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Combined effect of larval and pupal parasitoid use for *Anastrepha fraterculus* (Diptera: Tephritidae) control

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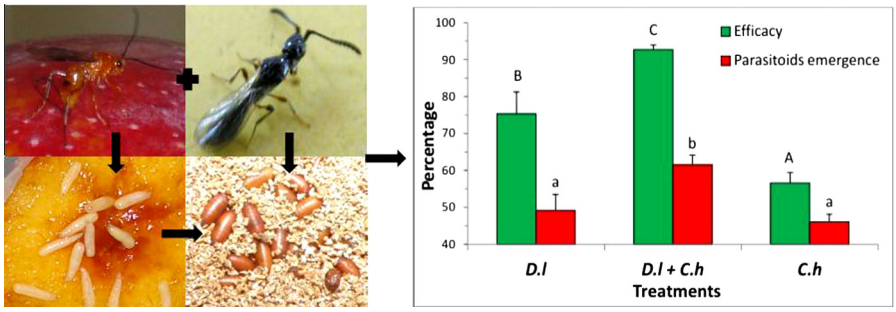
HIGHLIGHTS

- Combined use of *D. longicaudata* and *C. haywardi* against *A. fraterculus* was assessed.
- Host emergence was <10% when both parasitoids were released sequentially.
- *C. haywardi* contributed 19% of the total parasitism.
- Simultaneous release for augmentative biological control is encouraged.

GRAPHICAL ABSTRACT



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ABSTRACT

Anastrepha fraterculus (Wiedemann) is a serious pest of commercial fruit production in Argentina. Consequently, biological control is being taken into consideration as a key component of ongoing area-wide fruit fly management strategies. Two parasitoid species are currently considered for mass production and augmentative releases against pest species in the genus *Anastrepha* in the Americas: the braconid *Diachasmimorpha longicaudata* (Ashmead), a larval-prepupal koinobiont endoparasitoid, and the diapriid *Coptera haywardi* (Ogloblin), a native idiobiont pupal endoparasitoid. The prediction that the combined use of *D. longicaudata* and *C. haywardi* would be more efficient at suppressing *A. fraterculus* populations than using them individually was tested under natural environmental conditions. Particularly, the efficacy of both parasitoid species to kill their host, the proportion of superparasitism and the effect of intrinsic competition on effectiveness of host control were determined. Females of both parasitoids were singly and sequentially released inside a field cage. Peaches artificially inoculated with *A. fraterculus* larvae were exposed to *D. longicaudata*, whereas *A. fraterculus* pupae inside Petri dishes were exposed to *C. haywardi*. While used separately, effectiveness rates of *D. longicaudata* and *C. haywardi* were around 75% and 56%, respectively. However, the total efficacy increased to 93% when they were used sequentially. *Coptera haywardi* was able to attack hosts that had escaped to *D. longicaudata* parasitism, contributing by around 19% of the total parasitism in *A. fraterculus*. Both parasitoid species would induce host mortality through superparasitism. The simultaneous use of both parasitoids in fruit-growing regions for the biological control of *A. fraterculus* in Argentina is recommended.

1. Introduction

The South American fruit fly, *Anastrepha fraterculus* (Wiedemann), native of the Neotropical region, is one of the main pests

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of commercial fruit and vegetable crops in Argentina and its presence in fruit-growing regions is a phytosanitary barrier to the export of fresh fruits. This pestiferous tephritid causes major economic losses either by direct damage to fruits, i.e., presence of host larvae or oviposition activity of female flies, or indirect losses, i.e., export restrictions imposed by countries free of *A. fraterculus* (Guillén and Sánchez, 2007). *Anastrepha fraterculus* is found in the most humid and warmest areas of central and northern Argentina, where it coexists with the exotic Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), another tephritid species of quarantine importance (Ovruski and Schliserman, 2012).

Classical, augmentative, and conservative biological control approaches are the feasible biorational strategies for the management and suppression of pestiferous fruit flies (Sivinski, 1996; Purcell, 1998; Aluja and Rull, 2009; Vargas et al., 2012). Results of open-field pilot trials using augmentative releases of hymenopterous parasitoids against damaging *Anastrepha* species in the New World have resulted in increased parasitism in target areas (Aluja, 1999; Ovruski et al., 2000; Montoya et al., 2007). However, most of those biocontrol programs have been performed by using a single exotic parasitoid species, the braconid *Diachasmimorpha longicaudata* (Ashmead), essentially because it is readily mass-reared and it is able to successfully attack to different *Anastrepha* species of economic importance (Burns et al., 1996; Sivinski et al., 1996; Montoya et al., 2000a,b, 2007; Carvalho, 2005). During the last two decades, fruit fly biological control programs, particularly augmentative schemes considering multiple species releases, has received special attention, aiming to increase effectiveness of existing diverse natural enemy guilds (Purcell, 1998; Sivinski et al., 1998; Aluja, 1999; Baeza-Larios et al., 2002; Wang and Messing, 2004; Rendon et al., 2006; Garcia-Medel et al., 2007; Wang et al., 2008; Cancino et al., 2012, 2014). Releasing multiple parasitoid species simultaneously, instead of a single species, may result in efficient suppression of a fruit fly pest population particularly when no niche overlap exists (Knipling, 1992; Cancino et al., 2014). Nevertheless, when niche overlap exists, but the conditions for coexistence among parasitoid species are suitable (De Moraes et al., 1999; Borer et al., 2004), multiple releases may still be beneficial (Garcia-Medel et al., 2007). This may arise in particular when released parasitoids have either more efficient or different host foraging strategies rather than a naturally occurring parasitoid species of the same guild (Pedersen and Mills, 2004; Miranda et al., 2015). Thus, a parasitoid that can find the host at low densities may be a superior searcher that might not interfere with other parasitoid species adapted to seek for hosts present in high densities (Garcia-Medel et al., 2007). Nevertheless, it should be noted that when the mortalities inflicted by multiple natural enemies are fewer than the sum of their individual capacities to suppress host populations, the outcome is a non-additive effect (Ferguson and Stiling, 1996; van Lenteren et al., 2006). On the basis of the remarks made in the foregoing paragraphs, understanding and predicting potential competitive outcomes is important for the design of biological control programs when several parasitoid species may be simultaneously used (Murdoch and Briggs, 1996). Using unsuitable combinations of parasitoid species should be avoided altogether when choosing potential candidates for augmentative releases (Pedersen and Mills, 2004).

Within natural enemy guilds, larval-pupal parasitoids are the most commonly used in biological control programs to suppress pestiferous fruit fly populations (Wong et al., 1991, 1992; Sivinski, 1996; Purcell, 1998; Ovruski et al., 2000; Montoya et al., 2007; Vargas et al., 2012; Miranda et al., 2015). One of the most widely used species in biological control programs is precisely *D. longicaudata*, which is a larval-pupal, koinobiont, solitary endoparasitoid, native to Southeast Asia (Montoya et al., 2000a). This braconid parasitoid has been mainly released against various *Bactrocera* spp. throughout the Pacific region (Vargas et al., 2012). Moreover, *D. longicaudata* has been introduced quite extensively in the Americas as a classical biological control agent (Ovruski et al., 2000). In the 1960s, it was introduced into Argentina for the first time for the biological control of *C. capitata* and *A. fraterculus*. In the 1990s, *D. longicaudata* was reintroduced to promote the use of biological control in the fruit fly integrated management programs performed by the National Fruit Fly Control and Eradication Program (ProCEM) of the National Agri-Food and Animal Health and Quality Service of Argentina (Ovruski et al., 2003). Nevertheless, the permanent establishment of *D. longicaudata* on *A. fraterculus* was only confirmed approximately 40 years after its first releases (Schliserman et al., 2003).

Biological control has been incorporated as an auxiliary tool for *C. capitata* control and eradication practices currently deployed in the San Juan province of central-western Argentina (Ovruski and Schliserman, 2012). Consequently, *D. longicaudata* is being mass-reared at the "BioPlanta San Juan" facility with the objective of augmentative-releasing this species either against *C. capitata* under semiarid conditions in ecologically isolated fruit-growing valleys of San Juan, or against *A. fraterculus* in Citrus-growing areas of northwestern and northeastern Argentina (Suarez et al., 2012). Recently, *D. longicaudata* adults were recovered from Medfly-infested fig, grape, rose, orange, tangerine, and persimmon fruits as a result of sporadic releases of this exotic parasitoid species in several fruit-producing valleys of San Juan (Suarez et al., 2014). However, *D. longicaudata* alone does not sufficiently suppress pestiferous fruit fly populations in Argentina; therefore the use of multiple parasitoid species for fruit flies biological control would be justified (Ovruski and Schliserman, 2012).

