

Host availability affects the interaction between pupal parasitoid *Coptera haywardi* (Hymenoptera: Diiapridae) and larval–pupal parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae)

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Abstract

The use of multiple species in biological control programmes is controversial when interactions among them are not fully understood. We determined the response of the pupal parasitoid *Coptera haywardi* (Oglobin) to different availability of *Anastrepha ludens* (Loew) pupae previously parasitized or not by larval–pupal *Diachasmimorpha longicaudata* (Ashmead). The two types of pupae were exposed at different ages and proportions to different numbers of *C. haywardi* females for 48 h. The performance of *C. haywardi* adults emerging from parasitized and unparasitized pupae was measured. *Coptera haywardi* prefers to attack unparasitized *A. ludens* pupae rather than pupae parasitized by *D. longicaudata*. However, when the availability of unparasitized pupae was low or the number of foraging females was high, *C. haywardi* competed against early immature stages of the *D. longicaudata*, or hyperparasitized, feeding directly on the advanced-immature developmental stages of the early acting species. Adults of *C. haywardi* emerging as hyperparasitoids were no different in size, fecundity and longevity from those emerging as primary parasitoids. Our data suggest that simultaneous use of these species in augmentative biological control projects may be feasible but should be carefully planned in order to avoid any detrimental effect of its interaction.

Keywords: competition, hyperparasitism, host discrimination, multiple agents, augmentative biological control

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Introduction

In biological control programmes, there is a hypothesis that releasing multiple parasitoid species that attack different host

stages would improve pest control because a later acting species would attack the fraction of host population that escaped from early acting species (Knipling, 1977; Sivinski, 1996). However, both mathematical models and empirical evidence indicate that those parasitoid species that attack earlier stages of a common host exhibit competitive advantages over those parasitoids that parasitize later host stages, reducing the effectiveness of later acting species (Pedersen & Mills, 2004; Wang & Messing, 2004). Contrasting results indicate that a superior competitor can take advantage of parasitized host

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and feed on immatures of both the host and of the early acting parasitoid (Briggs, 1993). Furthermore, interaction between species could be altered by the coevolutionary history of species. Thus, when native and introduced species interact, there is no recognition between them, and defensive responses are not stimulated eliminating the advantage of an early acting species (Wang et al., 2008; Harvey et al., 2013).

A low host availability may transform a stable coexistence of two parasitoid species into a competence by increasing superparasitism (i.e., parasitism of a host by more than one parasitic individual of the same species) and hyperparasitism (i.e., the capability of a parasitoid to attack another parasitoid on or within the natural host), consequently interfering in the control of target pests (Godfray, 1994; Harvey et al., 2013; Xu et al., 2013). There are two types of hyperparasitoids: (a) obligatory hyperparasitoids, which can develop only on or within a primary parasitoid, and (b) facultative hyperparasitoids, which can develop on an herbivore or in a parasitoid (Sullivan, 1987; Sullivan & Völkl, 1999). The latter trait is considered detrimental in species used as natural enemies in biological pest-control programmes (e.g., Pérez-Lachaud et al., 2004; Wang & Messing, 2004).

Some evidence indicates that there is no cumulative insect pest control when multiple species of parasitoids are released, suggesting possible negative interactions between natural enemies (Briggs, 1993; Denoth et al., 2002). Conversely, other studies indicate that the addition of two or more biocontrol agents may substantially increase host mortality compared with single releases (May & Hassell, 1981; Stiling & Cornelissen, 2005; Bader et al., 2006). The controversy of using single or multiple species of parasitoids to control an insect pest goes further when using augmentative releases that favour the probability of interactions between the parasitoids. Augmentative releases of braconids attacking larvae have been successfully used to control pest tephritid flies (Wong et al., 1991; Sivinski et al., 1996; Montoya et al., 2000a). However, when larvae develop in large fruits that function as refuges, they are less likely to be parasitized by these guild of parasitoids (Sivinski et al., 1991, 1997; Hawkins, 1992; Montoya et al., 2007). Therefore, the control reached with larval parasitoids can be reinforced adding pupal parasitoids that attack pupal host that escaped from larval parasitism, since larvae pupate in the soil, they are no longer protected by the host fruit (Cancino et al., 2012). Thus, understanding the effect of competition and multiparasitism is relevant for augmentative release decisions to minimize undesirable interactions.

Diachasmimorpha longicaudata (Ashmead) (Hymenoptera: Braconidae), a solitary larval endoparasitoid is one of the natural enemies most frequently used to control fruit flies. This parasitoid originated in the Indo-Australian region and was introduced to Mexico during the 1950s (Jiménez y Jiménez, 1956). In Mexico, *D. longicaudata* has been reported to parasitize various species of the genus *Anastrepha* (Aluja et al., 1990). Because it can be easily reared in the laboratory (Wong & Ramadan, 1987; Cancino & Yoc, 1993), *D. longicaudata* is an ideal candidate for pest-control programmes using augmentative releases (Sivinski et al., 1996; Montoya et al., 2000a, 2007). *Coptera haywardi* (Ogloblin) (Hymenoptera: Diapriidae), a native pupal parasitoid, is a good candidate for use in biological pest control because it is the only parasitoid reported to attack *Anastrepha* spp. pupae from southern Mexico to northern Argentina (Sivinski et al., 1998; López et al., 1999; Ovruski et al., 2000). Additionally, this parasitoid exhibits

great discrimination ability against young (3–5-day-old) *Anastrepha ludens* (Loew) pupae previously parasitized by *D. longicaudata* (Cancino et al., 2012). However, some preliminary observations suggest that under specific conditions, *C. haywardi* can also attack pupae already parasitized by *D. longicaudata* using hyperparasitism as a strategy (Guzmán-Salinas & Montoya, 2008).

