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Superparasitism of Ceratitis capitata and Anastrepha fraterculus (Diptera: Tephritidae) by Diachasmimorpha longicaudata (Hymenoptera: Braconidae)

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SUPERPARASITISM OF *CERATITIS CAPITATA* AND *ANASTREPHA FRATERCULUS* (DIPTERA: TEPHRITIDAE) BY *DIACHASMIMORPHA LONGICAUDATA* (HYMENOPTERA: BRACONIDAE)

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ABSTRACT

Diachasmimorpha longicaudata is the most widely used braconid in biological control programs of fruit flies around the world. The objective of this study was to investigate the influence of different parasitoid-host ratios on *D. longicaudata* parasitism of *Ceratitis capitata* and *Anastrepah fraterculus*. Larvae of these fruit fly species were exposed to 5 different parasitoid-host ratios (4:1, 2:1, 1:1, 1:2 and 1:4). Subsequently, the numbers of oviposition scars and of parasitoid larvae per host were evaluated in the pupal stage. In both tephritid species, a larger number of scars and parasitoid larvae were observed at the 4:1 and 2:1 ratios. Results suggest that females of *D. longicaudata* can superparasitize both hosts. The differences between the tested parasitoid-host ratios suggest that there is an optimum parasitoid-host ratio for multiplying this natural enemy. These results are important for improving mass rearing programs of *D. longicaudata*.

Key Words: *Anastrepha fraterculus*, *Ceratitis capitata*, *Diachasmimorpha longicaudata*, superparasitism

RESUMO

Diachasmimorpha longicaudata é o braconídeo mais utilizado em programas de controle biológico de moscas-das-frutas no mundo. O objetivo deste trabalho foi verificar a influência de coespecíficos no parasitismo sobre *C. capitata* e *A. fraterculus*, em diferentes razões de parasitoides e hospedeiros. Avaliamos o número de marcas de punctura e o de larvas do parasitoide no interior de pupários das duas espécies hospedeiras, após as larvas de moscas serem expostas nas razões (4:1, 2:1, 1:1, 1:2, 1:4) de parasitoide/hospedeiro. Para ambas as espécies de tefritídeos houve maior número de marcas e de larvas nos pupários nas razões 4:1 e 2:1, onde havia menor densidade de larvas por parasitoide. Os resultados mostram que fêmeas de *D. longicaudata* podem realizar superparasitismo em ambos hospedeiros. As diferenças entre as razões testadas sugerem uma proporção adequada para otimização dos métodos de multiplicação do inimigo natural. Os resultados tem importância como subsídio para a condução de criações massais em programas de liberação de *D. longicaudata*.

Palavras Chave: *Anastrepha fraterculus*, *Ceratitis capitata*, *Diachasmimorpha longicaudata*, superparasitismo

Understanding parasitoids-hosts interactions is essential for improving the management of pest populations in biological control programs. Most parasitoids are able to recognize hosts parasitized by conspecifics or by themselves (an ability known as "host discrimination") and reject such hosts. However, they often lay a second clutch of eggs in or on them (an act called "superparasitism") (van Dijken & Waage 1987; van Alphen & Visser 1990).

Superparasitism was initially believed to reflect parasitoid error, since there is no apparent benefit to laying eggs in a host already parasitized by the same species. It is now recognized, however, that superparasitism may promote nat-

ural selection through intraspecific competition (Bakker et al. 1985).

Diachasmimorpha longicaudata (Ashmead) (Hymenoptera: Braconidae) is a larval-prepupal endoparasitoid of tephritid fruit fly pests in tropical and subtropical countries (van Nieuwenhove et al. 2012). It was originally collected in the Indo-Philippine region attacking *Bactrocera* species (Sivinski et al. 2006). This parasitoid species is currently considered to be one of the most significant biological agents for augmentative release programs against fruit fly pests in Latin America (González et al. 2007).

Fruit flies (Diptera: Tephritidae) are the primary pests of fruit crops in Brazil (Nava et al.