One of the neotropical parasitoid species that has potential for fruit fly biological control is *C. haywardi* (Ogloblin) (Sivinski et al., 1998; Baeza-Larios et al., 2002; Aluja et al., 2009; Cancino et al., 2009, 2014), a solitary idiobiont pupal endoparasitoid that attacks its host after pupation in the soil (Ovruski et al., 2000). Although it was originally discovered in the Yungas forest of northwestern Argentina attacking *A. fraterculus* and *A. schultzi* Blanchard pupae (Loiacono, 1981), *C. haywardi* is widely distributed in the Neotropical region on several *Anastrepha* species (López et al., 1999; Ovruski et al., 2000; Garcia and Montilla, 2001; Guillén et al., 2002; Aguiar-Menezes et al., 2003). Moreover, it is a parasitoid that can successfully develop in irradiated pupae of *C. capitata* (Menezes et al., 1998). Currently, *C. haywardi* is being reared under artificial conditions using both *A. fraterculus* and *C. capitata* pupae as host at the Biological Control Division of the Pilot Plant of Industrial Microbiological Processes and Biotechnology (PROIMI), in Tucumán (Núñez-Campero et al., 2012), to develop new strategies for controlling both fruit fly pest species in Argentina.

The braconid *D. longicaudata* is widely established into Latin America, occurring from Mexico to Argentina, while the diaprid *C. haywardi* is a native parasitoid species of America (Ovruski et al., 2000; Ovruski and Schliserman, 2012). Both *D. longicaudata* and *C. haywardi* have the ability to attack several fruit flies of economic importance in the Neotropic (Cancino et al., 2014). Moreover, certain biological traits of both *D. longicaudata* and *C. haywardi*, as well as the development of techniques for mass rearing of these two fruit fly parasitoid species in México (Montoya et al., 2007; Aluja et al., 2009; Cancino et al., 2009, 2014), have been considered suitable for selecting them for augmentative biological control in the framework of integrated fruit fly management programs on an area-wide basis in Argentina. Some of these relevant characteristics have previously been mentioned for *D. longicaudata* by Ovruski and Schliserman (2012), such as its capacity for successful development on the larvae of either *A. fraterculus* or *C. capitata* and its host-finding ability at different host-densities on a wide variety of fruit species at canopy and

ground level. It adds to those traits its adaptability to different environmental conditions of the Argentinean fruit-growing regions into which it has been released (Schliserman et al., 2003; Oroño and Ovruski, 2007; Suarez et al., 2014). The diapriid *C. haywardi* is particularly interesting as a fruit fly biological control agent in Argentina due to its following features: (1) its specificity for parasitizing Tephritidae (Sivinski et al., 1998), (2) its ability to locate and attack pupae of both *C. capitata* and *A. fraterculus* (Baeza-Larios et al., 2002; Núñez-Campero et al., 2012), (3) its good performance in finding tephritid pupae under different soil conditions (Guillén et al., 2002), and (4) because its population and reproductive parameters are similar to those recorded for other fruit fly parasitoid species already used with positive results against tephritid species in augmentative biological control programs (Núñez-Campero et al., 2012). In addition, a very important trait of *C. haywardi* recently pointed out by Cancino et al. (2012) is its ability to discriminate *Anastrepha ludens* (Loew) pupae that were previously parasitized by *D. longicaudata* as larvae. However, there is little biological information on *C. haywardi* in association with *A. fraterculus* in Neotropics.

Based on the above, we predicted that the combined use of *D. longicaudata* and *C. haywardi* females would be more efficient in suppressing *A. fraterculus* populations than using either species individually. This prediction was based in particular on the fact that the capacity of *C. haywardi* to discriminate among hosts parasitized by heterospecifics, such as *D. longicaudata*, could result in an additive effect for controlling pestiferous fruit fly populations when both parasitoid species are used together (Cancino et al., 2014). Specifically, the following issues were examined under natural environmental conditions: (1) the relative efficacy of *D. longicaudata* and *C. haywardi* females in both single- and multiple-species cohorts to reduce an *A. fraterculus* population; (2) the effect on *A. fraterculus* larvae/pupae attacked by conspecifics by both parasitoid species by recording superparasitised hosts; and (3) the potential consequence of the competitive interaction between larvae of both parasitoid species occupying the same host larva (= intrinsic competition) (Wang et al., 2008; Cusumano et al., 2012) on effectiveness of *A. fraterculus* control.

This study is part of a renewed effort to incorporate an exotic parasitoid with the native parasitoids for the control of damaging tephritid species in Argentina (Ovruski and Schliserman, 2012). Given this issue, results are discussed in terms of the possibilities to simultaneously use one parasitoid species with a long co-evolutionary history (*C. haywardi*) and another with a very recent history of interaction (*D. longicaudata*) with the same host (*A. fraterculus*), in augmentative and/or conservative fruit fly biological control programs contemplated or underway in Argentina and eventually anywhere in subtropical America.

2. Material and methods

2.1. Insect rearing

Parasitoids and fruit flies were reared at the Laboratorio de Investigaciones Ecoetológicas de Moscas de la Fruta y sus Enemigos Naturales (LIEMEN) of the Planta Piloto de Procesos Microbiológicos Industriales y Biotecnología (PROIMI), in San Miguel de Tucumán, Argentina. Both *D. longicaudata* and *C. haywardi* were reared under laboratory conditions using *A. fraterculus* larvae (9-11 d-old) and pupae (2-3 d-old) as parasitoid hosts, respectively, at 25 ± 1 °C, 75 ± 5% RH, and a 12:12 (L:D) h photoperiod. Parasitoid colonies were held in 30 cm-cubical Plexiglas cages that were covered on both lateral walls by a double organdy cloth screen. Parasitoids were provided with water and honey every other day. Rearing procedures for *C. haywardi* were carried out as described by Núñez-Campero et al. (2012) whereas those of *D. longicaudata* were previously described by Ovruski et al. (2011). *A. fraterculus* was reared following methods described by Braga-Sobrinho et al. (2006, 2010), and Vera et al. (2007).

2.2. Study site

The study was conducted in the Horco Molle Experimental Reserve under field conditions. This Reserve is a protected area of wild vegetation belonging to the Universidad Nacional de Tucumán in the district of Yerba Buena, Tucumán, Argentina, located at 26° 38′–26° 57′ S and 65° 26′–65° 20° W. During experiments, maximum and minimum temperatures and relative humidity were recorded with a Data Logger (HOBO U10, onset®) inside each field cage. Temperatures and relative humidities did not sharply vary in both field cages during each testing day (Appendix 1).

2.3. Experimental procedure

2.3.1. Adult parasitoid performance

Experiments were conducted inside a cylindrical nylon field cage (3 m diameter × 3 m height) surrounded by trees that provided shade. The cage was protected from rain by a translucent fiber glass roof that allowed natural light to go through. The field cage was internally divided into three smaller cylindrical organdy cages (0.7 m diameter × 2 m height). A small potted sour orange tree (1 m height) was placed inside each organdy cage to simulate a natural environment and provide shelter and rest for parasitoids. The field-cage experiment simultaneously included three treatments. The first trial involved only *D. longicaudata* adults, which were released in an organdy cage; the second included only *C. haywardi* adults, which were released in another organdy cage; and a third trial involved adults of both parasitoid species that were sequentially released in the third organdy cage. The assays were conducted to assess, under a free-foraging condition, the following issues: first the individual capabilities of both *D. longicaudata* and *C. haywardi* in parasitizing *A. fraterculus* larvae and pupae, respectively; second, the efficacy of combining the abovementioned two parasitoid species to increase *A. fraterculus* mortality rate; and third, the effect of the interaction between *D. longicaudata* and *C. haywardi* on sex ratio of parasitoid offspring.