The objectives of the present study were to determine the level of hyperparasitism by *C. haywardi* on the primary parasitoid *D. longicaudata* through: (1) studying the patterns of oviposition and emergence of *C. haywardi* in *A. ludens* pupae of different age and previously parasitized by *D. longicaudata*, (2) examining the emergence of the two parasitoid species when variable proportions of parasitized and unparasitized pupae are exposed to different numbers of females of *C. haywardi*, and (3) to analyse some parameters of fitness of *C. haywardi* adults emerging as hyperparasitoids. The results can be used to infer the feasibility of using simultaneously both parasitoid species in augmentative biological pest-control projects.

Materials and methods

Biological material and workplace

The experiments were carried out in the Biological Control Laboratory within the Methods Development Unit of the Moscafrut Program (SAGARPA-IICA), located at Metapa de Dominguez, Chiapas, Mexico. The bioassays were conducted using 8-day-old *A. ludens* larvae and 5–7-day-old *D. longicaudata* females provided by Moscafrut Facility where these species are mass reared (Cancino et al., 2010; Domínguez et al., 2010). Seven-day-old *C. haywardi* females were obtained from the rearing colony of the Biological Control Laboratory. The bioassays were conducted at $22 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ relative humidity.

Exposure of pupae of different ages to C. haywardi under non-choice conditions

Anastrepha ludens unparasitized or parasitized pupae by *D. longicaudata* were obtained from mass rearing facilities. Parasitized pupae present scars left by the ovipositor of *D. longicaudata* (Montoya et al., 2000b). Pupae from 1–11-day old were separately exposed to *C. haywardi* females. Eight groups of 50 pupae of every age and condition studied (i.e., parasitized/unparasitized) were exposed during 48 h to the attack of four *C. haywardi* females of 7-day old and sexually mature. Twenty-four hours before the test, *C. haywardi* females received oviposition experience being exposed to 3-day-old *A. ludens* pupae. The experiment was conducted in plastic trays (26.5 × 16.5 × 7 cm) filled with moist vermiculite to simulate soil. The trays were fitted with lids with an 18 × 6.5 cm window covered with tulle mesh to prevent parasitoid escape while providing ventilation.

The following parameters were measured: (1) percentage of adult emergence of *D. longicaudata* and *C. haywardi*, (2) number of *C. haywardi* oviposition scars per pupa (determined on 10% of the attacked pupae) and (3) the number of immature *C. haywardi* parasitoids per pupa (recorded after dissecting the pupae). The experiments were repeated eight times. *Anastrepha ludens* and *D. longicaudata* pupae that were not exposed to *C. haywardi* were used as controls.

Exposure of parasitized and unparasitized *A. ludens* pupae to female *C. haywardi* under choice conditions

This experiment tested the effect of the simultaneous presence of pupae that had or had not been parasitized by *D. longicaudata* during their larval stage, on the performance of different numbers of *C. haywardi* females. Based on the results of the previous experiment on pupal age, where we observed when hyperparasitism of *C. haywardi* begins (see fig. 1), we used 7-day-old *A. ludens* pupae previously parasitized by *D. longicaudata*, and unparasitized 3-day-old *A. ludens* pupae were used as a control (Cancino *et al.*, 2012). The proportions of previously parasitized and unparasitized pupae were: (1) 100% unparasitized *A. ludens* pupae ($n = 50$) (control), (2) 80% unparasitized *A. ludens* pupae and 20% pupae previously parasitized by *D. longicaudata*, (3) 60% unparasitized *A. ludens* pupae and 40% parasitized pupae, (4) 40% unparasitized *A. ludens* pupae and 60% parasitized pupae, (5) 20% unparasitized *A. ludens* pupae and 80% parasitized pupae, and (6) 100% *A. ludens* pupae previously parasitized by *D. longicaudata*. Each group was exposed to two, four, six or eight 7-day-old *C. haywardi* females with oviposition experience.

Similar to the previous experiment, the pupae were subjected to attack by *C. haywardi* females in plastic trays (26.5 × 6.5 × 7 cm) filled with moist vermiculite to simulate soil. The following parameters were tested: (1) the number of *C. haywardi* oviposition scars on 10% of the pupae (five pupae in each replicate); (2) the number of immature *C. haywardi* per pupa (obtained after dissection); and (3) the percentage of adult emergence of *A. ludens*, *D. longicaudata* and *C. haywardi*. Eight replicates were used, including control groups of unparasitized and previously parasitized *A. ludens* pupa that were not exposed to *C. haywardi*.

Fitness parameters of *C. haywardi* adults emerging from previously parasitized pupae

The body size, average fertility and longevity were determined in 17 *C. haywardi* pairs (males and females) that emerged from pupae previously parasitized by *D. longicaudata*. Once they reached sexual maturity (7-day), 20 3-day-old *A. ludens* pupae were offered daily to each female until death (Cancino *et al.*, 2012). The following parameters were tested: (1) fertility, expressed as the number of male and female descendants per female per day, and (2) longevity, expressed as the number of days that the males and females survived with water and food.

The body size was determined measuring the length of antennae, wings, femur and the width of the abdomen. All measurements were taken using a stereomicroscope NIKON, SMZ745T with camera ProgRes[®] CT3 equipped with software ProgRes[®] CapturePro 2.9.0.1. Male and female *C. haywardi* adults that emerged from unparasitized *A. ludens* pupae by *D. longicaudata* were used as controls.

