Biocontrol Science and Technology, 2007; 17(10): 1079–1085

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SHORT COMMUNICATION

The effect of four fruit species on the parasitization rate of *Anastrepha fraterculus* (Diptera: Tephritidae, Trypetinae) by *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae, Opiinae) under laboratory rearing conditions

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Abstract
In the laboratory, the effect of host fruit species *Citrus paradisi*, *C. aurantium*, *Prunus persica*, and *Psidium guajava* on *A. fraterculus* parasitism by *Diachasmimorpha longicaudata* was studied. The number of ovipositor-probing events and the probing-time were documented to evaluate the role of fruit chemistry, and epicarp and mesocarp thickness, respectively. The relationship between the parasitization rate and fruit size in particular plant species was analyzed by applying a simple regression. Results showed that guava and peach yielded significantly more parasitoids than both *Citrus* spp. Probably, the parasitization rate of *D. longicaudata* on *A. fraterculus* would be influenced in part by chemical and physical factors from fruit species.

Keywords: *Argentina, braconidae, biological control, fruit flies, parasitoids*

The native South American fruit fly, *Anastrepha fraterculus* (Wiedemann), is a serious fruit pest in Argentina (Ovruski et al. 2003a) and in several countries of South America (Norrbom 2004). Although *A. fraterculus* represents a cryptic species group (Vera et al. 2006), Alberti et al. (2002) concluded that Argentinian populations of the north and central-eastern regions, and also from south Brazil are conspecific. Polyphagous in feeding habits, *A. fraterculus* is mainly distributed throughout the citrus growing areas of northwest Argentina, where it coexists with the exotic Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Even though the Argentinian form of *A. fraterculus* is mainly found in fruit of the families Myrtaceae and Rosaceae, it can, and in fact does, infest citrus under natural conditions (Ovruski et al. 2003a).

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ISSN 0958-3157 print/ISSN 1360-0478 online
DOI: 10.1080/09583150701661620

Diachasmimorpha longicaudata (Ashmead), a solitary larval-pupal endoparasitoid original from the Malaysia–Philippine region, is a generalist species of various genera of fruit flies feeding on a wide variety of host plant families (Purcell 1998). It is currently mass released in several tropical and subtropical countries for biological control of tephritid flies (Ovruski et al. 2000; Sime et al. 2006). In 1999, *D. longicaudata* was introduced into Argentina via Mexico with the purpose of renewing the employment of exotic parasitoids against both *A. fraterculus* and *C. capitata* (Ovruski et al. 2003b). Although there was a previous introduction between 1960 and 1970, the permanent establishment of *D. longicaudata* on *A. fraterculus* has been recently confirmed in the provinces of Misiones (Schliserman et al. 2003) and Salta (Oroño & Ovruski 2007). Given that *D. longicaudata* may be an important factor in regulating *A. fraterculus* populations in northern Argentina, the aim of this study is to provide preliminary information on the effect of four exotic host fruit species on the rate of *A. fraterculus* parasitization under laboratory conditions.

This study was performed in the Insectary of the Planta Piloto de Procesos Microbiológicos Industriales y Biotecnología (PROIMI), Biological Control Division, located in San Miguel de Tucumán, Argentina. The colony of *D. longicaudata* used for experiments originated from a strain already adapted to laboratory conditions using *Anastrepha ludens* (Loew) larvae as a host and was obtained from the Biological Control Laboratory of the Mexico's Moscamed-Moscafrut National Program in Metapa de Dominguez, Chiapas, México (Montoya et al. 2000). Prior to the experiments, this parasitoid strain was successfully reared at the PROIMI's laboratory for at least four generations using late-third instar *A. fraterculus* larvae at $26 \pm 1^{\circ}$C, 75 ± 5% RH, and 14:10 (L:D) h photoperiod. Adult parasitoids were reared in cubic Plexiglas cages (30 cm^3) covered by a fiberglass screen (1-mm mesh) at a capacity of 300 pairs per cage and fed on honey and water (Ovruski et al. 2003b). The general *A. fraterculus* rearing procedure was carried out as described by Jaldo et al. (2001).