For treatment 1 (T1) (*D. longicaudata* adults alone), three ripe peaches (*Prunus persica* L.), each one artificially inoculated with 100 laboratory-reared third-instar (9-10-d old) larvae of *A. fraterculus,* were used as oviposition units. 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 and Ovruski, 2011). The fruit were obtained directly from unsprayed, uninfested trees from the Experimental Reserve. Several branches of peach trees, each containing 5–10 unripe fruit, were covered with a cloth mesh. One day before each assay, 10 ripe fruit were harvested and transported to PROIMI's laboratory. However, 3 fruit with both similar size and ripeness degree were selected and subsequently used in the assay. Each fruit was inoculated by removing the stone and filling this space with naked larvae (without artificial diet). To this end, fruit was halved by using a sterilized scalpel. Once the fruit was inoculated, both right and left fruit portions were joined together with 2.5 cm wide Parafilm strips (Parafilm 'M', Parafilm® Laboratory Film, Pechiney Plastic Packaging, Chicago, IL). Then, approximately 50% of the total fruit volume was occupied by the host. Each inoculated peach was individually hung from the ceiling of the field cage and positioned at canopy level on a potted sour orange tree to form a central circle (50 cm in diameter) about 1 m above ground. All of the fruits were equidistant from each other, and their positions were randomized. Fruit was suspended

from the cage roof using a similar method to that described by Garcia-Medel et al. (2007). Once inoculated peaches were hung, 60 naïve, 5-7-d-old, mated *D. longicaudata* females were released inside the organdy cage. Parasitoids were released at the central point of the circle formed by the test fruit. Parasitoid females were allowed to forage freely for 24 h starting at 09:00 h. The host-parasitoid ratio was 5 host larvae per 1 *D. longicaudata* female as proposed by Van Nieuwenhove et al. (2012). Once the 24-h period was over, both fruit and female parasitoids were removed from the cage. At the laboratory, fruit were individually placed into plastic cups (250 ml) with sterilized Vermiculite® on the bottom to allow pupation and covered with pieces of organdy on the top. Fruit were kept for 24 h in containers to allow larvae to leave the fruit and develop into pupae. After that time, each fruit was removed from the container and dissected to retrieve live *A. fraterculus* larvae remaining in the fruit. These larvae were placed into abovementioned plastic cups. The pupae were moistened weekly to avoid desiccation and held inside cups until adult flies or parasitoids emerged. Cups were kept in a room at 25 ± 1 °C, 75 ± 5% RH and 10:14 h (L: D) photoperiod. Both the number of dead larvae per fruit and the number of dead pupae per cup were recorded.

For treatment 2 (T2) (*C. haywardi* adults alone), three Petri dishes (10 cm in diameter, 1 cm in depth), each one containing 100 laboratory-reared *A. fraterculus* 2-3-d old pupae were used as oviposition units. Pupae were buried at a depth of 5 mm inside each Petri dish. For which purpose, pupae were covered lightly with sterilized and moistened local soil. This artificial burial depth was selected for two major reasons: first, because tephritid larvae burrow 5 cm or more depending on the soil type and other conditions (Hodgson et al., 1998); and second, because *C. haywardi* achieves higher parasitism rates in buried host pupae at 5 mm than in pupae buried at greater depth (Baeza-Larios et al., 2002; Guillén et al., 2002). Each Petri dish was placed on the cage floor beneath respective fruit in such a way that they also formed a central circle of 50 cm in diameter. Once Petri dishes were placed on the floor, 60 naïve, 6-7-d-old, mated *C. haywardi* females were released inside the organdy cage. The pupal age to expose hosts to parasitoids was chosen because the highest number of *C. haywardi* offspring may be achieved using 2-3-d old *A. fraterculus* pupae as hosts (Núñez-Campero et al., 2012). Parasitoids were released at the central point of the circle formed by the dishes. Parasitoid females were allowed to forage freely for 24 h starting at 09:00 h. The host-parasitoid ratio was 5:1. Once the 24-h period was over, each Petri dish was removed from the cage. At the laboratory, pupae recovered from individual Petri dishes were sifted from the pupation medium and kept into plastic cups (250 ml), with new sterilized moist vermiculite until all of the flies and parasitoids emerged. The cups were kept in a room at 25 ± 1 °C, 75 ± 5% RH and 10:14 h (L: D) photoperiod. The number of dead pupae per cup was recorded.

For treatment 3 (T3) (*D. longicaudata* and *C. haywardi* adults together), three peaches each one inoculated with 100 third-instar (9-10-d old) larvae of *A. fraterculus* were first exposed to 60 naïve, 5-7-d-old, mated *D. longicaudata* females. After exposure, fruit were removed and transported to the laboratory following the method outlined in trial 1. Then, 2-3-d old pupae recovered from inoculated peaches were exposed to *C. haywardi* females inside the field-cage as was the case for treatment 2.

A cylindrical nylon field cage adjacent to Experimental cage was used to perform a Control test for each treatment. This second field cage was used to provide better security conditions in control treatments, in order to avoid any further possible contamination from both the parasitoids used in treatments and wild native parasitoids. The control tests involved inoculated fruit or/and pupae not exposed to parasitoids and were conducted to determine natural *A. fraterculus* larval and pupal mortality rates, as well as adult fly emergence rate. Treatments and control tests were replicated 11 times on different days. For each replicate, a new parasitoid cohort was always released into the cage, and either new inoculated peaches were hung from the cage roof or new Petri dishes were placed on the cage floor. The adult parasitoid emergence and the real or total host mortality inflicted by the parasitoid (effectiveness) were estimated from each treatment.

2.3.2. Determination of parasitism strategies

In a second experiment the host was not allowed to develop until adult emergence, but was dissected 96 h after its last exposure to parasitoid females. After this period, first-instar larvae of each parasitoid species can be found without difficulty (Córdova, 2008). Samples of 12 pupae per replicate of each abovementioned treatment were removed from cups (i.e. 132 for each treatment and 396 in total). Host pupa dissections were performed as described by Van Nieuwenhove and Ovruski (2011). Samples were categorized according to three parasitism strategies as follows: mono-, super-, and multi-parasitism. The first strategy involved a single 1st-instar larva, alive or dead, belonging to one parasitoid species inside host pupa. The second consisted in two or more 1st-instar larvae, alive or dead, belonging to a single parasitoid species inside host pupa. The third involved 1st-instar larvae, alive or dead, belonging to both *D. longicaudata* and *C. haywardi* inside host pupa. A parasitoid larva was considered dead when it either did not move or was damaged (Aluja et al., 2013). First-instar larvae of *D. longicaudata* and *C. haywardi* were recognized according to morphological features described by Ibrahim et al. (1994), Córdova (2008), respectively. However, previous dissections of 20 host larvae parasitized by *D. longicaudata* and 20 others parasitized by *C. haywardi* were made to ease distinction of 1st-instar larva of both parasitoid species. The experiment was carried out to determine and compare the percentage of parasitized host pupae on the basis of the different parasitism strategies under single- or multiple-species cohort releases.

2.4. Biological parameters calculation

Individual parasitoid emergence for each parasitoid species was calculated as the number of adult parasitoids emerged divided by the total number of host larvae (or pupae) exposed to parasitoid females × 100. Overall parasitoid emergence for both parasitoid species together was calculated as the number of *D. longicaudata* adults emerged plus the number of *C. haywardi* adults emerged on the total number of host larvae exposed to parasitoid females × 100. The Abbot's corrected formula, which involves adult fly emergence rates for both treatment and control tests, was used to determine the effectiveness of the parasitoid species for killing the host (Rosenheim and Hoy, 1989). Sex ratio was estimated as the proportion of female offspring over male offspring. The percentage of parasitized host pupae was calculated as the number of pupae that contained parasitoid larvae (1st-instars), either alive or dead, divided by total number of host larvae (or pupae) originally exposed to parasitoid females in the assay × 100.