Statistical analysis

For binary data of parasitism, a generalized linear model (GLM) with binomial distribution and logit link function were used. Small integer counts recorded for two of the response variables (i.e., number of scars and number of immatures) were also analysed using GLM with Poisson errors, a log-link function and type III significance tests (Crawley, 1993; Agresti, 1996). Contrasts were used to test for differences

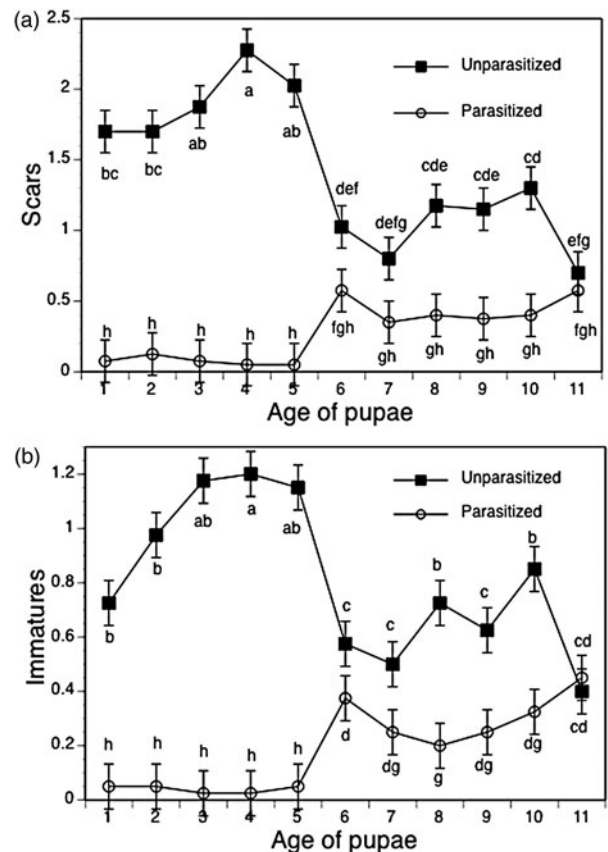


Fig. 1. (a) Number of scars (mean \pm SE) on pupae after being hyperparasitized by *Coptera haywardi*. (b) Number of immatures (mean \pm SE) on pupae after being hyperparasitized by *C. haywardi*.

in levels within a variable. For the continuous response variables, such as body size, *t* test was used. Longevity was analysed through the non-parametric *log-rank* test (Francis *et al.*, 1993). Analyses were performed using the software JMP v.7, SAS Institute, Cary, NC, USA and Statgraphics Centurion XV (Statgraphics, 2008).

Results

Pupal age

The analysis of the patterns of oviposition indicates that the number of scars vary depending on the condition of exposed pupae, parasitized/unparasitized, and according to the age of pupae unparasitized young pupae received higher numbers of ovipositions ($\chi^2 = 195.2$, $df = 10$, $P < 0.0001$; $\chi^2 = 31.5$, $df = 10$, $P = 0.0005$, respectively). The interaction between factors was significant indicating that females presented a different pattern of oviposition in parasitized and unparasitized pupae according to the pupae age ($\chi^2 = 81.1$, $df = 10$, $P < 0.0001$) (fig. 1a).

The number of immatures developed inside the pupae was also affected by the pupal condition, parasitized/unparasitized, exposed to *C. haywardi* and for the age of pupae. This result correlates with the number of scars and followed a similar pattern ($\chi^2 = 186.3$, $df = 1$, $P < 0.0001$; $\chi^2 = 51.7$, $df = 10$, $P < 0.0001$, respectively). The interaction between factors was also significant ($\chi^2 = 109.1$, $df = 10$, $P < 0.0001$) (fig. 1b).

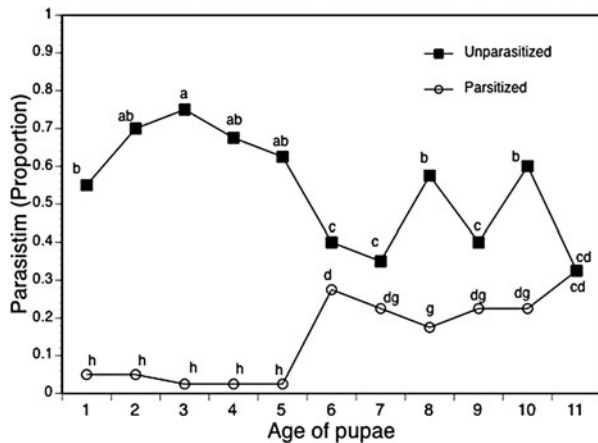


Fig. 2. Emergence (mean proportion) of *Coptera haywardi* from *Anastrepha ludens* pupae of different ages parasitized or not by *Diachasmimorpha longicaudata*.

The number of *C. haywardi* adults emerging from *A. ludens* pupae previously parasitized by *D. longicaudata* was significantly lower than the number of *C. haywardi* adults emerging from unparasitized *A. ludens* pupae ($\chi^2 = 1247.1$, $df = 1$, $P < 0.0001$). The effects of *C. haywardi* attack and of pupal age on the emergence of *D. longicaudata* were also significant ($\chi^2 = 73.9$, $df = 10$, $P < 0.0001$). Again, the interaction term was significant, indicating that pupal age also influenced the attack ($\chi^2 = 123.1$, $df = 10$, $P < 0.0001$) (fig. 2).

