

# Influence of Anastrepha fraterculus (Diptera: Tephritidae) Larval Instars on the Production of Diachasmimorpha longicaudata (Hymneoptera: Braconidae) Progeny and their Sex Ratio

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## INFLUENCE OF ANASTREPHA FRATERCULUS (DIPTERA: TEPHRITIDAE) LARVAL INSTARS ON THE PRODUCTION OF DIACHASMIMORPHA LONGICAUDATA (HYMNEOPTERA: BRACONIDAE) PROGENY AND THEIR SEX RATIO

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#### Abstract

The aim of this study was to determine the optimal larval age for exposing Anastrepha fraterculus (Wiedemann) to Diachasmimorpha longicaudata (Ashmead) females to maximize parasitoid female offspring production under laboratory conditions in order to develop an efficient mass-rearing process for this biocontrol agent. Five age ranges of *A. fraterculus* larvae were assessed: 1-3 d-old (first instar), 4-6 d-old (second instar), 7-8 d-old (early third instar), 9-10 d-old (middle third instar), and 11-12 d-old (late third instar). The number of *D. longicaudata* initiating ovipositor probing on a device with third instars of *A. fraterculus* was significantly higher than with others containing younger instars. Although female biased parasitoid offspring was recorded in all treatments using third instars as hosts, significantly more *D. longicaudata* females emerged from *A. fraterculus* pupae originated from middle and late third instars. However, the percentage of unemerged host puparia also increased significantly in both those host instar groups. Superparasitism, recorded as the number of first instar parasitoid head capsules per host, increased significantly as *A. fraterculus* larvae matured from second instars to late third instars. Nevertheless, the maximum average yield of parasitoid progeny was achieved using 9-12 d-old *A. fraterculus* larvae as hosts.

Key Words: fruit flies, parasitoids, sex ratio, superparasitism, biological control, Argentina

#### RESUMEN

Se determinó la edad óptima de la larva de Anastrepha fraterculus (Wiedemann) para ser expuesta a Diachasmimorpha longicaudata (Ashmead) con el objetivo de maximizar la producción de descendientes hembras en la cría del parasitoide y así lograr un eficiente proceso de cría masiva del agente. Fueron evaluados 5 rangos de edad de larvas de A. fraterculus: 1-3 días (1er estadío), 4-6 días (2do estadío), 7-8 días (3er estadío temprano), 9-10 días (3er estadío medio) y 11-12 días (3er estadío tardío). En las pruebas con el ovipositor se observó un número significativamente mayor de hembras de D. longicaudata en la unidad de oviposición con larvas de A. fraterculus del 3er estadío que en dispositivos con larvas de estadíos más tempranos. En todos los tratamientos que usaron larvas huéspedes del 3er estadío, la descendencia del parasitoide tuvo predominancia de hembras. Sin embargo, el máximo porcentaje de puparios no eclosionados, también se registró en tratamientos que utilizaron larvas huéspedes del 3er estadío medio y tardío. El superparasitismo, registrado como el número de cápsulas cefálicas de larvas del 1er estadío del parasitoide por pupa del huésped, manifestó un significativo incremento en larvas de A. fraterculus del 3er estadío tardío. No obstante, cuando se utilizaron larvas de 9-12 días de edad como huéspedes se alcanzó la máxima producción de progenie del parasitoide.

Translation provided by the authors.

Diachasmimorpha longicaudata (Ashmead) is a solitary, koinobiont larval-prepupal endoparasitoid of several fruit-infesting tephritid flies (Montoya et al. 2000a). It is native to Southeast Asia (Wharton 1989) but has been used in augmentative biological control programs against the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) in Mexico and Guatemala (Cancino et al. 1995), the Caribbean fruit fly *Anastrepha suspensa* (Loew) in Florida (Sivinski et al. 1996), the Oriental fruit fly *Bactrocera dorsalis* (Hendel) in Hawaii (Purcell 1998), and the Mexican fruit fly *Anastrepha ludens* (Loew) in Mexico (Montoya et al. 2000a). In 1999, *D. longicaudata* was introduced to Argentina via Mexico with the purpose of renewing biological control programs against tephritid pests such as the exotic *C. capitata* and the native South American fruit fly *Anastrepha fraterculus* (Wiedemann) (Ovruski et al. 2003). Although a previous introduction was carried out

