.

Host:parasitoid ratios	Exposure times (min)	% Parasitoid Emergence	% Female offspring	% Additional host mortality	Superparasitism <sup>1</sup>
2:1	120	26.8 $\pm$ 5.6 a	<b>84.9 <math>\pm</math> 10.5 ab</b>	<b>57.7 <math>\pm</math> 5.8 a</b>	<b>9.9 <math>\pm</math> 1.4 a</b>
	90	29.0 $\pm$ 8.9 a	<b>85.7 <math>\pm</math> 8.3 a</b>	<b>49.6 <math>\pm</math> 9.2 a</b>	<b>8.5 <math>\pm</math> 1.6 a</b>
	60	32.5 $\pm$ 5.5 a	<b>76.1 <math>\pm</math> 7.1 bc</b>	<b>44.7 <math>\pm</math> 9.7 a</b>	<b>8.1 <math>\pm</math> 2.0 a</b>
	30	42.7 $\pm$ 6.2 b	69.1 $\pm$ 9.6 cde	34.4 $\pm$ 6.0 b	<b>6.2 <math>\pm</math> 1.8 b</b>
4:1	120	49.7 $\pm$ 8.6 bcd	<b>74.8 <math>\pm</math> 7.5 c</b>	28.3 $\pm$ 6.9 b	3.9 $\pm$ 0.9 c
	90	57.5 $\pm$ 7.2 def	71.6 $\pm$ 6.0 cd	24.5 $\pm$ 5.1 bc	4.7 $\pm$ 1.9 bc
	60	<b>65.0 <math>\pm</math> 10.6 efg</b>	67.3 $\pm$ 5.1 cde	16.8 $\pm$ 4.9 c	3.5 $\pm$ 1.4 c
	30	<b>72.7 <math>\pm</math> 8.2 g</b>	60.7 $\pm$ 5.9 e	8.5 $\pm$ 3.5 de	2.3 $\pm$ 1.2 cd
6:1	120	53.2 $\pm$ 5.5 cde	69.1 $\pm$ 4.5 cde	26.9 $\pm$ 5.0 bc	2.9 $\pm$ 1.0 cd
	90	56.1 $\pm$ 2.8 de	62.9 $\pm$ 5.7 de	23.4 $\pm$ 4.9 c	2.5 $\pm$ 1.1 cd
	60	<b>66.2 <math>\pm</math> 4.7 fg</b>	62.6 $\pm$ 3.5 de	14.6 $\pm$ 5.5 e	2.3 $\pm$ 1.4 cd
	30	<b>74.3 <math>\pm</math> 5.6 g</b>	61.8 $\pm$ 4.4 e	7.3 $\pm$ 3.6 de	1.3 $\pm$ 1.1 d
8:1	120	46.4 $\pm$ 7.6 bc	66.5 $\pm$ 7.4 de	23.9 $\pm$ 6.5 c	2.1 $\pm$ 1.2 d
	90	51.7 $\pm$ 4.2 bcde	65.7 $\pm$ 5.8 de	21.2 $\pm$ 6.7 c	2.3 $\pm$ 1.1 d
	60	55.9 $\pm$ 5.1 de	63.8 $\pm$ 5.3 de	16.9 $\pm$ 4.5 c	1.5 $\pm$ 1.0 d
	30	<b>67.1 <math>\pm</math> 6.9 fg</b>	60.9 $\pm$ 4.9 e	9.2 $\pm$ 4.1 de	1.3 $\pm$ 0.6 d
10:1	120	45.3 $\pm$ 4.3 bc	68.2 $\pm$ 4.6 cde	21.4 $\pm$ 5.6 c	1.5 $\pm$ 0.5 d
	90	50.1 $\pm$ 7.0 bcd	67.5 $\pm$ 3.6 cde	16.5 $\pm$ 4.2 ce	1.7 $\pm$ 0.7 d
	60	51.4 $\pm$ 4.7 bcd	64.6 $\pm$ 5.8 e	11.0 $\pm$ 3.9 de	1.3 $\pm$ 0.6 d
	30	62.4 $\pm$ 5.5 ef	60.1 $\pm$ 3.9 e	4.1 $\pm$ 2.3 d	1.1 $\pm$ 0.9 d

<sup>1</sup>Number of *D. longicaudata* 1st instar larvae per *A. fraterculus* pupa. Mean value followed in the same column by the same letter indicates no significant difference (Tukey's test,  $P = 0.05$ ). Means highlighted in bold indicate significantly higher values.



portion of parasitoid female offspring was noticeably greater at 2:1 host/parasitoid ratio and at longer host exposure times (90-120 min) than in higher host densities and shorter exposure times (Table 1). However, the sex ratio of parasitoid offspring (percent females) was not lower than 60% in all of the other treatments (Table 1). Additional host mortality significantly decreased at the 30-min exposure time when parasitoid females were exposed to *A. fraterculus* larvae at higher host densities than at the 2:1 host/parasitoid ratio (Table 1). The number of *D. longicaudata* 1st instar larvae recorded per dissected host pupa increased appreciably at the lowest host density associated with the four exposure times assessed in this experiment (Table 1). There was a marked negative correlation between the number of 1st instar parasitoid larvae and the percentage of parasitoid emergence ( $r = -0.6250$ ,  $N = 240$ ,  $P < 0.0001$ ) (Fig. 1). Conversely, a strong positive correlation was found between superparasitism and the additional host mortality rate ( $r = 0.7856$ ,  $N = 240$ ,  $P < 0.0001$ ) (Fig. 2).