These data indicate that *C. haywardi* parasitized mostly young unparasitized hosts. In parasitized pupae by *D. longicaudata*, the interactions between the immature of the two species of parasitoids of the two species changed over time. In young pupae (1–4 days), young parasitoids of both species were observed feeding simultaneously within the puparium. Older *C. haywardi* larvae (6–11 days) fed on third-instar larvae, prepupae and pupae of *D. longicaudata* (table 1). Developmental stages of immature *D. longicaudata* parasitoids were identified following methods of Carabajal-Paladino et al. (2010).

Proportions of pupae and female density

Emergence of *C. haywardi* was affected by two factors: the proportion of parasitized/unparasitized pupae exposed and female density. Emergence was higher when the proportion of parasitized pupae was lower and when the density of females increased ($\chi^2 = 717.4$, $df = 5$, $P < 0.0001$; $\chi^2 = 25.9$, $df = 3$, $P < 0.0001$, respectively). The interaction between these factors was significant ($\chi^2 = 74.0$, $df = 15$, $P < 0.0001$). Additionally, emergence of *D. longicaudata* was influenced by the proportion of parasitized pupae and by the density of females ($\chi^2 = 642.0$, $df = 4$, $P < 0.0001$; $\chi^2 = 41.9$, $df = 3$, $P < 0.0001$, respectively). The interaction between these two factors was significant ($\chi^2 = 116.1$, $df = 12$, $P < 0.0001$) (fig. 3). Furthermore, the parasitism by *C. haywardi* reduces the emergence of *D. longicaudata* (Wilcoxon test of the emergence proportion of the exposed and unexposed pupa of *D. longicaudata*, $Z = -10.76$, $df = 156$, $P < 0.0001$) (mean \pm SE: 0.29 ± 0.0119 exposed; 0.36 ± 0.015 unexposed).

Fitness parameters

The survival of *C. haywardi* females emerging from unparasitized *A. ludens* pupae did not differ significantly from that

of females emerging from pupae previously parasitized by *D. longicaudata* (\log -rank $\chi^2 = 0.06$, $df = 1$, $P = 0.805$) (fig. 4a). However, the survival of hyperparasitic males emerging from pupae previously attacked by *D. longicaudata* (fig. 4b) was significantly higher than that of males emerging from unparasitized *A. ludens* pupae (\log -rank $\chi^2 = 14.81$, $df = 1$, $P < 0.0001$).

The fertility of the two groups of females did not differ significantly ($t = 0.732$, $df = 52$, $P = 0.467$). Females produced an average of 0.25–6.1 offspring per day of exposure, and their fertility was higher during the first days of exposure. There were non-significant differences on none body sizes measures between females (t test, $\alpha = 0.05$). In the case of males, wings and abdomen length values were higher in males emerging from hyperparasitic conditions (t test, $\alpha = 0.05$) (table 2).

Discussion

We found that *C. haywardi* is a primary parasitoid of *A. ludens* that compete with early *D. longicaudata* immatures in parasitized *A. ludens* pupae; however, it is also able to hyperparasitize on advanced-immature stages of *D. longicaudata* (table 1). This facultative hyperparasitism strategy was observed only under conditions of high host competition and under the presence of advanced immature stage of *D. longicaudata*. Furthermore, those *C. haywardi* adults that emerged from hyperparasitized hosts presented fitness parameters very similar to those emerged from unparasitized ones.

Our results indicate that when *C. haywardi* attacks young pupae previously parasitized by *D. longicaudata*, interspecific competition takes place, and under this condition, *D. longicaudata* has advantage over immatures of *C. haywardi*. Conversely, when *C. haywardi* attacked 6-day-old or older fly pupae previously parasitized by *D. longicaudata*, it is susceptible to be attacked by first- and second-instar larvae of *C. haywardi* that becomes then a hyperparasitoid. It is possible that advanced developmental stages (third-instar larvae, prepupae and pupae) of *D. longicaudata* had consumed most of the host, forcing *C. haywardi* larvae to feed on the immatures of *D. longicaudata*. Thus, *C. haywardi* can be considered an indirect facultative hyperparasitoid because it initially attacks a phytophagous host, but it is able to hyperparasitize depending on the age of *D. longicaudata* larvae (Sullivan & Völkl, 1999).

Facultative hyperparasitoids can occupy different trophic levels because they can develop as primary parasitoids or as parasitoids of other parasitoid species attacking the same host (Powell et al., 1996). Parasitoid strategies exhibit a continuum, where the two extremes are represented by obligate primary parasitism and obligate secondary parasitism (hyperparasitism) (Ehler, 1990). A facultative secondary parasite occupies an intermediate position, which is advantageous when unparasitized hosts are scarce. Some facultative parasitoids develop as koinobionts and can survive in two hosts that are not taxonomically related (Godfray, 1994). This strategy is possible when the different primary parasitoids feed on the same host. Therefore, the host and the primary parasitoid exhibit physiological similarities as shown in the present study. Pupal parasitoids may use facultative hyperparasitism because of interspecific competition with larval parasitoids (Grandgirard et al., 2002). It has been proposed that early attack of larval parasitoids represents a competitive advantage. However, reports of attacks by *Pachycrepoides dubius* (Ashmead) (Hymenoptera: Pteromalidae) on *Delila radicum* (L.) pupae previously parasitized by the larval parasitoid

Table 1. Interaction between immatures of *Coptera haywardi* and *Diachasmimorpha longicaudata* within *Anastrepha ludens* pupae parasitized as larvae by *D. longicaudata* and hyperparasitized by *C. haywardi* at different ages (dissected 72 h after hyperparasitized).