2.5. Statistical analysis

To meet parametric assumptions, percentage data were transformed to arcsine square root prior to analyses (Zar, 1999); nevertheless untransformed means (±SE) are shown in figures to ease interpretation. Parasitoid emergence and effectiveness were subjected to a two-way univariate mixed-model ANOVAs with type III error at $P = 0.05$. This type of analysis allowed us to identify significant effects of parasitoid species (*D. longicaudata* and *C. haywardi*), their condition (alone or together), and their interaction (parasitoid species × condition) on both response variables

(effectiveness and parasitoid emergence). The fixed component of the models were parasitoid species, condition, and their interaction (parasitoid species∗condition), whereas the random component (time) with 11 levels (days 1–11) was blocked. Also, a two-way univariate mixed-model with type III error ($P = 0.05$) was used to examine the effect of each treatment [i.e. parasitoid species in presence of conspecific and heterospecific cohorts (*D. longicaudata*, *C. haywardi*, and *D. longicaudata* plus *C. haywardi*)], parasitism strategy (mono- super- and multi-parasitism) and their interaction on the percentage of parasitized host pupae. Mean comparisons were analyzed by Tukey's honestly significant difference (HSD) test at $P = 0.05$. The female offspring percentage for *D. longicaudata* and *C. haywardi* was compared using a *t*-test at $P = 0.05$. Statistical analyses were performed using STATISTICA, version 10.0 software (StatSoft, 2011).

3. Results

3.1. Efficacy and parasitoid emergence

Efficacy varied considerably between parasitoid species ($F_{1,10} = 8.82$, $P < 0.01$) as well as in presence or absence of one of the two parasitoid species ($F_{1,\ 0} = 120.59$, $P < 0.01$) and their interaction ($F_{1,10} = 8.14$, $P < 0.01$). More specifically, the efficacy of *D. longicaudata* on *A. fraterculus* was 2.0-times higher than that recorded for *C. haywardi* under an isolated condition, but it was 1.6-times lower than the value found when both parasitoid species were sequentially released (Fig. 1). Similarly, parasitoid emergence varied notably between parasitoid species ($F_{1,10} = 30.44$, $P < 0.01$), with species condition (alone or together) ($F_{1,10} = 28.74$, $P < 0.01$), and the interaction between fixed factors ($F_{1,10} = 65.76$, $P < 0.01$). The emergence of *D. longicaudata* and *C. haywardi* was similar when each species was separated from one another (Fig. 1). However, when both parasitoid species were sequentially released, parasitoid emergence was 2.4- and 3.2-times higher than those found for *D. longicaudata* and *C. haywardi*, respectively (Fig. 1). It is noteworthy that the emergence of *C. haywardi* decreased 2.5-times in presence of *D. longicaudata* (Fig. 2) relative to when the diapriid parasitoid was alone. However, the emergence percentage of *A. fraterculus* was less than 10% (Fig. 2). Percentage of host mortality in the controls were 4.9 ± 0.9%, 4.5 ± 1.5%, and 4.7 ± 0.7% for T1, T2, and T3, respectively.

3.2. Parasitism strategy (mono-, super-, and multi-parasitism)

The percentage of parasitized host pupae, in presence of conspecific and heterospecific cohorts, were significantly influenced by "type of parasitism" factor ($F_{5,2374} = 8.19$, $P < 0.01$) and the interaction with the "treatment species" factor ($F_{10,2374} = 6.01$, $P < 0.01$).

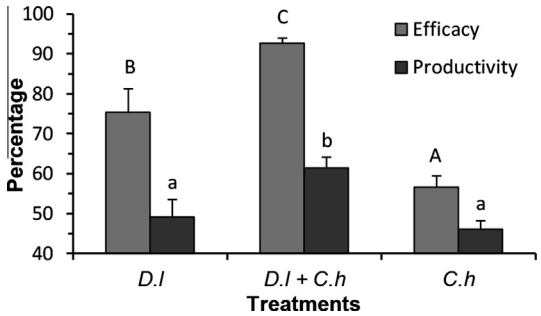

Fig. 1. Efficacy and productivity (mean ± SE) of *Diachasmimorpha longicaudata* (*Dl*) and *Coptera haywardi* (*Ch*) under conspecific and heterospecific conditions. Bars crowned by the same letter indicate no significant differences (Tukey HSD test, $P = 0.05$).

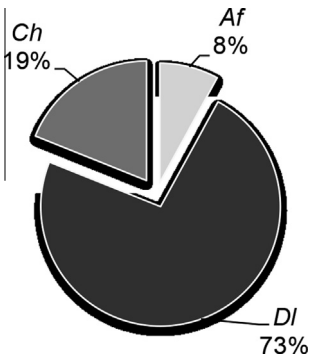

Fig. 2. Percentage of emerged adult insects under a heterospecific condition. Notations: *Dl*, *Diachasmimorpha longicaudata*; *Ch*, *Coptera haywardi*; *Af*, *Anastrepha fraterculus*.

No significant difference was observed for the "species treatment" factor ($F_{2,2374} = 0.01$, $P = 99.98$). In conspecific situation *D. longicaudata* exhibited a tendency towards super-parasitism 1.5-times higher than that observed for *C. haywardi*, however there was no statistical difference between the values of super-parasitism recorded for each parasitoid species (Fig. 3). Moreover, super-parasitism was remarkably (2.0-times) higher than mono-parasitism for *D. longicaudata* (Fig. 3). For *C. haywardi* super-parasitism was 1.6-times higher than mono-parasitism but there was no statistical difference between the two parasitism types (Fig. 3).

In presence of heterospecific cohorts, super-parasitism by *C. haywardi* was similar to mono-parasitism recorded for both parasitoid species (Fig. 3). However, for *C. haywardi* super-parasitism was 6.0-times lower than the super-parasitism caused by *D. longicaudata* (Fig. 3). Super-parasitism exhibited by *D. longicaudata* was 1.8-times higher than the multi-parasitism, but there was no statistical difference between both parasitism types (Fig. 3). Although multi-parasitism was approximately 2.0-times higher than both mono-parasitism and super-parasitism recorded for *C. haywardi* there was no significant difference among these three parasitism types (Fig. 3). It is noteworthy that under a heterospecific condition, the number of multi-parasitized hosts was slightly (1.3-times) higher than those only parasitized by *C. haywardi* (including jointly both mono- and super-parasitism), but also it was notably (2.9-times) lower than those only parasitized by *D. longicaudata*.

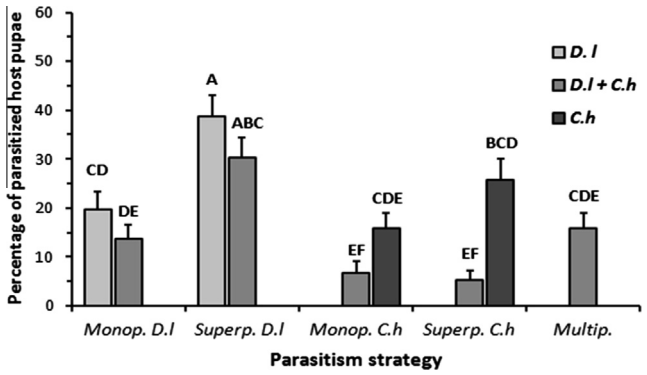

Fig. 3. Types of parasitism (mean ± SE) recorded for *Diachasmimorpha longicaudata* (*Dl*) and *Coptera haywardi* (*Ch*) both in conspecific and heterospecific conditions. Bars crowned by the same letter indicate no significant differences (Tukey HSD test, $P = 0.05$). Notations: Monop., monoparasitism; Superp., superparasitism; Multip., multipleparasitism.

3.3. Sex ratio of parasitoid offspring

There was no significant difference between the female offspring percentage for both *D. longicaudata* and *C. haywardi* in any of the trials ($t = 0.996$, $df = 20$, $P = 0.3309$ for *C. haywardi*; $t = 0.447$, $df = 20$, $P = 0.6595$ for *D. longicaudata*) (Table 1). Furthermore, *D. longicaudata* always exhibited a female-biased sex ratio whereas *C. haywardi* displayed a male-biased sex ratio (Table 1).

4. Discussion

Results of the present study showed that *D. longicaudata* performed better than *C. haywardi* from the single species exposure treatment. This fact clearly indicated that the two parasitoid species had a dissimilar effect on mortality rate of *A. fraterculus* assuming that *D. longicaudata* would have a greater 'killing power' than *C. haywardi*. Nevertheless, both *D. longicaudata* and *C. haywardi* performed similarly in terms of specific emergence rate when they were individually tested on *A. fraterculus*. Emergence percentages recorded for *D. longicaudata* from *A. fraterculus* pupae compared to the data observed in this work (around 50%) were previously reported by Ovruski et al. (2011), Van Nieuwenhove and Ovruski (2011), and Van Nieuwenhove et al. (2012). In addition, 50 million adult parasitoids of *D. longicaudata* are weekly released in among five field areas in Mexico, where it is possible to obtain in some areas 70% of *Anastrepha's* fruit fly populations control with a 50% average of parasitism as was reported by Montoya et al. (2000a, 2007).