during the 1960's, the permanent establishment of D. longicaudata on A. fraterculus was only recently confirmed in northeast (Schliserman et al. 2003) and northwest (Oroño & Ovruski 2007) Argentina. Consequently, the use of D. longicaudata in augmentative releases to suppress populations of A. fraterculus in citrus-growing areas of northwest Argentina might be successful and ecologically suitable. Knowing which factors influence offspring production is essential to successfully rear D. longicaudata for augmentative releases (Montoya et al. 2011). Based on this analysis, a colony of D. longicaudata was established on A. fraterculus at our laboratory in 2005 (Ovruski et al. 2011). The aim of the present study was to determine the optimal larval age for exposing A. fraterculus to D. longicaudata females to maximize parasitoid offspring production and thus develop an efficient mass-rearing process for this biocontrol agent.

## MATERIALS AND METHODS

*D. longicaudata* and *A. fraterculus* colonies were kept at  $25 \pm 1$  °C;  $75 \pm 5\%$  RH, under a 12:12 h L:D photoperiod in the laboratory of Biological Control Division at the Planta Piloto de Procesos Microbiológicos Industriales y Biotecnología (PROIMI), located in San Miguel de Tucumán, Argentina. Parasitoids were obtained from a strain reared at the PROIMI laboratory using *A. fraterculus* larvae for over 30 generations (Ovruski et al. 2011). The *A. fraterculus* rearing procedures were carried out as described by Vera et al. (2007).

Five age ranges of A. fraterculus larvae were compared to determine the optimal larval age for exposing hosts to D. longicaudata females: 1-3 dold (first instar), 4-6 d-old (second instar), 7-8 dold (early 3rd-instar), 9-10 d-old (middle 3rd-instar), and 11-12 d-old larvae (late 3rd-instar). A. *fraterculus* instars were determined using the size and shape of the cephalopharyngeal skeleton under a stereomicroscope, as described by Frías et al. (2008). Forty host larvae of each instar group, provided with artificial diet (hydrolyzed brewer's yeast + wheat germ + sugar + agar + water), were exposed to 10 mated D. longicaudata females for 1 h inside oviposition devices placed on the floor of cubical Plexiglas cages (20 cm). The oviposition unit was composed of an organdy screen-covered Petri dish (8) cm diameter and 0.8 cm deep). The parasitoid/ host ratio was based on data for optimizing D. longicaudata mass rearing on A. ludens at the Moscafrut Metapa facility in Mexico (Montoya et al. 2000b). Parasitoid females were 6-7 d-old and had no prior oviposition experience. After exposure to the parasitoids, host larvae were transferred onto plastic travs  $(15 \times 11 \times 1.5 \text{ cm})$  filled with fresh food. These trays were then placed inside plastic boxes  $(33 \times 23 \times 11 \text{ cm})$  containing a 2 cm-vermiculite layer on the bottom as a pupation medium and covered with organdy on the top. The 11-12 d-old larvae were removed from the oviposition device and transferred directly to the plastic boxes described above. All dead larvae were counted and removed. Puparia were sifted from the pupation medium and transferred into plastic cups (8 cm diam  $\times$  5 cm deep) containing fresh vermiculite on the bottom. The cups were then tightly covered with a piece of organdy cloth. The number of emerged flies and parasitoids during this period was recorded for a period of 40 d. After this period, sub samples of 10 unemerged puparia were removed and dissected to determine the presence or absence of recognizable immature parasitoid stages (larvae, prepupae, or pupae) or pharate-adult parasitoid cadavers. Instars of *D. longicaudata* were determined using the size and shape of mandibles as described by Ibrahim et al. (1994). The number of first instar parasitoid head capsules per dissected host pupa was also examined. Control tests (no parasitoid exposure) for all larval age ranges were made to determine natural A. fraterculus larvae and pupae mortalities, as well as the rate of fly emergence. Treatments and control tests were replicated 12 times. All assays were carried out at the laboratory under the environmental conditions described previously. Parasitoid and fly emergence percentages were based on the total number of recovered pupae in each sample. Parasitoid progeny sex ratio was calculated as the proportion of female offspring. The percentage of larval mortality was calculated as the fraction of dead host larvae from the total number of larvae exposed to parasitoids. The percentage of pupal mortality was estimated as the fraction of non-eclosed puparia from the total number of emerged flies and parasitoids, and unemerged puparia (Montoya et al. 2000b).