Age of <i>C. haywardi</i> pupae (days)	Developmental stage of immature <i>D. longicaudata</i> at the time of exposure to <i>C. haywardi</i> ¹	Activity of immature <i>C. haywardi</i> parasitoids (first and second instar) 72 h after exposure
1	Eggs	Competing with <i>D. longicaudata</i>
2	Eggs and first-instar larvae	Competing with <i>D. longicaudata</i>
3	First-instar larvae	Competing with <i>D. longicaudata</i>
4	Second-instar larvae	Competing with <i>D. longicaudata</i>
5	Second- or third-instar larvae	Feeding on <i>D. longicaudata</i>
6	Third-instar larvae	Feeding on <i>D. longicaudata</i>
7	Prepupae	Feeding on <i>D. longicaudata</i>
8	Prepupae	Feeding on <i>D. longicaudata</i>
9	Pupae (male or female)	Feeding on <i>D. longicaudata</i>
10	Pupae (male or female)	Feeding on <i>D. longicaudata</i>
11	Pupae (male or female)	Feeding on <i>D. longicaudata</i>

¹The developmental stages of immature *D. longicaudata* parasitoids were identified according to the descriptions of Carabajal-Paladino (2010).

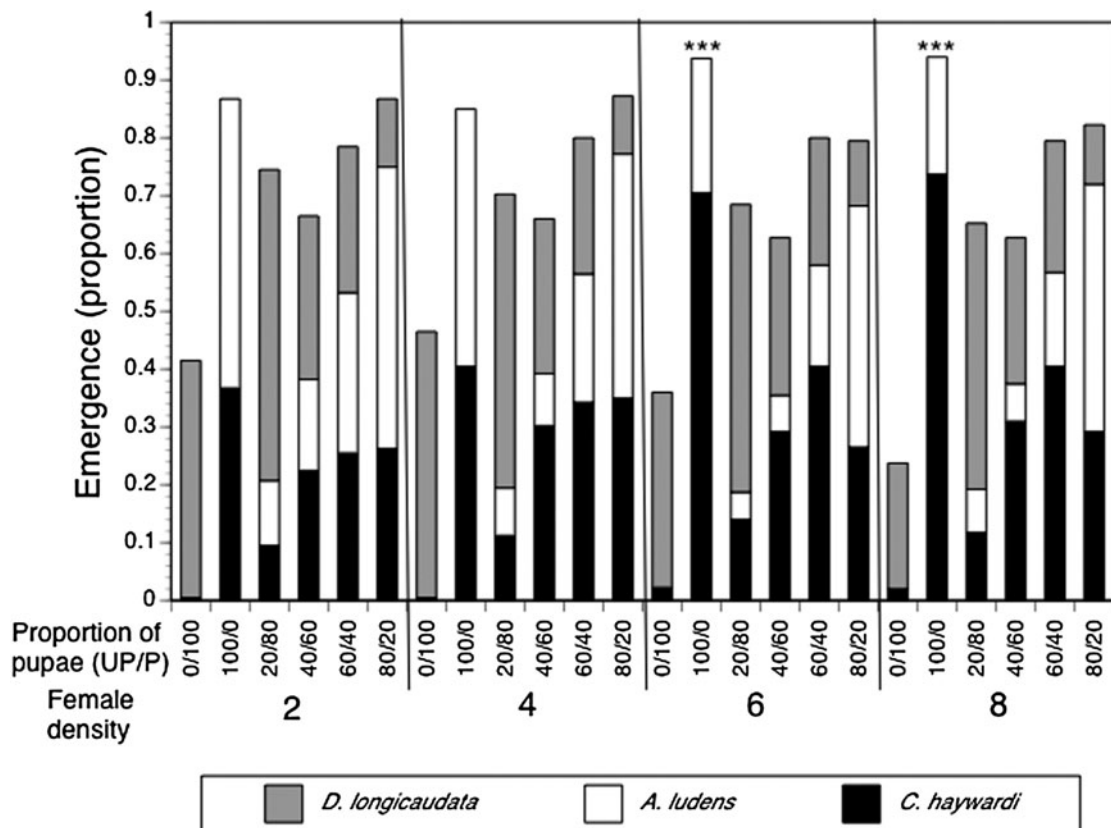


Fig. 3. Emergence (mean proportion) of the three species: *Anastrepha ludens*, *Coptera haywardi* and *Diachasmimorpha longicaudata* from *Anastrepha ludens* pupae (unparasitized and parasitized by *D. longicaudata* (UP/P) available at different proportions to different densities of females of *C. haywardi*).

Trybliographa rape (Westwood) (Hymenoptera: Figitidae) indicate that hyperparasitism can be an advantageous strategy for pupal parasitoids (Grandgirard *et al.*, 2002).