The host fruit species used during experiment were: *Citrus paradisi* Macfadyn (grapefruit, 'March' cultivar), *C. aurantium* L. (sour orange, rootstock, wild cultivar) (Rutaceae), *Prunus persica* (L.) Batsch (peach, wild cultivar named 'cuaresmillo') (Rosaceae), and *Psidium guajava* L. (common guava, wild cultivar) (Myrtaceae). All fruits were obtained directly from unsprayed and uninfested trees from 'Parque Sierra de San Javier', a protected area of wild vegetation belonging to the Universidad Nacional de Tucumán, which is located in Horco Molle, Tucumán. All these exotic species are *A. fraterculus* host plants growing in patches of wild vegetation in NW Argentina (Ovruski et al. 2003a). All the fruit were individually weighed and the diameter and longitude of both epicarp and mesocarp were measured. Grapefruits, sour oranges, peaches, and guavas weighed and measured on average ($\pm$SEM), 325.5 ± 7.9 g, 9.9 ± 0.9 cm, 0.75 ± 0.08 cm, and 0.96 ± 0.02 cm; 159.6 ± 5.6 g, 7.1 ± 0.8 cm, 0.83 ± 0.01 cm, and 0.37 ± 0.02 cm; 40.7 ± 1.9 g, 4.0 ± 0.1 cm, 0.03 ± 0.01 cm and 0.09 ± 0.01 cm; 47.0 ± 1.4 g, 4.6 ± 0.1 cm, 0.02 ± 0.01 cm and 0.08 ± 0.01 cm, respectively.

To simulate naturally infested fruit, with a standard number of fruit-flies, a portion of fruit pulp was removed and replaced with larvae and diet. This was accomplished by making a cross-section in each fruit of the four host plant species using a sterilized scalpel. The apical portion, which included the peduncle, was smaller than the basal portion and represented about 25% of the fruit total volume. Then, approximately 50% of the total endocarp (pulp) volume from the remainder basal portion of the each

fruit was removed. Thus, the created cavity, which was similar in size in all fruit species, was inoculated with 30 laboratory-reared early-third instar *A. fraterculus* larvae plus diet (hydrolyzed brewer's yeast + wheat germ + water). After this, the cavity was covered with the apical section that was previously trimmed, and attached to the remainder of the fruit with parafilm (Parafilm 'M') (Parafilm Laboratory Film, Pechiney Plastic Packaging, Chicago, IL). A single inoculated fruit was then placed onto an inverted 50-ml plastic cup to keep it within the central part of the cubic Plexiglas cage (20 cm^3) away from the walls, and it was exposed for 24 h to one mated female *D. longicaudata*, 5–7-days-old, deprived of any host before testing. Lawrence et al. (1978) reported a density of 30 *A. suspensa* larvae: 1 *D. longicaudata* female as optimal for 24 h oviposition experiments. Each infested fruit was placed in a plastic container (25 cm^3) with sterilized sand on the bottom as pupation substrate after exposure to the parasitoid. The pupae obtained from each fruit were separated and kept in small plastic vials (15 cm^3) with sterilized and moisted sand until parasitoids and flies emerged. A complete random design was analyzed applying four treatments with 22 replicates (one fruit per replicate). Parasitism percentage, pupal viability and sexual proportion were calculated. The number and sex of the parasitoids, number of flies, and the number of unemerged puparia were checked. Unemerged puparia were dissected to check for the presence of pupae and/or pharate adult parasitoids. Percent parasitism was calculated by dividing the total number of emerged and unemerged parasitoids into the total number of larvae exposed in the fruit. Pupal viability was determined as the total number of pupae that yielded flies and parasitoids divided into the sum of emerged and unemerged pupae.

Both the number of ovipositor-probing events and control probing-time were documented to assess both the role of fruit chemistry and thickness of fruit epicarp and mesocarp. Probing-time was recorded with the help of a stop watch when the parasitoid contacted the host substrate (fruit). A 'probe' was confirmed when a female elevated its metasoma and inserted the tip of its ovipositor through the peel of fruit for at least 3 s. Previously, Duan and Messing (1999) reported a probing-period of 3 s as a sound for the opiine *Diachasmimorpha tryoni* (Cameron) the recognise the host substrate. An observation test ended either when 30 min elapsed or when the female left the fruit before the 30-min period. Observations started at 10:00 h and ended at 10:30 h. A one-way analyses of variance was used for statistically compared data. Means were separated with a Tukey HSD test ($P = 0.05$). Simple regression analysis using a linear model between parasitism percentage and fruit size were also performed. Data were transformed before analysis. The number of oviposition attempts and probing-period were transformed using ln $+0.1$ while percentages were changed to arcsine square root. All untransformed means ($\pm$SEM) were used for data presentation.