Correspondingly, the emergence percentages recorded in this study for *C. haywardi* were similar to those reported for this diapriid parasitoid from *A. ludens* pupae either collected in the field (López et al., 1999) or tested in laboratory (Guillén et al., 2002), and from *C. capitata* pupae exposed in field cages (Baeza-Larios et al., 2002). Nevertheless, Sivinski et al. (1998), Aluja et al. (2009), and Núñez-Campero et al. (2012) reported emergence percentages close to 75, 70, and 86% by *C. haywardi* in 3-d-old pupae of *A. suspensa*, *A. ludens*, and *A. fraterculus*, respectively. Those values are 1.6- and 1.9-times higher than the percentage recorded for this diapriid species in the present work. This dissimilarity might be caused by several factors, such as different experimental procedures (i.e., host/parasitoid proportion, host exposure time, parasitoid female age), different environmental conditions of the study (i.e. laboratory controlled conditions), uneven quality of host pupae (i.e. sizes and diets used for host larval rearing), and different fly species as hosts (*A. suspensa* (Loew) and *A. ludens*). Contrary to data provided by the authors cited above, Cancino et al. (2009) reported emergence values for *C. haywardi* reared on *A. ludens* 1.4- and 1.6-times lower than that recorded in this work. Probably, this difference was due to the fact that the aim of the Cancino et al. (2009) study was focused on evaluating the effect of different irradiation doses on adult emergence rates of *C. haywardi*. Interestingly, the combined action of *D. longicaudata* and *C. haywardi* enhanced effectiveness on mortality rate of the target pest, compared to the instances when each parasitoid species was used individually. The significant additive effect of both parasitoid species on *A. fraterculus* mortality which reached nearly 93% is noteworthy. This result clearly supports Cancino et al. (2014) finding which indicated that *C. haywardi* is suitable for complementing initial parasitism by *D. longicaudata* attacking *Anastrepha*. The aforementioned authors found that the contribution of *D. longicaudata* to the mortality rate of the two *Anastrepha* species (*A. ludens* and *A. serpentina*) studied was higher than that by *C. haywardi* in a single fruit species of the three host fruit species tested. In the present study, a single host fruit species, namely peach, was assessed and the host fly mortality due to *D. longicaudata* was 4 times higher than that caused by *C. haywardi*. Variation in the difference found between the two parasitoid species regarding mortality inflicted on the host is likely to be the result of two scenarios. Firstly, *D. longicaudata* has shown some limitations to parasitize *Anastrepha* larvae into different fruit species (Leyva et al., 1991; López et al., 1999; Montoya et al., 2007; Ovruski et al., 2012; Cancino et al., 2014). Parasitism caused by *D. longicaudata* can decrease according to fruit characteristics, such as fruit surface area, rind thickness, and pulp depth (Ovruski et al., 2012). Secondly, *C. haywardi* seems to avoid hosts previously parasitized by *D. longicaudata* (Cancino et al., 2012). Consequently, host larvae that escape from the attack of *D. longicaudata* could attract subsequent *C. haywardi* parasitism on pupae (Cancino et al., 2014). The fact that the pupal parasitoid *C. haywardi* can exploit a different host stage than that used by *D. longicaudata*, joined to its ability to discriminate pupae previously parasitized by conspecific and heterospecific parasitoids, leads to low levels of both intrinsic and extrinsic competition between them. According to Harvey et al. (2013) and Wang et al. (2015) two or more parasitoid species could coexist when they share the same host species and even the same stage due to their different life-history traits, considering the ability to discriminate previously parasitized hosts, in order to dilute the competition among them.

Peach evaluated in the present study is a fruit with good conditions allowing host larvae access to *D. longicaudata*, e.g. thin epicarp, soft and shallow pulp, and small surface area. Therefore, the individual effect of each parasitoid species on *A. fraterculus* mortality rate in a sequential host exposure test may vary depending of fruit characteristics. Based on these issues, a series of new experiments involving both *C. haywardi* and *D. longicaudata* with the most common *A. fraterculus* host plants growing in Argentina's central and northern regions has been arranged.

Data on intrinsic competition in the heterospecific test recorded under the conditions of this study showed that *D. longicaudata* dominated *C. haywardi*. This result suggests that the former was a superior competitor in multiparasitised hosts. This would be associated with the fact that *D. longicaudata* had a competitive advantage over *C. haywardi* due to the order in which the two parasitoids attacked. Previous studies by Sivinski et al. (1998) on heterospecific parasitism between *C. haywardi* and *D. longicaudata* found that the former parasitoid species oviposited in *A. suspensa* pupae that had been previously parasitized by the opine, but *C. haywardi* did not complete development. Similar data were recorded by Cancino et al. (2012) using *A. ludens* as target host. However, the remarkable reduction of both emergence and efficacy of *C. haywardi* recorded in the present study may be caused by either intrinsic competition with *D. longicaudata* or a significant decline of non-parasitized host number.

Results of this study demonstrated that *D. longicaudata* displayed a strong tendency to superparasitize *A. fraterculus* larvae. This agrees with prior studies by Lawrence (1988) on *A. suspensa*, Montoya et al., 2000b and Gonzalez et al. (2007) on *A. ludens*, and Van Nieuwenhove et al. (2012) on *A. fraterculus*. As suggested by Montoya et al. (2000b), superparasitism in *D. longicaudata* might be an adaptive behavior that would facilitate the survival of parasitoid female larvae inside host fly larvae. However,

Table 1
Sex ratio of both *D. longicaudata* and *C. haywardi* offspring recorded from treatments involving a single parasitoid species release or combined parasitoid species releases. $^{(ns)}$ No significance differences were found between female offspring in both *D. longicaudata* and *C. haywardi* in any of the treatments (*t*-test, $P = 0.05$).

Treatments	*D. longicaudata*			*C. haywardi*		
	% Female$^{(ns)}$	% Male	Sex ratio (F:M)	% Female$^{(ns)}$	% Male	Sex ratio (F:M)
T1 (Dl)	65.1	35.0	2.3:1	–	–	–
T2 (Ch)	–	–	–	33.1	66.9	0.6:1
T3 (Dl + Ch)	62.3	37.8	2.1:1	38.4	61.6	0.7:1

superparasitism may increase host mortality levels (Montoya et al., 2000b), resulting in an increased effectiveness on the host by parasitoid female action (Van Nieuwenhove and Ovruski, 2011). Although in *C. haywardi* there was not as marked a tendency to superparasitize host larvae as that of *D. longicaudata*, the study showed a level of superparasitism significantly similar to monoparasitism under both conspecific and heterospecific conditions. Probably, the high level of efficiency exhibited by both *D. longicaudata* and *C. haywardi* in the present study (Fig. 1) is not only due to reproductive host-killing through oviposition, but also to non-reproductive host-killing by means of the action of superparasitism. In addition, another host mortality factor probably caused by *D. longicaudata* and *C. haywardi* might be the host stinging activity without oviposition. Approximately 3–5% of dead *A. fraterculus* pupae with one or more scars on the puparial cuticle due to previous attacks by *C. haywardi* and *D. longicaudata,* respectively, had no immature parasitoids inside when they were dissected (S.M. Ovruski, unpublished data). The scars may occur by oviposition or oviposition attempts in the host by females of both *D. longicaudata* and *C. haywardi* (Cancino et al., 2012). Another mortality factor caused by many synovigenic parasitoids is host feeding behaviour (Jervis and Kidd, 1986). However, this behavior was not observed in *C. haywardi* when females had direct contact with host pupae (L.P. Bezdjian, unpublished data).