Adult *C. haywardi* emerging from hyperparasitized pupae were similar in those fitness parameters measured to those emerging from unparasitized *A. ludens* pupae, since body size, longevity and fertility of the two types of adults did not

differ significantly. Similar results were observed in pupal hyperparasitoid species, such as *Pachycrepoides vindemniae* (Rondani) (Hymenoptera: Pteromalidae) (Wang & Messing, 2004), which can successfully develop on four different braconid species: *D. longicaudata*, *Fopius arisanus* (Sonan), *Diachasmimorpha kaussii* (Viereck) and *Psytalia concolor* (Szépligeti). *Pachycrepoides vindemniae* has also been

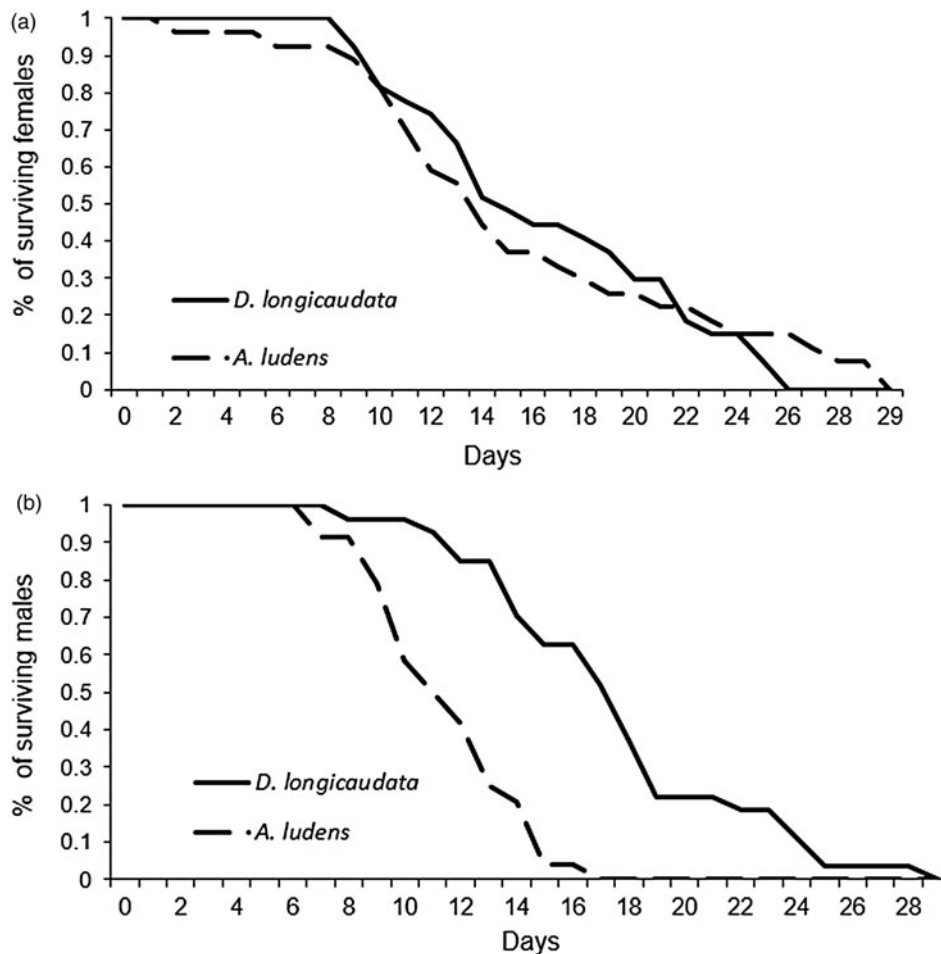


Fig. 4. Percentage of survival of females (a) and males (b) of *Coptera haywardi* emerging from *Anastrepha ludens* pupae previously parasitized or not by *Diachasmimorpha longicaudata*.

Table 2. Size attributes in mm (mean \pm SE) of *Coptera haywardi* males and females of emerging from hyperparasitized and non-hyperparasitized conditions.

	Males			Females		
	Hyperparasitized	Non-parasitized	<i>t</i>	Hyperparasitized	Non-parasitized	<i>t</i>
Antennae	3.12 \pm 0.027	3.15 \pm 0.025	NS	1.79 \pm 0.021	1.82 \pm 0.022	NS
Wings	3.02 \pm 0.019	2.97 \pm 0.022	*	2.85 \pm 0.01	2.8 \pm 0.014	NS
Femur	0.91 \pm 0.006	0.91 \pm 0.006	NS	0.87 \pm 0.008	0.87 \pm 0.008	NS
Abdomen length	1.79 \pm 0.018	1.66 \pm 0.023	*	1.79 \pm 0.023	1.81 \pm 0.03	NS

t = *t* test; * = significant differences between columns; NS = not significant, α = 0.05.

reported to attack pupae previously parasitized by other parasitoids of fruit flies, such as *Diachasmimorpha tyroni* (Cameron), *Psytalia humilis* (Silvestri) (Hymenoptera: Braconidae) and *Dirhinus giffardii* (Silvestri) (Hymenoptera: Chalcididae), but detailed information concerning these interactions lacks (Wang & Messing, 2004).

In the present study, the reduction in various parameters of *C. haywardi* analysed (i.e., the number of oviposition scars and the number of immature parasitoids and adult emergence) indicated that *A. ludens* pupae previously attacked by *D. longicaudata* was not a first option for oviposition. However,

when all of the available pupae had been previously parasitized by *D. longicaudata*, *C. haywardi* females preferentially attacked upon 6–7-day-old pupae. Adult emergence was higher in these hosts category than in the others (6 and 6.25%, respectively) (fig. 1a). A large decrease in the emergence of *D. longicaudata* after being attacked by *C. haywardi* in experiments using *Anastrepha suspensa* (Loew) as the primary host has been reported previously (Sivinski et al., 1998).