#### DISCUSSION

Results from the present study clearly showed that a relatively low ratio of hosts exposed to parasitoids for a short time was critical to achieve parasitoid emergence rates exceeding 70%, and to achieve a ♀:♂ sex ratio of at least 61:39. This finding indicated that increasing both the ratio of host larvae to female parasitoids further than 4:1 and the exposure time of *D. longicaudata* to hosts beyond 30 min did not significantly enhance overall parasitoid progeny yield. Similarly, Montoya et al. (2000b) did not find major differences between the percentage of emergence of *D. longicaudata* adults at 1:4 and 1:8 parasitoid/host

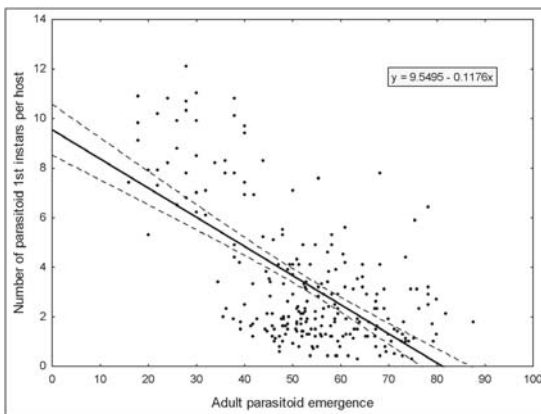


Fig. 1. Relationship between number of *Diachasmimorpha longicaudata* 1st instar larvae per *Anastrepha fraterculus* pupa (= superparasitism) and percentage of *D. longicaudata* emergence.

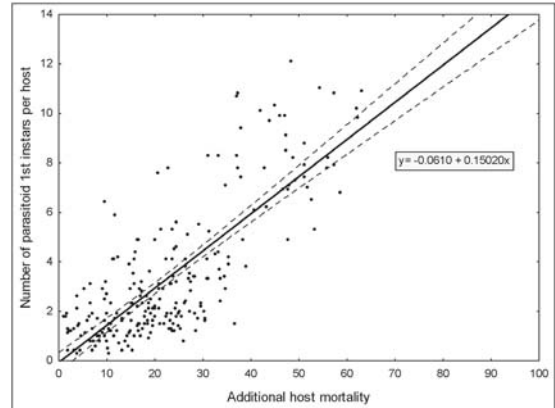


Fig. 2. Relationship between number of *Diachasmimorpha longicaudata* 1st instar larvae per *Anastrepha fraterculus* pupa (= superparasitism) and percentage of additional *A. fraterculus* mortality (larval and pupal mortalities).

ratios using irradiated *A. ludens* larvae as hosts for the parasitoids.

This study also demonstrated that significant yield reductions of parasitoid progeny at the lower densities of *A. fraterculus* larvae that were exposed to *D. longicaudata* females, as well as at longer host exposure times, involved major increases of host mortality. These results could be mainly attributed to two negative effects of the parasitoid female oviposition activity. First, the direct killing of *A. fraterculus* larvae produced by an excessive number of ovipositor insertions into their bodies. González et al. (2007) reported that the mortality of the *A. ludens* larvae may be due to a decrease in host quality resulting from the damage generated by *D. longicaudata* oviposition. Secondly, increased host mortality induced by superparasitism. This assumption would be supported by correlation data recorded in the present study between the number of 1st instar parasitoid larvae per host pupa and both the percentage of *D. longicaudata* emergence and the rate of additional host mortality. Moreover, when dissections of unemerged host pupae from all treatments were made to detect *D. longicaudata* 1st instar larvae, noticeably higher incidences of superparasitized pupae were found at the lowest host densities as well as at longer host exposure times. These results would confirm previous observations by Montoya et al. (2000b), who noted that at low *A. ludens* larvae densities, and in the presence of conspecifics, *D. longicaudata* females increase their oviposition activity. Nonetheless, it is a well-known fact that *D. longicaudata* is a parasitoid with a strong tendency to superparasitism not only under laboratory conditions (González et al. 2007) but also under natural field conditions (González et al. 2010).

Although a female-biased sex ratio was achieved at all host/parasitoid ratios and host ex-

posure times assessed in the present study, the greater parasitoid female progeny proportions was found in association with the higher levels of superparasitism. This finding agrees with observations by González et al. (2007) on *D. longicaudata* parasitizing *A. ludens* under mass rearing conditions. These authors reported that moderate to high levels of superparasitism resulted in a sex ratio biased to females. As suggested by Montoya et al. (2011) superparasitism could be an effective reproductive strategy for ensuring survival chances of at least 1 *D. longicaudata* female larva against host immune defence mechanisms.

Although the results of this study may be used to optimize the *D. longicaudata* rearing method by using *A. fraterculus* as host for biological control purposes in Argentina, understanding all the factors that influence offspring production is important for successful parasitoid rearing. Therefore, additional studies in laboratory mostly focused on the evaluation of the female parasitoid cage density (Paranhos et al. 2008), female parasitoid age (Wong & Ramadan 1992), demographic parameters (Vargas et al. 2002; Viscarret et al. 2006), and the quality of the host larvae, e.g. size (Cancino & Montoya 2004; López et al. 2009) may be needed to achieve more efficient production of *D. longicaudata* on *A. fraterculus* larvae.

#### ACKNOWLEDGMENTS

We would like to express our gratitude to Jorge Cancino-Díaz and Pablo Montoya (Mexican MOSCAMED Program, Metapa de Domínguez, Chiapas, México) for allowing us to introduce *D. longicaudata* specimens from México into Argentina. Financial support was provided by Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (CONICET) (Grant PIP/2005 No. 5129) and by Agencia Nacional de Promoción Científica y Tecnológica de Argentina through Fondo Nacional de Ciencia y Tecnología (FONCYT) (Grant PICT/2010 No. 0393).

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