The number of female ovipositor probes in the oviposition device was recorded when the parasitoids were released into the cages. Behavioral observations were made every 15 min and each observation lasted 20 s. An ovipositor probe was confirmed each time a female parasitoid inserted its ovipositor through the organdy screen of the oviposition device (Duan & Messing 2000).

Data on parasitoid emergence, sexual ratio of parasitoid offspring, number of first instar parasitoid head capsules per host, and number of female ovipositor probes were compared statistically using one-way analyses of variance (ANOVA) (P < 0.05). Fly emergence and larval and pupal mortalities recorded from the experimental treatments and controls were compared by a two-way ANOVA (P < 0.05). Data on the stages of parasitoid cadavers recorded from dissected unemerged host puparia were subjected to multivariate analyses of variance (MANOVA) (P < 0.05). Fisher LSD tests (P = 0.05) were used to determine significant differences between means from both the ANOVA and MANOVA. Proportional data were transformed to arcsine square root before analyzed. Only untransformed means (±SE) are presented in the text. A Pearson correlation test (P < 0.05) was used to determine the degree of association between the sexual ratio of parasitoid offspring and the number of first instar parasitoid head capsules per host pupa.

## RESULTS

The age of host larvae affected parasitoid emergence ( $F_{(4,55)}$  = 69.7, P < 0.0001) and offspring sexual proportion ( $F_{(4,55)}$  = 82.6, P < 0.0001). Older *A. fraterculus* larvae yielded a higher proportion of adult parasitoids than younger host larvae (Fig. 1). Also, parasitoid offspring sex ratio increased significantly with older A. fraterculus larvae. Exposure to middle and late A. fraterculus third instars produced the highest proportion of female offspring, whereas exposure to second instar larvae produced a higher proportion of male offspring (Fig. 1). The proportions of dead larvae and unemerged puparia differed between the ages of host larvae exposed to parasitoids (F  $_{\scriptscriptstyle (4,\,110)}$ = 27.918, P < 0.0001 for larval mortality, and  $F_{_{(4,)}}$ percentage of dead host larvae decreased significantly from younger larvae to older larvae, while the proportions of unemerged puparia increased (Fig. 2). The mortality of unexposed host larvae (control) was significantly lower compared to the larvae exposed to parasitoids (F  $_{(4,110)}$  = 21.205, P < 0.0001) (Fig. 2). The percentages of unemerged puparia recorded in the parasitoid treatments were also significantly higher than in the controls  $(F_{(4, 110)} = 20.275, P < 0.0001)$  (Fig. 2). The high quality of the host larvae used in the study was confirmed by the emergence of adult flies re-



Fig. 1. Effect of the age of *A. fraterculus* larvae on the proportion of emerged *D. longicaudata* adults (mean  $\pm$  SE) and on progeny sex ratio (% emerged females of the total parasitoid offspring). Bars with the same letter indicate no significant differences (Fisher LSD test, *P* = 0.05).