Parasitism percentage and pupal viability were significantly higher in medium-size fruits (*Prunus persica* and *Psidium guajava*) than in large-size fruits (*C. aurantium* and *C. paradisi*) [$F(3, 84) = 110.76$, $P < 0.0001$ and $F(3, 84) = 24.96$, $P < 0.0001$, respectively] (Table I). On the other hand, the proportion of female progenies that emerged from host pupae did not vary significantly among fruit species [$F(3, 84) = 0.50$, $P < 0.6809$] (Table I). Of all the parasitoids collected from the four fruit species (254 parasitoids), 44.3 and 33.2% were recorded from *P. guajava* and *P. persica*, respectively. Regarding the mean number of parasitoids harbored per fruit, *C. paradisi* and *C. aurantium* yielded the smallest number (3.2 ± 0.5 and 2.6 ± 0.4 parasitoids,

Table I. Comparison of mean (± SEM) percentage parasitism, proportion of emerged *D. longicaudata* females, and pupal viability in four *A. fraterculus* host fruit species.

Fruit species	% Parasitism	% Emerged females	% Pupal viability
Citrus aurantium	16.2±1.8a	58.5±9.4a	41.4±2.2a
Citrus paradisi	12.9±2.0a	54.6±8.2a	47.1±1.6a
Prunus persica	54.1±1.9b	55.0±4.8a	58.8±1.8b
Psidium guajava	62.9±3.9b	58.3±2.9a	61.4±2.6b

Means within a column followed by the same letter are not significantly different (Tukey HSD test, $P=0.05$).

respectively), and *P. guajava* and *P. persica* yielded the highest number (11.5±0.7 and 8.6±0.5 parasitoids, respectively).

Fruit size was not significantly related to parasitization rate when it was compared within particular fruit species [grapefruit, $y=2.490+0.293x$ (±0.214) Log fruit weight, $F(1, 20)=1.883$, $P<0.185$, $R^2=0.040$; sour orange, $y=2.179+0.189x$ (±0.219), $F(1, 20)=0.741$, $P<0.399$, $R^2=0.035$; peach, $y=1.740–0.238x$ (±0.217), $F(1, 20)=1.206$, $P<0.285$, $R^2=0.010$; guava, $y=1.771–0.097x$ (±0.222), $F(1, 20)=0.191$, $P<0.667$, $R^2=0.009$].

Diachasmimorpha longicaudata females exhibited a number of ovipositor-probing events significantly different between *Citrus* spp., *P. persica*, and *P. guajava* [$F(3, 84)=48.51$, $P<0.0001$]. The number of ovipositor attempts was 2.6, 2.3, and 1.5 times significantly higher in common guava that those in wild sour orange, Marsh grapefruit, and wild peach, respectively (Table II). There were significant differences in the duration of ovipositor-probing period between *Citrus* spp. and both common guava and wild peach [$F(3, 84)=390.8$, $P<0.0001$]. As regards Marsh grapefruit, probing-time significantly lasted 4.5-, 4.9-, and 1.1-times more than in common guava, wild peach, and wild sour orange, respectively (Table II).

Results of this study demonstrated that *D. longicaudata* females showed a clear attraction for common guava and wild peach over Marsh grapefruit and wild sour orange, albeit fruit were not offered in a choice situation. Moreover, the results also showed that *A. fraterculus* infesting common guava and wild peach was more heavily parasitized than in both *Citrus* spp. analyzed in this study. These data suggest that the odor from common guava and wild peach may increase attractiveness to *D. longicaudata* females; also, and its physical characteristics, such as thin pericarp and fleshy mesocarp, could facilitate detection of and oviposition in *A. fraterculus* larvae. On the contrary, epicarp and mesocarp of Marsh grapefruit and wild sour orange are

Table II. Mean (±SEM) number and duration of ovipositor attempts by *D. longicaudata* females in four *A. fraterculus* host fruit species.

Fruit species	Number of ovipositor attempts	Duration (s) of ovipositor attempts
Citrus aurantium	7.8±0.5a	50.1±1.1a
Citrus paradisi	6.9±0.9a	58.8±2.1b
Prunus persica	12.1±1.2b	12.2±0.6c
Psidium guajava	17.8±0.9b	13.2±1.0c

Means within a column followed by the same letter are not significantly different (Tukey HSD test, $P=0.05$).

31 to 36 times and 10–12 times thicker than epicarp and mesocarp of common guava and wild peach, respectively. Perhaps, it reduces *D. longicaudata* female effectiveness to spot host larvae successfully in *C. paradisi* and *C. aurantium*.