Several studies on interactions between different parasitoid species attacking one same host (Godfray, 1994; Bautista and Harris, 1997; Cusson et al., 2002; Wang and Messing, 2002, 2003, 2004; Javad Ardeh et al., 2005; Quicke, 2015) remark that multiparasitism would be more frequent than the superparasitism. Data shown in this study is in agreement with this statement, because that superparasitism was 3.0-times lower than multiparasitism. Furthermore, superparasitism and monoparasitsm under a heterospecific situation was 4.9- and 2.4-times lower, respectively, than those recorded in a conspecific condition. These results would suggest that *C. haywardi* may have greater ability to discriminate previously exploited hosts by conspecifics than by heterospecifics. Interestingly, Cancino et al. (2012) found that 30% of the total number of *C. haywardi* females oviposited into host previously parasitized by *D. longicaudata* whereas only 12% of *C. haywardi* females did so in pupae previously parasitized by conspecifics. However, the aforementioned authors reported that *C. haywardi* females were less likely to oviposit into hosts previously attacked by *D. longicaudata*. Further studies on inter-specific discrimination by *C. haywardi* adults are therefore warranted to test in detail predictions stemming from the present findings.

5. Conclusion

Multi-parasitized hosts did not influence *D. longicaudata* parasitism rates, but the specific parasitism of *C. haywardi* decreased in the presence of *D. longicaudata*. However, *C. haywardi* managed to attack hosts that had escaped from *D. longicaudata*. Thereby, *C. haywardi* contributed by around 19% of the total parasitism on *A. fraterculus*.

Superparasitism would be an important additional mortality factor, which needs to be accounted for when evaluating *D. longicaudata* and *C. haywardi* performance.

Interestingly, no harmful effect was found on the offspring sex ratio of parasitoids when they were released either sequentially or individually.

Since both parasitoids combined led to a higher efficiency, the effect of the simultaneous use of *D. longicaudata* and *C. haywardi* in augmentative biological control programs would contribute to an increase of *A. fraterculus* mortality rate. Nevertheless, due to the negative effect on *C. haywardi* emergence caused by prior *D. longicaudata* parasitism into the same host, it would be advisable to focus further studies on interactions between this exotic parasitoid species and other neotropical parasitoid species, such as braconids and figitids, before deciding on their combined use by means of releases in Citrus-growing areas of northern Argentina.

Combined releases of *D. longicaudata* and *C. haywardi* may be more advantageous in fruit-growing regions where no evidence of endemic *Anastrepha* parasitoid species has ever been found. Thus, both parasitoid species may be released under semiarid conditions in ecologically isolated fruit-producing irrigated-valleys of San Juan and La Rioja, located in central-western Argentina. In such semiarid areas, fruit crops and orchards are found in vegetation patches under artificial irrigation constituting ecological islands. Therefore, combined releases of *D. longicaudata* and *C. haywardi* in such patches might facilitate the assessment of the effect of both parasitoids on pest population under field conditions. Nevertheless, studies on the bioclimatic requirements of both *D. longicaudata* and *C. haywardi*, as well as post-release monitoring and assessment of parasitoids efficacy, are still needed in these irrigated fruit-producing areas. Finally, it is noteworthy that the results of this study are relevant to the fruit fly biological control framework of the New World taking into account that both parasitoid species occur throughout the Neotropical region attacking several pestiferous *Anastrepha* species.

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Appendix 1

See Table A1.

Table A1
Mean ($T_{\bar{x}}$), minimum (T_{min}), and maximum (T_{Max}) temperatures and relative humidity ($RH_{\bar{x}}$, RH_{min}, and RH_{Max}) in the study area during testing dates (midsummer) in 2012.

Testing dates	Treatment field cage						Control field cage					
	T_{Max} (°C)	T_{min} (°C)	$T_{\bar{x}}$ (°C)	RH_{Max} (%)	RH_{min} (%)	$RH_{\bar{x}}$ (%)	T_{Max} (°C)	T_{min} (°C)	$T_{\bar{x}}$ (°C)	RH_{Max} (%)	RH_{min} (%)	$RH_{\bar{x}}$ (%)
Feb. 8	29.7	27.5	28.6	71	57	64.0	29.6	27.4	28.5	70	55	62.5
Feb. 10	32.7	26.4	29.6	67	55	61.0	32.7	26.4	29.6	69	52	60.5
Feb. 13	27.2	24.1	25.7	70	49	59.5	27.0	24.0	25.5	73	49	61.0
Feb. 15	32.6	27.7	30.2	70	55	62.5	32.7	27.7	30.2	72	54	63.0
Feb.17	29.2	24.9	27.1	73	56	64.5	29.1	25.0	27.1	72	58	65.0
Feb. 20	26.5	21.8	24.2	74	57	65.5	26.3	21.8	24.1	71	56	63.5
Feb. 22	28.6	25.6	27.1	73	54	63.5	28.6	25.5	27.1	76	52	64.0
Feb. 24	30.5	26.7	28.6	65	47	56.0	30.5	26.7	28.6	64	49	56.5
Feb. 27	29.2	23.9	26.6	73	61	67.0	29.2	24.0	26.6	71	60	65.5
Feb. 29	27.5	24.9	26.2	75	61	68.0	27.6	25.1	26.4	77	64	70.5
Mar. 2	29.9	27.5	28.7	59	49	54.0	30.0	27.6	28.8	61	50	55.5

References

Aguiar-Menezes, E.L., Menezes, E.B., Loiácono, M.S., 2003. First record of *Coptera haywardi* Loiácono (Hymenoptera: Diapriidae) as a parasitoid of fruit-infesting Tephritidae (Diptera) in Brazil. Neotropical Entomol. 32, 355–358.

Aluja, M., 1999. Fruit fly (Diptera: Tephritidae) research in Latin America: myths, realities and dreams. Anais da Sociedade Entomologica do Brasil 28, 565–594.

Aluja, M., Rull, J., 2009. Managing pestiferous fruit flies (Diptera: Tephritidae) through environmental manipulation. In: Aluja, M., Leskey, T.C., Vincent, C. (Eds.), Biorational Tree-Fruit Pest Management. CAB International, Wallingford, UK, pp. 171–213.

Aluja, M., Sivinski, J., Ovruski, S., Guillén, L., López, M., Cancino, J., Torres-Anaya, A., Gallegos-Chan, G., Ruíz, L., 2009. Colonization and domestication of seven species of native New World hymenopterous larval-prepupal and pupal fruit fly (Diptera: Tephritidae) parasitoids. Biocontrol Sci. Tech. 19, 49–79.

Aluja, M., Ovruski, S.M., Sivinski, J., Córdova-García, G., Schliserman, P., Nuñez-Campero, S.R., Ordano, M., 2013. Inter-specific competition and competition-free space in the Tephritid parasitoids *Utetes anastrephae* and *Doryctobracon areolatus* (Hymenoptera: Braconidae: Opiinae). Ecol. Entomol. 38, 485–496.

Baeza-Larios, G., Sivinski, J., Holler, T., Aluja, M., 2002. The ability of *Coptera haywardi* (Ogloblin) (Hymenoptera: Diapriidae) to locate and attack the pupae of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), under seminatural conditions. Biol. Control 23, 213–218.

Bautista, R.C., Harris, E.J., 1997. Effects of multiparasitism on the parasitization behavior and progeny development of oriental fruit fly parasitoids (Hymenoptera: Braconidae). J. Econ. Entomol. 90, 757–764.

Borer, E.T., Murdoch, W.W., Swarbrick, S.L., 2004. Parasitoid coexistence: linking spatial field patterns with mechanism. Ecology 85, 667–678.

Braga-Sobrinho, R., Caceres, C., Islam, A., Wornoayporn, V., Enkerlin, W., 2006. Improving mass rearing technology for South American fruit fly (Diptera: Tephritidae). Caatinga 19, 310–316.

Braga-Sobrinho, R., Guimaraes, J.A., Araujo, E.L., Moreira, M.A.B., 2010. Suitable larval diets for *Anastrepha fraterculus* (Diptera: Tephritidae). In: Sabater Muñoz, B., Navarro Llopis, V., Urbaneja, A. (Eds.), Book of Abstracts of the 8th International Symposium on Fruit Flies of Economic Importance. Universitat Politécnica de Valencia, Valencia, España, pp. 222.

Burns, E.D., Diaz, J., Holler, T.C., 1996. Inundative release of the parasitoid *Diachasmimorpha longicaudata* for the control of the Caribbean fruit fly *Anastrepha suspensa* (Loew). In: McPheron, B.A., Steck, G.F. (Eds.), Fruit Fly Pests: A World Assessment of Their Biology and Management. Saint Lucie Press, Delray Beach, Florida, USA, pp. 377–381.