Fig. 2. Influence of *A. fraterculus* instars on the percentage (mean  $\pm$  SE) of larval and pupal mortality recorded in experimental treatments (hosts exposed to *D. longicaudata* females) and controls (unexposed hosts). Plot markers followed by the same small letter indicate no significant differences (Fisher LSD test, P = 0.05). Different capital letters (either bold or italic) indicate significant differences between treatments and controls (ANOVA, P = 0.05).

corded in the controls, which was significantly higher (> 92%) than for the treatments (from 3 to 53%) ( $F_{_{(4,110)}} = 49.364, P < 0.0001$ ). Significantly more *D. longicaudata* females (*F* 

Significantly more *D. longicaudata* females (*F*  $_{^{(4,55)}}$  = 172.76, *P* < 0.0001) were observed probing the oviposition unit containing third instars of *A. fraterculus* than devices containing first or second host instars (Fig. 3). Significant differences (Wilks'  $\lambda$  = 0.0339, *F*  $_{^{(32,178,61)}}$  = 8.384, *P* < 0.0001) were recorded among the five ages of *A. fraterculus* larvae regarding the stages of parasitoid cadavers found after the unemerged host puparia had been dissected (Table 1).

First instar head capsules of *D. longicaudata* were mainly found in parasitized unemerged pupae. The number of first instar parasitoid head capsules per host increased significantly ( $F_{(4,55)} = 32.823$ , P < 0.0001) as the *A. fraterculus* larvae



Fig. 3. Probing responses of *D. longicaudata* females (mean  $\pm$  SE) on artificial oviposition devices containing different instars of *A. fraterculus* (mean  $\pm$  SE). Bars with the same letter indicate no significant differences (Fisher LSD test, *P* = 0.05).

	Percentage (mean $\pm$ SE) of immature parasitoid cadavers <sup>b</sup>						Percentage $(mean + SE)$
Host instarª	Egg	1st instar	2nd instar	3rd instar	prepupa	pupa	of pharate adult cadavers <sup>b</sup>
L	0 a	0 a	0 a	0 a	0 a	0 a	0 a
$L_2$	$45.9 \pm 10.7 \; \mathrm{b}$	$40.8 \pm 4.9$ b	2.1 ± 2.1 a	1.4 ± 1.5 a	1.4 ± 1.4 a	0 a	0 a
$L_{3E}$	$2.9 \pm 2.9$ ac	$37.7 \pm 4.4 \text{ b}$	$8.7 \pm 3.8 \mathrm{~b}$	$6.0 \pm 3.4 \mathrm{~b}$	$18.6 \pm 3.1 \text{ b}$	$19.3 \pm 4.4 \mathrm{~b}$	$6.9 \pm 3.2 \text{ b}$
$L_{3M}$	8.7 ± 4.2 c	49.8 ± 4.5 c	2.1 ± 1.4 ab	0.9 ± 0.9 a	$22.1 \pm 2.5 \text{ c}$	$12.9 \pm 4.3$ b	3.5 ± 1.9 ab
$L_{3L}$	$1.3 \pm 1.3$ ac	$72.2 \pm 3.3 \; d$	0 a	1.1 ± 1.1 a	$20.2 \pm 2.7 \text{ bc}$	$3.1 \pm 2.1 \text{ a}$	$2.2 \pm 1.5$ ab

 

 TABLE 1. COMPOSITION OF THE STAGES OF PARASITOID CADAVERS IN THE DISSECTED UNECLOSED PUPARIA OF ANAS-TREPHA FRATERCULUS PARASITIZED BY DIACHASMIMORPHA LONGICAUDATA.

 ${}^{\rm s}L_{\rm 1}, 1 {\rm st \ instar; \ }L_{\rm 2}, 2 {\rm nd \ instar; \ }L_{\rm _{3E}}, {\rm early \ 3rd-instar; \ }L_{\rm _{3M}}, {\rm middle \ 3rd-instar; \ }L_{\rm _{3L}}, late \ 3rd-instar.$ 

<sup>b</sup>Values in the same column with the same letter are not significantly different (Fisher LSD test, P = 0.05).

matured from second instars to late third instars (Fig. 4). This increase was significantly associated with an increase in parasitoid female offspring (r = 0.7776, N = 60, P = 0.0002).