2010). In the Brazilian state of Rio Grande do Sul, *Ceratitis capitata* (Wiedemann) and *Anastrepha fraterculus* (Wiedemann) are especially important. The latter causes damage to temperate-zone fruit crops (Kovaleski & Ribeiro 2003) and native Myrtaceae (Gattelli et al. 2008).

The objective of this study was to investigate the influence of *D. longicaudata* conspecifics on parasitism of *C. capitata* and *A. fraterculus* tephritids, at different parasitoid-host ratios. These 2 host species are used in Brazil's Moscamed Biofactory for the multiplication of *D. longicaudata* for release in biological control programs.

MATERIALS AND METHODS

The study was conducted in the Biology, Ecology and Biological Control of Insects Laboratory (BIOECOLAB), at the Universidade Federal do Rio Grande do Sul, Brazil. Laboratory conditions were maintained at 25 ± 2 °C, 65 ± 10% RH and 14:10 L:D.

C. capitata and *A. fraterculus* were bred using a modified version of the methodology proposed by Terán (1976). Adults were kept in 45 L × 30 W × 30 H cm wooden cages with the sides covered with voile mesh, provided distilled water, and fed *ad libitum* with a solid diet that consisted of sugar crystals, wheat germ, corn gluten, and brewer's yeast (3:1:1:1) (adapted from Jaldo et al. 2001). A 250 mL orange plastic tube was used as a substrate for *C. capitata* oviposition, following the methodology of FAO/IAEA/USDA (2003). The oviposition substrate for *A. fraterculus* was a 15 cm ∅ plastic Petri dish with an 11 cm ∅ opening protected with voile mesh covered by a thin layer of black silicone. This dish was filled with water and placed on the upper surface of the cage where *A. fraterculus* larvae were bred. Newly hatched larvae were placed onto blocks of artificial diet, based on carrot and corn flour, according to the BIOECOLAB protocol (Meirelles 2011).

Parasitoids were bred following Carvalho et al. (1998). Third instar larvae of *C. capitata* and *A. fraterculus* (approximately 12 days old) were used as hosts. Forty-50 fruit fly larvae were exposed to parasitoids for a 1-h period, once a day, in parasitism chambers. Superparasitism was evaluated using 5 ratios (4:1, 2:1, 1:1, 1:2 and 1:4) of parasitoid females to host larvae (third-instar larvae of *A. fraterculus* or *C. capitata*, adapted from Montoya et al. 2000). The experiments were conducted in voile mesh cages (30 L × 30 W × 30 H cm). Larvae of both fly species were placed separately on blocks of artificial diet in parasitism chambers and exposed to the parasitoids in the cages. The parasitism chambers were made with 2 rectangular acrylic plates (10 × 8 cm), one of which had an opening (4 × 6 cm) covered with voile mesh where parasitoid females had access to the larvae (maxi-

mum 40 individuals per chamber). The parasitism chambers were placed on a wall of the cage in front of the light source, and offered individually to 4 to-5–day-old mated *D. longicaudata* females for 3 h. Females were previously (24 h before the experiment) exposed for 3 h to third-instar larvae of the respective host to gain experience in the parasitism chamber.

After exposure the groups of larvae from each replicate were kept in plastic containers with sterilized sand substrate for pupation. Seventy-two hours after exposure to parasitoids the pupae of fruit flies were examined under a stereomicroscope (50X) to count the number of punctures or oviposition scars, and then dissected to count the number of *D. longicaudata* larvae, following Montoya et al. (2000) and González et al. (2007). Superparasitism was recorded when a fruit fly pupa contained more than one parasitoid larva. We analyzed a minimum of 120 pupae for each host and each parasitoid-host ratio.

Differences in the number of oviposition scars and parasitoid larvae per pupa for each fruit fly species were analyzed through the Kruskal-Wallis test ($P < 0.05$) and Dunn's paired comparisons (Ayres et al. 2007). The number of superparasitized pupae between species and parasitoid-host ratios were compared using the Mann-Whitney test ($P = 0.05$) (Ayres et al. 2007).