Cancino, J., Ruiz, L., López, P., Sivinski, J., 2009. The suitability of *Anastrepha* spp. and *Ceratitis capitata* larvae as hosts of *Diachasmimorpha longicaudata* and *Diachasmimorpha tryoni*: effects of host age and radiation dose and implications for quality control in mass rearing. Biocontrol Sci. Tech. 19, 81–94.

Cancino, J., Liedo, P., Ruiz, L., López, G., Montoya, P., Barrera, J., Sivinski, J., Aluja, M., 2012. Discrimination by *Coptera haywardi* (Hymenoptera: Diapriidae) of hosts previously attacked by conspecifics or by the larval parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). Biocontrol Sci. Tech. 22, 899–914. http://dx.doi.org/10.1080/09583157.2012.696088.

Cancino, J., Montoya, P., Barrera, J.F., Aluja, M., Liedo, P., 2014. Parasitism by *Coptera haywardi* and *Diachasmimorpha longicaudata* on *Anastrepha* flies with different fruits under laboratory and field cage conditions. Biocontrol 59, 287–295.

Carvalho, R.D.S., 2005. Avaliaçaõ das liberações inoculativas do parasitóide exótico *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) em pomar diversificado em Conceição do Almeida, BA. Neotropical Entomol. 34, 799–805.

Córdova, G., 2008. Mecanismos de defensa de las moscas de la fruta Anastrepha oblicua y Ceratitis capitata (Díptera: Tephritidae) ante el ataque de parasitoides nativos (Magister Tesis). Universidad Veracruzana, Xalapa, Veracruz, Mexico, pp. 44–50.

Cusson, M., Laforge, M., Regniere, J., Beliveau, C., Trudel, D., Thireau, J., Ballemare, G., Keirstead, N., Stolz, D., 2002. Multiparasitism of *Chorisoneura fumiferana* by the Ichneumonid *Tranosema rostrale* and the Tachinid *Aclia inlerrupta*: Occurrence in the field and outcome of competition under laboratory conditions. Entomol. Exp. Appl. 102, 125–133.

Cusumano, A., Peri, E., Vinson, S.B., Colazza, S., 2012. Interspecific extrinsic and intrinsic competitive interactions in egg parasitoids. Biocontrol 57, 719–734.

De Moraes, C., Cortesero, A.M., Stapel, J.O., Lewis, W.J., 1999. Intrinsic and extrinsic competitive interactions between two larval parasitoids of *Helitohis virescens*. Ecol. Entomol. 24, 402–410.

Ferguson, K.I., Stiling, P., 1996. Non-additive effects of multiple natural enemies on aphid populations. Oecologia 108, 375–379.

García, J., Montilla, R., 2001. *Coptera haywardi* Loiácono (Hymenoptera: Diapriidae) parasitoides de pupas de Anastrepha spp. (Díptera: Tephritidae) en Venezuela. Entomotropica 16, 191–195.

Garcia-Medel, D., Sivinski, J., Diaz-Fleischer, E., Ramirez-Romero, R., Aluja, M., 2007. Foraging behavior by six fruit fly parasitoids (Hymenoptera: Braconidae) released as single-or multiple-species cohorts in field cages: Influence of fruit location and host density. Biol. Control 43, 12–22.

Godfray, H.J.C., 1994. Parasitoids: Behavioural and Evolutionary Ecology. Princeton University Press, Princeton, New Jersey.

Gonzalez, P.I., Montoya, P., Perez-Lachaud, G., Cancino, J., Liedo, P., 2007. Superparasitism in mass reared *Diachasmimorpha longicaudata* (Asmead) (Hymenoptera: Braconidae), a parasitoid of fruit flies (Diptera: Tephritidae). Biol. Control 40, 320–326.

Guillén, D., Sánchez, R., 2007. Expansion of the national fruit fly control programme in Argentina. In: Vreysen, M.J.B., Robinson, A.S., Hendrichs, J. (Eds.), Area-Wide Control of Insect Pests: From Research to Field Implementation. Springer, Dordrecht, The Netherlands, pp. 653–660.

Guillén, L., Aluja, M., Equihua, M., Sivinski, J., 2002. Performance of the two fruit fly (Diptera: Tephritidae) pupal parasitoids (*Coptera haywardi* [Hymenoptera: Diapriidae] and *Pachycrepoideus vindemiae* [Hymenoptera: Pteromalidae]) under different environmental soil conditions. Biol. Control 23, 219–227.

Harvey, J.A., Poelman, E.H., Tanaka, T., 2013. Intrinsic inter- and intraspecific competition in parasitoid wasps. Annu. Rev. Entomol. 58, 333–351.

Hodgson, P.J., Sivinski, J., Quintero, G., Aluja, M., 1998. Depth of pupation and survival of fruit fly (*Anastrepha* spp: Tephritidae) pupae in a range of agricultural habitats. Environ. Entomol. 27, 1310–1314.

Ibrahim, A.G., Palacio, L.P., Rohani, I., 1994. Biology of *Diachasmimorpha longicaudata*, a parasitoid of carambola fruit fly (Diptera: Tephritidae). J. Trop. Agric. Sci. 12, 139–143.

Javad Ardeh, M., de Jong, P.W., van Lenteren, J.C., 2005. Intra-and Interspecific host discrimination in arrhenotokous and thelyotokous *Eretmocerus* spp. Biol. Control 33, 74–80.

Jervis, M.A., Kidd, N.A.C., 1986. Host-feeding strategies in hymenopteran parasitoids. Biol. Rev. 61, 395–434.

Knipling, E.F., 1992. Principles of Insect Parasitism Analysed from New Perspectives. USDA-ARS, Agriculture Handbook N, 693.

Lawrence, P.O., 1988. Intraspecific competition among first instars of the parasitic wasp *Biosteres longicaudatus*. Oecologia 74, 607–611.

Leyva, J.L., Browning, H.W., Gilstrap, F.E., 1991. Effect of host fruit species, size, and color on parasitization of *Anastrepha ludens* (Diptera: Tephritidae) by *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). Environ. Entomol. 20, 1470–1474.

Loiacono, M.S., 1981. Notas sobre Diapriinae neotropicales (Hymenoptera, Diapriidae). Revista de la Sociedad Entomológica de Argentina 40, 237–241.

López, M., Aluja, M., Sivinski, J., 1999. Hymenopterous larval–pupal and pupal parasitoids of *Anastrepha* flies (Diptera: Tephritidae) in Mexico. Biol. Control 15, 119–129.

Menezes, E., Sivinski, J., Holler, T., Aluja, M., Jeronimo, F., Ramirez, E., 1998. Development of *Coptera haywardi* (Hymenotera: Diapriidae) in irradiated and unirradiated pupae of caribbean fruit fly and the Mediterranean fruit fly (Diptera: Tephritidae). Florida Entomologist 81, 567–570.

Miranda, M., Sivinski, J., Rull, J., Cicero, L., Aluja, M., 2015. Niche breadth and interspecific competition between *Doryctobracon crawfordi* and *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae), native and introduced parasitoids of Anastrepha spp. fruit flies (Diptera: Tephritidae). Biol. Control 82, 86–95.

Montoya, P., Liedo, P., Benrey, B., Barrera, J.E., Cancino, J., Sivinski, J., 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., Barrera, J.F., Sivinski, J., Aluja, M., 2000b. Functional response and superparasitism by *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae), a parasitoid of fruit flies (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 93, 47–54.

Montoya, P., Cancino, J., Zenil, M., Santiago, G., Gutierrez, J.M., 2007. The augmentative biological control component in the Mexican national campaign against *Anastrepha* spp. fruit flies. In: Vreysen, M.J.B., Robinson, A.S., Hendrichs, J. (Eds.), Area-Wide Control of Insects Pests: From Research to Field Implementation. The Netherlands, Springer, Dordrecht, pp. 661–670.

Murdoch, W.W., Briggs, C.J., 1996. Theory for biological control: recent developments. Ecology 77, 344–366.

Núñez-Campero, S.R., Ovruski, S., Aluja, M., 2012. Survival analysis and demographic parameters of the pupal parasitoid *Coptera haywardi* (Hymenoptera: Diapriidae), reared on *Anastrepha fraterculus* (Diptera: Tephritidae). Biol. Control 61, 40–46.