#### DISCUSSION

The biased offspring sex ratio towards females of *D. longicaudata* as *A. fraterculus* larvae aged could be influenced by an interaction between following biological factors: 1) suitable host age and high quality of host larvae that might stimulate parasitoids to lay more female eggs; 2) high level of superparasitism; 3) differential survival of each sex during parasitoid post-embryonic development in superparasitized hosts; 4) higher number of fertilized eggs laid in the presence of conspecific females foraging on the same artificial oviposition device.

Regarding the first statement, the results of this study clearly demonstrated that all the instars of *A. fraterculus* were not equally suitable for *D. longicaudata*, and that parasitoids develop successfully in older instars. As suggested by Montoya et al. (2011), *D. longicaudata* females could be able



Fig. 4. Mean ( $\pm$  SE) number of first instar *D. longi*caudata head capsules per host pupa found in the five assayed instars of *A. fraterculus*. Plot markers followed by the same letter indicate no significant differences (Fisher LSD test, *P* = 0.05).

to regulate their offspring sex ratio in response to host conditions. Chemical cues emanated from host larvae can be exploited in the process of host acceptance by D. longicaudata (Wong & Ramadan 1992). Chemosensory cells located at the tip of the ovipositor might allow *D. longicaudata* females to detect changes in pH and hormone levels in the host larva haemolymph (Greany et al. 1977). Thus, the internal physiological conditions of maturer instars would thus elicit egg fertilization in D. longicaudata females (Wong & Ramadan 1992). The behavioral data of the present study showed that a very high level of ovipositor-probing responses (>85%) only occurred in devices containing third instar larvae of A. fraterculus. It seems that distinct physical and/or chemical cues are be involved in the host acceptance of *D. longicaudata* females. Arthur (1981) pointed out that host size, shape, movement and surface texture, the presence of kairomones associated with host larval frass, body fat, and labial and mandibular glands, as well as haemolymph, may elicit host acceptance behaviors by female parasitoids. It has been demonstrated that the cues of third instar larvae of A. suspensa and C. capitata that elicit ovipositor-probing behavior in gravid D. longicaudata are primarily vibration and/or sound produced by larvae feeding or crawling inside the substrate (Lawrence 1981; Duan and Messing 2000).

The second aforementioned assumption is supported by the close association found between parasitoid female offspring production and the number of first instar parasitoid head capsules per host. Middle and late *A. fraterculus* instars yielded significantly more *D. longicaudata* female offspring that younger instars, but they also recorded the highest rates of superparasitization. This data is in agreement with the observations of Wong et al. (1990) on *Diachasmimorpha tryoni* (Cameron), another fruit fly larval endoparasitoid. These authors found that both progeny female-biased sex ratio and superparasitism increased when late third-instars of *C. capitata* were used as hosts in the *D. tryoni* mass-rearing process. Similarly, Wang & Messing (2003) also recorded more *D. tryoni* females emerged from superparasitized host puparia. Also, González et al. (2007) demonstrated that under mass-rearing conditions, the *D. longicaudata* offspring sex ratio always favoured females when moderate to high levels of superparasitism were recorded in third-instars (8-9-d old) of *A. ludens*. As suggested by Montoya et al (2000b), superparasitism in *D. longicaudata* might be an adaptative behavior that could increase the chance of parasitoid female offspring survival inside host larvae.

According to the third factor, differential sex mortality during parasitoid larval development has been mentioned as a probable cause influencing offspring sex ratios in solitary parasitoid species (Heimpel & Lundgren 2000). Montova et al. (2011) previously proposed this hypothesis to explain parasitoid female biased sex ratios recorded in A. ludens pupae superparasitized by D. longicaudata. Ovipositing D. longicaudata females would be expected to lay eggs of the better sex competitor (probably parasitoid female) under conditions of superparasitism (Montoya et al. 2011). Supernumerary D. longicaudata larvae might be eliminated through physiological suppression mechanisms (Montoya et al. 2000b), although physical competition could also cause the death of *D. longicaudata* first instar larvae (Lawrence 1988).