RESULTS

Superparasitism by *D. longicaudata* was observed in both fruit fly hosts. There was a significant difference between the number of oviposition scars recorded at different parasitoid-host ratios in pupae of *C. capitata* ($H = 170.1525$; df = 4; $P < 0.0001$), and *A. fraterculus* ($H = 123.4763$; df = 4; $P < 0.0001$). The same pattern was observed for the number of *D. longicaudata* larvae in pupae of the 2 host species (*C. capitata*: $H = 131.0551$; df = 4; $P < 0.0001$; *A. fraterculus*: $H = 139.7015$; df = 4; $P < 0.0001$) (Table 1).

The number of superparasitized pupae did not differ between the 2 hosts at the 4:1 ($U = 6,405.5$; $P = 0.1143$) and 2:1 ($U = 6,642.0$; $P = 0.0983$) parasitoid-host ratios. However, *A. fraterculus* showed a higher superparasitism rates at the 1:1 ($U = 5,128.0$; $P < 0.0001$), 1:2 ($U = 5,564.0$; $P < 0.0001$), and 1:4 ($U = 5,550.5$; $P < 0.0001$) parasitoid-host ratios (Table 2).

The number of oviposition scars per pupa ranged from 1 to 66 in *C. capitata*, with a mean (± SD) of 6.67 ± 0.289. In *A. fraterculus,* this number ranged from 1 to 53 and the mean (± SD) was 11.72 ± 0.323. The mean number of *D. longicaudata* oviposition scars was higher in *A. fraterculus* than in *C. capitata* at the 4:1 ($H = 38.3865$; df = 1; $P < 0.05$), 1:1 ($H = 79.5814$; df = 1; $P < 0.05$), 1:2 ($H = 131.7541$; df = 1; $P < 0.05$), and 1:4 ratios ($H = 48.8724$; df = 1; $P < 0.05$). The number

TABLE 1. Mean number (± SD) of oviposition scars in *C. capitata* and *A. fraterculus* pupae, and mean numbers of first-instar larvae of *Diachasmimorpha longicaudata* per host, 72 h after exposure, at different ratios of parasitoid females to host larvae (values in parentheses indicate the number of exposed host larvae).

| Parasitoid-host ratio | | Number of | | | |
| | | Oviposition scars | | *D. longicaudata* larvae | |
C. capitata	*A. fraterculus*	*C. capitata*	*A. fraterculus*	*C. capitata*	*A. fraterculus*
4:1 (120)	4:1 (121)	9.43 ± 0.89 b	17.02 ± 0.96 a	6.94 ± 0.78 a	9.35 ± 0.65 a
2:1 (123)	2:1 (123)	13.20 ± 0.82 a	13.75 ± 0.82 ab	7.56 ± 0.66 a	7.0 ± 0.53 a
1:1 (127)	1:1 (120)	5.42 ± 0,33 b	11.38 ± 0.48 b	1.70 ± 0.20 b	4.50 ± 0.26 b
1:2 (133)	1:2 (136)	3.19 ± 0.18 c	10.63 ± 0.47 b	1.25 ± 0.13 b	4.29 ± 0.23 b
1:4 (143)	1:4 (124)	3.17 ± 0.28 c	6.08 ± 0.33 c	0.87 ± 0.11 b	1.86 ± 0.13 c

Means with different letters within a column are significantly different (Kruskal-Wallis, $\alpha = 0.05$).

of oviposition scars in the 2 species did not differ significantly at the 2:1 ratio ($H = 0.3654$; df = 1; $P = 0.54$) (Table 1).

The mean number of *D. longicaudata* larvae was higher in *A. fraterculus* than in *C. capitata* at the 4:1 ($H = 12.3466$, df = 1, $P < 0.05$), 1:1 ($H = 71.9833$, df = 1, $P < 0.05$), 1:2 ($H = 95.744$, df = 1, $P < 0.05$), and 1:4 parasitoid-host ratios ($H = 37.9866$, df = 1, $P < 0.05$). There was no significant difference at the 2:1 ratio ($H = 0.0092$, df = 1, $P = 0.92$) (Table 1).