When two species of parasitoids use different stages of the same host, their coexistence is possible because their life histories are different. Commonly, the larval parasitoids would have

more hosts to oviposit and thus, their fecundity will be higher than in pupal parasitoids that would confront lower host availability (Price, 1972; Bonsall *et al.*, 2004). Furthermore, the generalist–specialist continuum strategies also play important role that may permit the coexistence of two species of parasitoids since generalists would possess more available hosts than the specialists (Price, 1972; Bonsall *et al.*, 2004). However, the coexistence could be broken when the availability of hosts fall and the probability of finding a host gets reduced. For example, switching from primary parasitism to a hyperparasitism in two coexisting species may be triggered by host availability and the dispersal abilities of females. For example, in two solitary secondary hyperparasitoids, *Lysibia nana* (Gravenhorst) and the wingless *Gelis agilis* (Fabricius), which concur in cocoons of a primary parasitoid, *Cotesia glomerata* L., *G. agilis* is the winner when competition for a host takes place (Harvey *et al.*, 2011). Thus, the wingless condition reduces the searching capacity and hence the availability of hosts for *G. agilis*. In our study however, both species are highly mobile; thus, the low hyperparasitism by *C. haywardi* females could be explained by their high host choosiness behaviour (Cancino *et al.*, 2012).

Our findings suggest that hyperparasitism of *C. haywardi* when used as biocontrol agent could be relevant if the proportion of parasitized pupa by the primary parasitoids is higher, which can be influenced by the density of the larval parasitoid, but also by the host fruit size that contained the larvae initially attacked by this species. Large fruit can protect the host larvae and facilitate their escape from their natural enemies, bring about large numbers of unparasitized pupae available for the pupal parasitoid (Sivinski *et al.*, 1998; Montoya *et al.*, 2007, 2016). The hyperparasitism by *C. haywardi* was neglected when the proportion of unparasitized hosts was high, confirming that this species possess a high discrimination ability (Cancino *et al.*, 2012).

The impact of facultative hyperparasitoids and their interactions on biological pest-control programmes are complex and poorly understood but are generally thought to be detrimental (Rosenheim, 1998; White *et al.*, 1998; Brodeur, 2000). Some studies have shown that facultative hyperparasitoids can prevent primary parasitoids from reaching their full potential as natural enemies, thus interfering with the success of biological pest-control programmes (Mills & Gutierrez, 1999). Facultative hyperparasitoids can play an important role in biological pest control; however, their exact impacts are uncertain. When a female discovers a host that has been previously parasitized by a primary parasitoid, it may oviposit on or within the host; consequently, the offspring will develop as secondary parasitoids of the primary parasitoid (Ehler, 1979; Rosenheim *et al.*, 1995; Heinz & Nelson, 1996). This phenomenon is classified as intra-guild predation rather than competition. Some authors consider this phenomenon to be common in parasitoid guilds (Hawkins, 1992). However, the prevalence of this type of predation among parasitoids is low.

This study adds evidences of the complexity of interspecific interactions of parasitoids. There are intrinsic factors such as the development stage of early acting species that clearly reduce the window of susceptibility for being hyperparasitized. Extrinsic factors such as host availability may trigger hyperparasitism in the later acting species; hence, the probability of negative interactions between these two species is scarce. Thus, *C. haywardi* can be considered as a low-risk facultative hyperparasitoid of *D. longicaudata* that could be used as a

complementary biocontrol agent in augmentative biological-control programmes against *Anastrepha* fruit flies.

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References

- Agresti, A. (1996) *An Introduction to Categorical Data Analysis*. New York, John Wiley.
- Aluja, M., Guillen, J., Liedo, P., Cabrera, M., Ríos, E., de la Rosa, G., Celedonio, H. & Mota, D. (1990) Fruit infesting tephritids (Diptera: Tephritidae) and associated parasitoids in Chiapas, Mexico. *Entomophaga* **35**, 39–48.
- Bader, A.E., Heinz, K.M., Wharton, R.A. & Bográn, C.E. (2006) Assessment of interspecific interactions among parasitoids on the outcome of inoculative biological control of leafminers attacking chrysanthemum. *Biological Control* **39**, 441–452.
- Bonsall, M.B., Jansen, V.A.A. & Hassell, M.P. (2004) Life history trade-offs assemble ecological guilds. *Science* **306**, 111–113.
- Briggs, C.J. (1993) Competition among parasitoid species on a stage-structured host and its effect on host suppression. *The American Naturalist* **141**, 372–397.
- Brodeur, J. (2000) Host specificity and trophic relationships of hyperparasitoids. pp. 163–183 in Hochberg, M.E. & Ives, R.A. (Eds) *Parasitoids Population Biology*. Princeton, New Jersey, Princeton University Press.
- Cancino, J. & Yoc, M. (1993) Methods proposed to apply quality control in the mass rearing of *Diachasmimorpha longicaudata*. pp. 37–47 in Nicoli, G., Benuzzi, M. & Leppla, N. (Eds) *Quality Control of Mass Reared Arthropods*, Rimini, Italy, IOBC.
- Cancino, J., Ruiz, L., López, P. & Moreno, F.M. (2010) Cría masiva de parasitoids. pp. 291–306 in Montoya, P., Toledo, J., Hernández, E. (Eds) *Moscas de la Fruta: Fundamentos y Procedimientos para su Manejo*. Mexico, D.F., S y G Editores.
- Cancino, J., Liedo, P., Ruiz, L., López, G., Montoya, P., Barrera, J.F., 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 Science and Technology* **22**, 899–914.
- Carabajal-Paladino, L.Z., Papeschi, A.G. & Cladera, J.L. (2010) Immature stages of development in the parasitoid wasp, *Diachasmimorpha longicaudata*. *Journal of Insect Science* **10**, 56. Available online: insectscience.org/10.56.
- Crawley, M.J. (1993) *GLIM for Ecologists*. Oxford, Blackwell.
- Denoth, M., Frid, L. & Myers, J.H. (2002) Multiple agents in biological control: improving the odds?. *Biological Control* **24**, 20–30.
- Domínguez, J., Artiaga-López, T., Solís, E. & Hernández, E. (2010) Métodos de colonización y cría masiva. pp. 259–276 in Montoya, P., Toledo, J. & Hernández, E. (Eds) *Moscas La Fruta: Fundamentos y Procedimientos para su Manejo*. Mexico, D.F., S y G Editores.
- Ehler, L.E. (1979) Utility of facultative secondary parasitoids in biological control. *Environmental Entomology* **4**, 353–354.