Regarding the fourth assumption, a female-biased sex allocation pattern has been frequently recorded in *D. longicaudata* under crowded mass rearing situations on *B. dorsalis* (Wong & Ramadan 1992; Vargas et al. 2002) and *A. ludens* (Montoya et al. 2000b, 2011; González et al. 2007), as well as under experimental laboratory rearing conditions on *Bactrocera papayae* Drew & Hancock (Petcharat & Petcharat 1997) and *C. capitata* (Ovruski et al. 2003; Paranhos et al. 2008).

Another issue to consider is that the difference in host mortality between experimental and control treatments could be due to parasitism. Although no parasitoids were recorded from the treatment including 1-3 d-old host larvae (L1), the high larval mortality detected in A. fratercu*lus* first instars might be caused when ovipositing parasitoid females insert their ovipositor in unsuitable larvae. Previous assays with first instar A. fraterculus parasitized individually by D. longicaudata showed a 90% mortality among these larvae (S.O., unpublished data). Dissections of puparia from second instar hosts exposed to parasitoids revealed that about 86% of the unemerged parasitized host pupae only contained cadavers of eggs and first instar parasitoids. As suggested previously by Lawrence et al. (1978), hormonal interference may influence the survival rate of immature stages of *D. longicaudata*. It has been demonstrated that first instar D. longicaudata need a suitable hormonal environment inside the host to advance to the second instar stage, which occurs when the host has pupated (Lawrence 1982, 1986). The high percentage of puparia that did not yield adult flies observed in the treatment using third instar larvae could be interpreted as a negative effect of superparasitism. This is in agreement with studies by Montoya et al. (2000b) on *D. longicaudata* parasitizing third instars of *A. ludens*, who suggested that high host mortality could be due to the effect of superparasitism.

As a conclusion, the results of the present study demonstrate that exposure to 9-12-d-old A. fraterculus larvae significantly increases D. longicaudata female emergence to a maximum of 80% under laboratory conditions. Moreover, these results also reveal that although the intensity of superparasitism was higher using middle and latethird instars of A. fraterculus as hosts, the maximum average yield of parasitoids (52.1%) was also achieved with these host instars. This information is important for optimizing female production in the mass rearing process of D. longicaudata on A. fraterculus. As pointed out by Montoya et al. (2011) regarding fruit fly biological control programs using D. longicaudata, an increased production of parasitoid females implies the mass release of more females, with a subsequent increase in the cost-effective relation of a biocontrol program.

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### References Cited

- ARTHUR, A. P. 1981. Host acceptance by parasitoids, pp. 97-120 In D. A. Nordlund, R. L. Jones, and W. J. Lewis [eds.], Semiochemicals. Their role in pest control. John Wiley & Sons, Inc., New York, USA.
- CANCINO, J. L., LÓPEZ, E., AND ÁGUIAR, C. É. 1995. Liberaciones inundativas de parasitoides como método alternativo de control de *Ceratitis capitata* en fincas cafetaleras en el Soconusco, Chiapas, México, pp. 51-53 *In* Actas de la Primera Conferencia Internacional sobre café orgánico. IFOAM/AMAE, Universidad Autónoma de Chapingo, México.
- DUAN, J. J., AND MESSING, R. H. 2000. Effects of host substrate and vibration cues on ovipositor-probing behavior in two larval parasitoids of Tephritid fruit flies. J. Insect Behav. 13(2): 175-186.
- FRÍAS, D., SELIVON, D., AND HERNÁNDEZ-ORTIZ, V. 2008. Taxonomy of immature stages: new morphological characters for Tephritidae larvae identification, pp.