There was a positive correlation between the number of oviposition scars and the number of parasitoid larvae per pupa, in both *C. capitata* ($r = 0.8348$, $r^2 = 0.6969$, $P < 0.0001$) and *A. fraterculus* ($r = 0.8455$, $r^2 = 0.7149$, $P < 0.0001$).

Discussion

Similar to our results with *C. capitata* and *A. fraterculus,* Montoya et al. (2003) had reported superparasitism of *A. ludens* by *D. longicaudata*. The higher superparasitism rates observed in *A. fraterculus* compared to *C. capitata* may have

TABLE 2. Mean numbers (± SD) of pupae containing more than one larva of the parasitoid *Diachasmimorpha longicaudata* (superparasitism) after 72 h of exposure, at 5 different ratios of parasitoid females to host larvae.

| Parasitoid-host ratio | Host pupae with more than one larva | |
	C. capitata	*A. fraterculus*
4:1	0.76 ± 0.04 a	0.88 ± 0.03 a
2:1	0.79 ± 0.04 a	0.91 ± 0.02 a
1:1	0.61 ± 0.04 b	0.93 ± 0.02 a
1:2	0.56 ± 0.04 b	0.94 ± 0.02 a
1:4	0.44 ± 0.04 b	0.81 ± 0.03 a

Means with different letters within a row are significantly different (Mann-Whitney test, $\alpha = 0.05$).

been influenced by the size of the host. Larger hosts (e.g., *A. fraterculus*) offer more resources to be exploited, allowing the development of larger and more competitive parasitoids with greater reproductive potential (Chau & Mackauer 2001). According to López et al. (2009), *D. longicaudata* superparasitizes more frequently (higher emergence, longevity, and search speed) when reared in larger hosts. Moreover, Ovruski et al. (2011) reported that *D. longicaudata* preferred *A. fraterculus* over *C. capitata* when given a choice.

In solitary parasitoids elimination of competition inside the pupae may occur by lethal combat between larvae or by physiological suppression (Godfray 1994). In our study combat between larvae was observed only once out of a total of 1,270 dissected pupae. Our observations are in accordance with Van Alphen & Visser (1990) and Montoya et al. (2000) who argued that *D. longicaudata* can efficiently remove excess conspecific larvae by physiological mechanisms.

The sex ratio of the offspring in our study was not determined because parasitoid pupae were dissected before emergence. However, González et al. (2007) and Montoya et al. (2011) reported a positive correlation between the number of oviposition scars and the percentage of females that emerged from *A. ludens* exposed to *D. longicaudata*. The positive correlation between the number of scars and the number of larvae in the pupae is similar to reports by Montoya et al. (2000) using *A. ludens* larvae as host.

Superparasitism is recognized as an adaptive feature of parasitoids (van Alphen & Visser 1990) and may be one of the factors that explain the success of *D. longicaudata* as a biological control agent of fruit flies. González et al. (2007) suggested that superparasitism in *D. longicaudata* selects for greater flying ability and a greater proportion of females, thereby increasing the reproductive success of the species. These authors also argued that the presence of more than one parasitoid larva can reduce the severity of the host's

immune response and increase the survival rate of the parasitoid. Nevertheless, Van Nieuwenhove et al. (2012) demonstrated that increasing the ratio of *A. fraterculus* larvae to parasitoid above 4:1, and the exposure time of *D. longicaudata* to hosts to more than 30 min, did not significantly enhance overall parasitoid progeny yield.

The results of our study have applications for optimizing the mass rearing of *D. longicaudata* in both host species tested, which occur in Brazil and are used to rear the parasitoid at the Biofactory Moscamed. Specifically, our results indicate that using 1:1, 1:2, and 1:4 parasitoid/host ratios should lead to a more efficient multiplication of the parasitoid. However, other factors that affect parasitoid rearing merit additional study, including the age of females, their previous experience, and the densities in the rearing cages.

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