Similar results to those found in this study were reported by Eben et al. (2000) studying the effect of fruit odor on *D. longicaudata* host-searching behavior. These authors found that *D. longicaudata* females were more strongly attracted to the odor of *Mangifera indica* than to that of *C. paradisi*, and that parasitism levels were higher on *Anastrepha ludens* (Loew) in mango fruit. In contrast, Leyva et al. (1991) recorded significantly stronger attraction of *D. longicaudata* females to grapefruit volatiles compared with mango volatiles. However, Leyva et al. (1991) also found that the successful oviposition of *D. longicaudata* females in *C. paradisi* was not significantly correlated with the number of females visiting fruit. Some chemical compounds are found in *P. guajava*, *Mangifera indica* L., *Prunus* spp. as well as in *Citrus* spp. (Carrasco et al. 2005). However, there may be other infochemicals more specific to different fruit species, which have a major attraction power to guide *D. longicaudata* females to the microhabitat of their host. However, little information is known about fruit volatiles responsible for attraction of *D. longicaudata* females, except data on peach (Greany et al. 1977), and on mango (Carrasco et al. 2005).

Probably, due to the favorable fruit characteristics described above, both common guava and wild peach keep various Neotropical parasitoid species. This was noted by Aguiar-Menezes et al. (1997) and Ovruski et al. (2004, 2005) on *A. fraterculus* in subtropical rainforests in southern Brazil and in northern Argentina, respectively. In contrast, it was also documented by these authors, natural parasitism in exotic *A. fraterculus* host fruit species, such as *Citrus paradisi*, *C. aurantium*, and *C. sinensis* L. (Osbeck) (sweet orange), is very low and few parasitoid species can be found.

Regression data obtained in this study showed that fruit size, measured simply as weight and diameter, would presumably not affect parasitization rate on *A. fraterculus* larvae, or at least it would substantially not be more significant than other factors such as fruit chemistry and thickness of the fruit tissues. Sivinski (1991) had previously suggested that fruit size would not play a major role in the determination of parasitization rate on *Anastrepha suspensa* (Loew) larvae. This author did not find a distinctive correlation when artificial fruit spheres from different sizes were offered to *D. longicaudata* in a choice situation. Moreover, Sivinski (1991) indicated that *D. longicaudata* females would probably prefer to seek host larvae in larger fruit, a fact which would reduce differences in parasitization rates within a fruit sample containing various fruit sizes. Similarly, Segura et al. (2007) found that *D. longicaudata* females showed a clear preference for larger artificial models.

The low pupal viability observed in *A. fraterculus* pupae collected from grapefruit and sour orange could be caused by physiological barriers for larvae development. According to Aluja et al. (2003) cryptic species of *A. fraterculus* may have not yet developed the ability to metabolize the toxic allelochemicals of the flavedo that citrus contain, resulting in a low egg viability and high larval mortality in these exotic fruits. Thus, it is possible to consider that physiological factors affecting host larvae survival may also explain why *D. longicaudata* had low parasitism rates in both grapefruit and sour orange.

In conclusion, data emerging from this study suggest that the parasitization rate of *D. longicaudata* on *A. fraterculus* larvae would be partly influenced by a complex mixture of chemical (odor), physical (thickness of epicarp and mesocarp), and maybe

physiological factors (possibly by fruit toxicity to fly larva) derived from host fruit type. In addition, visual cues such as fruit color or shape, beyond the scope of this study, may also facilitate *D. longicaudata* female orientation toward a host habitat as Leyva et al. (1991), Vargas et al. (1991), Messing and Jang (1992), and Segura et al. (2007) experimentally demonstrated. Thus, within the context of *A. fraterculus* biological control programs in the citrus-growing areas of northwestern Argentina, augmentative releases of *D. longicaudata* aimed at *A. fraterculus* populations in abandoned citrus orchards where wild guavas and peaches are highly abundant could exert a stricter control.

Acknowledgements

We would like to thank both Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (CONICET) (grant PIP no. 5129/05) and Agencia Nacional de Promoción Científica y Tecnológica, Secretaria de Ciencia y Técnica de la República Argentina (grant PICTO no. 0812909/04) for the financial support. Special thanks to Carolina Colin for assistance during laboratory work, Jorge Cancino-Diaz, Pablo Montoya (Mexican MOSCAMED Program, Metapa de Domínguez, Chiapas, México), Pablo Liedo (ECOSUR, Tapachula, Chiapas, México), and Martín Aluja (INECOL, Veracruz, Xalapa, México) for allowing us to introduce *D. longicaudata* specimens to Argentina from México, and Teresa Vera and Eduardo Willink (EEAIOC, Tucumán, Argentina) for providing us with the first *A. fraterculus* reared specimens.

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