29-44 *In* R. Sugayama, R. A. Zucchi, S. Ovruski, and J. Sivinski [eds.], Fruit Flies of Economic Importance from Basic to Applied Knowledge. Proc. 7th Int. Symp. Fruit Fly of Econ. Importance, Bahía, Brazil.

- GONZÁLEZ P. I., MONTOYA, P., PÉREZ-LACHAUD, G., CANCINO, J., AND LIEDO, P. 2007. Superparasitism in mass reared *Diachasmimorpha longicaudata* (Asmead) (Hymenoptera: Braconidae), a parasitoid of fruit flies (Diptera: Tephritidae). Biol. Control 40: 320-326.
- GREANY, P. D., TUMLINSON, J. H., CHAMBERS, D. L., AND BOUSH, G. M. 1977. Chemical mediated host finding by *Biosteres (Opius) longicaudatus*, a parasitoid of tephritid fruit fly larvae. J. Chem. Ecol. 3: 189-195
- HEIMPEL, G. E., AND LUNDGREN, J. G. 2000. Sex ratios of commercially reared biological control agents. Biol. Control 19: 77-93.
- IBRAHIM, A. G., PALACIO, L. P., AND ROHANI, I. 1994. Biology of *Diachasmimorpha longicaudata*, a parasitoid of carambola fruit fly (Diptera: Tephritidae). Trop. Agric. Sci. 12 (2): 139-143.
- LAWRENCE, P. L. 1981. Host vibration. A cue to host location by the parasite, *Biosteres longicaudatus*. Oecologia 48: 249-251.
- LAWRENCE, P. L. 1982. Biosteres longicaudatus: development dependence on host (Anastrepha suspensa) physiology. Exp. Parasitol. 53: 396-405.
- LAWRENCE, P. L. 1986. The role of 20-hydroxyecdysone in the moulting of *Biosteres longicaudatus*, a parasite of the Caribbean fruit fly, *Anastrepha suspensa*. J. Insect Physiol. 32: 329-337.
- LAWRENCE, P. L. 1988. Intraspecific competition among first instars of the parasitic wasp *Biosteres longicaudatus*. Oecologia 74: 607-611.
- LAWRENCE, P. L., GREANY, P. D., NATION, J. L., AND OBERLANDER, H. 1978. Influence of hydroprene on Caribbean fruit fly suitability for parasite development. Florida Entomol. 61: 93-99.
- MONTOYA. P., LIEDO, P., BENREY, B., CANCINO, J., BAR-RERA, J. F., SIVINSKI, J., AND ALUJA, M. 2000A. Biological control of *Anastrepha* spp. (Diptera: Tephritidae) in mango orchards through augmentative releases of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Biol. Control 18: 216-224.
- MONTOYA, P., LIEDO, P., BENREY, B., CANCINO, J., BAR-RERA, J. F., SIVINSKI, J. AND ALUJA, M. 2000b. Functional response and superparasitism by *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae), a parasitoid of fruit flies (Diptera: Tephritidae). Ann. Entomol. Soc. America 93(1): 47-54.
- MONTOYA, P., CANCINO, J., PÉREZ-LACHAUD, G., AND LIEDO, P. 2011. Host size, superparasitism and sex ratio in mass-reared *Diachasmimorpha longicaudata*, a fruit fly parasitoid. BioControl 56:11-17.
- OROÑO, L. E., AND OVRUSKI, S. M. 2007. Presence of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) in a guild of parasitoids attacking *Anastrepha fraterculus* (Diptera: Tephritidae) in northwestern Argentina. Florida Entomol. 90(2): 410-412.
- OVRUSKI, S. M., COLIN, C., SORIA, A., OROÑO, L., AND SCHLISERMAN, P. 2003. Introducción y establecimiento en laboratorio de *Diachasmimorpha tryoni* y *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae, Opiinae) para el control biológico de *Ceratitis capitata* (Diptera: Tephritidae, Dacinae) en la Argentina. Rev. Soc. Entomol. Arg. 62: 49-59.