Oroño, L., Ovruski, S., 2007. Presence of *Diachasmimorpha longicaudata* (hymenoptera: braconidae) in a guild of parasitoids attacking *Anastrepha fraterculus* (Diptera: Tephritidae) in northwestern Argentina. Florida Entomologist 90, 40–41.

Ovruski, S.M., Schliserman, P., 2012. Biological control of Tephritid fruit flies in Argentina: historical review, current status, and future trends for developing a parasitoid mass-release program. Insects 3, 870–888.

Ovruski, S.M., Aluja, M., Sivinski, J., Wharton, R., 2000. Hymenopteran parasitoids on fruit-infesting Tephritidae (Diptera) in Latin America and the southern United States: diversity, distribution, taxonomic status and their use in fruit fly biological control. Integr. Pest Manage. Rev. 5, 81–107.

Ovruski, S.M., Colin, C., Soria, A., Oroño, L.E., Schliserman, P., 2003. Introducción y producción en laboratorio de *Diachasmimorpha tryoni* y *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) para el control biológico de *Ceratitis capitata* (Diptera: Tephritidae) en la Argentina. Revista de la Sociedad Entomológica de Argentina 62, 49–59.

Ovruski, S.M., Bezdjian, L.P., Van Nieuwenhove, G.A., Albornoz-Medina, P., Schliserman, P., 2011. Host Preference by *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) reared on larvae of *Anastrepha fraterculus* and *Ceratitis capitata* (Diptera: Tephritidae). Florida Entomologist 94, 195–200.

Ovruski, S.M., Van Nieuwenhove, G., Bezdjian, L., Albornoz-Medina, P., Schliserman, P., 2012. Evaluation of *Diachasmimorpha longicaudata* (Hymenoptera:

Braconidae) as a mortality factor of *Ceratitis capitata* (Diptera: Tephritidae) infesting Citrus species under laboratory and field-cage conditions. Biocontrol Sci. Tech. 22, 187–202.
Pedersen, B.S., Mills, N.J., 2004. Single vs. multiple introduction in biological control: the roles of parasitoid efficiency, antagonism and niche overlap. J. Appl. Ecol. 41, 973–984.
Purcell, M.F., 1998. Contribution of biological control to integrated pest management of Tephritid fruit flies in the tropics and subtropics. Integr. Pest Manage. Rev. 3, 63–83.
Quicke, D.L.J., 2015. Biology, Systematics, Evolution and Ecology of Braconid and Ichneumonid Parasitoid Wasps. Wiley Blackwell, Chichester, UK, pp. 688.
Rendon, P., Sivinski, J., Holler, T., Bloem, K., López, M., Matinez, A., Aluja, M., 2006. The effects of sterile males and two braconid parasitoids, *Fopius arisanus* (Sonan) and *Diachasmimorpha kraussii* (Fullaway) (Hymenoptera), on caged populations of Mediterranean fruit flies, *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) at various sites in Guatemala. Biol. Control 36, 224–231.
Rosenheim, J.A., Hoy, M.A., 1989. Confidence intervals for the Abbott's formula correction of bioassay data for control response. J. Econ. Entomol. 82, 331–335.
Schliserman, P., Ovruski, S.M., Coll, O.R., 2003. The establishment of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) in Misiones, Northeastern Argentina. Florida Entomologist 86, 491–492.
Sivinski, J., 1996. The past and potential of biological control of fruit flies. In: McPheron, B.A., Steck, G.J. (Eds.), Fruit Fly Pests: A World Assessment of Their Biology and Management. Saint Lucie Press, Delray Beach, Florida, USA, pp. 369–375.
Sivinski, J., Calkins, C.O., Baranowski, R., Harris, D., Brambila, J., Diaz, J., Burns, R.E., Holler, T., Dodson, G., 1996. Suppression of a Caribbean fruit fly (*Anastrepha suspensa*) (Loew) (Diptera: Tephritidae) population through augmentative releases of the parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Biol. Control 6, 177–185.
Sivinski, J., Vulinec, K., Menezes, E., Aluja, M., 1998. The bionomics of *Coptera haywardi* (Oglobin) (Hymenoptera: Diapridae) and the other pupal parasitoids of Tephritid fruit flies (Diptera). Biol. Control 11, 193–202.
StatSoft, Inc. 2011. STATISTICA (data analysis software system), version 10.0. www.statsoft.com.
Suarez, L., Van Nieuwenhove, G., Murua, F., Bezdjian, L.P., Schliserman, P., Lara, N., Escobar, J., Ovruski, S., 2012. Offspring production in response to host exposure times in *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae), reared on the genetic sexing strain Vienna 8 of *Ceratitis capitata* (Diptera: Tephritidae). Florida Entomologist 95, 991–999.
Suarez, L., Murua, F., Lara, N., Escobar, J., Taret, G., Rubio, J., Van Nieuwenhove, G., Bezdjian, L.P., Schliserman, P., Ovruski, S.M., 2014. Biological control of *Ceratitis capitata* (Diptera: Tephritidae) in Argentina: releases of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) in fruit-producing semi-arid areas of San Juan. Nat. Sci. 06, 664–675.
van Lenteren, J.C., Bale, J., Bigler, F., Hokkanen, H.M.T., Loomans, A.J.M., 2006. Assessing risks of releasing exotic biological control agents of arthropod pests. Annu. Rev. Entomol. 51, 609–634.
Van Nieuwenhove, G.A., Ovruski, S.M., 2011. Influence of *Anastrepha fraterculus* (Diptera: Tephritidae) larval instars on the production of *Diachasmimorpha longicaudata* (Hymneoptera: Braconidae) progeny and their sex ratio. Florida Entomologist 94, 863–868.
Van Nieuwenhove, G.A., Bezdjian, L.P., Ovruski, S.M., 2012. 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. Florida Entomologist 95, 99–104.
Vargas, R.I., Leblanc, L., Harris, E.J., Manoukis, N.C., 2012. Regional suppression of *Bactrocera* fruit flies (Diptera: Tephritidae) in the Pacific through biological control and prospects for future Introductions into other areas of the world. Insects 3, 727–742.
Vera, M.T., Abraham, S., Oviedo, A., Willink, E., 2007. Demographic and quality control parameters of *Anastrepha fraterculus* (Diptera: Tephritidae) maintained under artificial rearing. Florida Entomologist 90, 53–57.
Wang, X.G., Messing, R.H., 2002. Newly imported larval parasitoid pose minor competitive risk to the extant egg-larval parasitoid of fruit flies in Hawaii. Bull. Entomol. Res. 92, 423–429.
Wang, X.G., Messing, R.H., 2003. Intra- and interspecific competition by *Fopius arisanus* and *Diachasmimorpha tryoni* (Hymenoptera: Braconidae), parasitoids of Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae). Biol. Control 27, 251–259.
Wang, X.G., Messing, R.H., 2004. Potential interactions between pupal and egg- or larval-pupal parasitoids of tephritid fruit flies. Environ. Entomol. 33, 1313–1320.
Wang, X.G., Bokonon-Ganta, A.H., Messing, R.H., 2008. Intrinsic inter-specific competition in a guild of tephritid fruit fly parasitoids: effect of co-evolutionary history on competitive superiority. Biol. Control 44, 312–320.
Wang, X.Y., Jennings, D.E., Jian, J., Duan, J.J., 2015. Trade-offs in parasitism efficiency and brood size mediate parasitoid coexistence, with implications for biological control of the invasive emerald ash borer. J. Appl. Ecol. 52, 1255–1263.
Wong, T.T.Y., Ramadan, M.M., McInnis, D.O., Mochizuki, N., Nishimoto, J.J., Herr, J.C., 1991. Augmentative releases of *Diachasmimorpha tryoni* (Hymenoptera: Braconidae) to suppress a Mediterranean fruit fly (Diptera: Tephritidae) population in Kula, Maui, Hawaii. Biol. Control 1, 2–7.
Wong, T.T.Y., Ramadan, M.M., Herr, J.C., McInnis, D.O., 1992. Suppression of a Mediterranean fruit fly (Diptera: Tephritidae) population with concurrent parasitoid and sterile fly releases in Kula, Maui, Hawaii. J. Econ. Entomol. 85, 1671–1681.
Zar, J.H., 1999. Biostatistical Analysis, Fifth ed. Prentice Hall, New Jersey.