- OVRUSKI, S. M., BEZDJIAN, L. P., VAN NIEUWENHOVE, G. A., ALBORNOZ-MEDINA, P., AND SCHLISERMAN, P. 2011. Host preference by *Diachasmimorpha longicaudata* (Hymneoptera: Braconidae) reared on larvae of *Anastrepha fraterculus* and *Ceratitis capitata* (Diptera: Tephritidae). Florida Entomol. 94(2): 195-200.
- PARANHOS, B. J., COSTA, M. L. Z., OVRUSKI, S. M., ALVES, R. M., LUMMER, L. B., AND WALDER, J. M. M. 2008. Offspring in response to parental female densities in the fruit fly parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae: Opiinae). Florida Entomol. 91(4): 628-635.
- PETCHARAT, J., AND PETCHARAT, J. 1997. Biology of *Diachasmimorpha longicaudata* Ashmead (Hymenoptera: Braconidae): a larval-pupal parasitoid of the oriental fruit fly *Bactrocera papayae* Drew& Hancock. Kaen Kaset Kaen Agricult. J. 25: 30-35.
- PURCELL, M. F. 1998. Contribution of biological control to integrated pest management of tephritid fruit flies in the tropics and subtropics. Int. Pest Manag. Rev. 3: 63-83.
- SCHLISERMAN, P., OVRUSKI, S. M., AND DECOLL, O. R. 2003. The recovery and permanent establishment of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) in Misiones, northeastern Argentina. Florida Entomol. 86(4): 491-492.
- SIVINSKI, J., CALKINS, C., BARANOWSKI, R., HARRIS, D., BRAMBILA, J., DIAZ, J., BURNS, J., HOLLER, T., AND DODSON, G. 1996. Suppression of a Caribbean fruit fly, Anastrepha suspensa (Loew), population through augmented releases of the parasitoid Diachasmimorpha longicaudata (Ashmead) (Hymenoptera: Braconidae). Biol. Control 6: 177-185.
- VARGAS, R. I., RAMADAN, M., HUSSAIN, T., MOCHIZUKI, N., BAUTISTA, R. C., AND STARK, J. D. 2002. Comparative demography of six fruit fly (Diptera: Tephritidae) parasitoids (Hymenoptera: Braconidae). Biol. Control 25: 30-40.
- VERA, M. T., ABRAHAM, S., OVIEDO, A., AND WILLINK, E. 2007. Demographic and quality control parameters of *Anastrepha fraterculus* (Diptera: Tephritidae) maintained under artificial rearing. Florida Entomol. 90: 53-57.
- WANG, X. G., AND MESSING, R. H. 2003. Intra- and interspecific competition by *Fopius arisanus* and *Diachasmimorpha tryoni* (Hymenoptera: Braconidae), parasitoids of tephritid fruit flies. Biol. Control 27: 251-259.
- WHARTON, R. A. 1989. Classical biological control of fruit infesting Tephritidae, pp. 303-313 In A. S. Robinson and G. Hooper [eds.], World Crop Pests: Fruit Flies, Their Biology, Natural Enemies, and Control. Vol. 3b. Elsevier Amsterdam, The Netherlands.
- WONG, T. T. Y., AND RAMADAN, M. M. 1992. Mass rearing biology of larval parasitoids (Hymenoptera: Braconidae: Opiinae) of tephritid flies (Diptera: Tephritidae) in Hawaii, pp. 405-426 In T. E. Anderson and N. C. Leppla [eds.], Advances in Insect Rearing for Research and Pest Management. Westview Press, San Francisco, USA.
- WONG, T. T. Y., M. M. RAMADAN, D. O. MCINNIS, AND MOCHIZUKI, N. 1990. Influence of cohort age and host age on oviposition activity and offspring sex ratio of *Biosteres tryoni* (Hymenoptera: Braconidae), a larval parasitoid of *Ceratitis capitata* (Diptera: Tephritidae). J. Econ. Entomol. 83: 779-783.