- Ehler, L.E. (1990) Introduction strategies in biological control of insects. pp. 111–134 in Mackauer, M., Ehler, L. & Roland, J. (Eds) *Critical Issues in Biological Control*. Andover, UK, Intercept.
- Francis, B., Green, M. & Payne, C. (1993) *Statistical System for Generalized Linear Interactive Modelling*. Oxford, UK, Clarendon Press.
- Godfray, H.C.J. (1994) *Parasitoids. Behavioral and Evolutionary Ecology*. pp. 9–11. Princeton, Princeton University Press.
- Grandgirard, J., Poinso, D., Krespi, L., Nénon, L. & Cortesero, A. (2002) Costs of secondary parasitism in the facultative hyperparasitoid *Pachycrepoideus dubius*: does host size matter? *Entomologia Experimentalis et Applicata* **103**, 239–248.
- Guzmán-Salinas, J. & Montoya, P. (2008) Efecto del tamaño del fruto hospedero en el parasitismo aditivo de tres especies de parasitoides atacando *Anastrepha ludens* (Diptera: Tephritidae). pp. 128–132 in *Memorias del XXXI Congreso Nacional de Control Biológico* Zacatecas, Mexico.
- Harvey, J.A., Pashalidou, F., Soler, R. & Bezemer, T.M. (2011) Intrinsic competition between two secondary hyperparasitoids results in temporal trophic switch. *Oikos* **120**, 226–233.
- Harvey, J.A., Poelman, E.H. & Tanaka, T. (2013) Intrinsic inter- and intraspecific competition in parasitoid wasps. *Annual Review of Entomology* **58**, 333–351.
- Hawkins, B.A. (1992) Parasitoid-host food webs and donor control. *Oikos* **65**, 159–162.
- Heinz, K.M. & Nelson, J.M. (1996) Interspecific interactions among natural enemy of *Bemisia* in an inundative biological control program. *Biological Control* **6**, 384–393.
- Jiménez y Jiménez, E. (1956) Las moscas de la fruta y sus enemigos naturales. *Fitofilo* **16**, 4–11.
- Knipling, E.F. (1977) The theoretical basis for augmentation of natural enemies. pp. 79–123 in Ridgway, R.L. & Vinson, S.B. (Eds) *Biological Control by Augmentation of Natural Enemies*. Volume 11 of the series Environmental Science Research. NY, Plenum Press.
- López, M., Aluja, M. & Sivinski, J. (1999) Hymenopterous larval-pupal and pupal parasitoids of *Anastrepha* flies (Diptera: Tephritidae) in Mexico. *Biological Control* **15**, 119–120.
- May, R.M. & Hassell, M.P. (1981) The dynamics of multiparasitoid–host interactions. *American Naturalist* **117**, 234–261.
- Mills, N.J. & Gutierrez, A. (1999) Biological control of insect pests. pp. 89–102 in Hawkins, B. & Cornell, H.V. (Eds) *Theoretical Approaches to Biological Control*. Cambridge, UK, Cambridge University Press.
- Montoya, P., Liedo, P., Benrey, B., Cancino, J., Barrera, J.F., Sivinski, J. & Aluja, M. (2000a) Biological control of *Anastrepha* spp. (Diptera: Tephritidae) in mango orchards through augmentative releases of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). *Biological Control* **18**, 216–224.
- Montoya, P., Liedo, P., Benrey, B., Barrera, J.F., Cancino, J. & Aluja, M. (2000b) Functional response and superparasitism by *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae), a parasitoid of fruit flies (Diptera: Tephritidae). *Annals of Entomological Society of America* **93**, 47–54.
- Montoya, P., Cancino, J., Zenil, M., Santiago, G. & Gutierrez, J. M. (2007) The augmentative biological control components in the Mexican National Campaign against *Anastrepha* spp. Fruit flies. pp. 661–670 in Vreysen, M., Robinson, A. & Hendrichs, J. (Eds) *Area-wide Control of Insect Pests*. The Netherlands, Springer.
- Montoya, P., Ayala, A., López, P., Cancino, J., Cabrera, H., Cruz, J., Martínez, M.A., Figueroa, I. & Liedo, P. (2016) Natural parasitism in fruit fly populations in disturbed areas adjacent to commercial mango orchards in Chiapas and Veracruz, Mexico. *Environmental Entomology* **42**, 328–337.
- Ovruksi, S., Aluja, M., Sivinski, J. & Wharton, R.A. (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. *Integrated Pest Management Reviews* **5**, 81–107.
- Pedersen, B.S. & Mills, N.J. (2004) Single vs. multiple introduction in biological control: the roles of parasitoid efficiency, antagonism and niche overlap. *Journal of Applied Ecology* **41**, 973–984.
- Pérez-Lachaud, G., Batchelor, T.M. & Hardy, I.C.W. (2004) Wasp eat wasp: facultative hyperparasitism and intra-guild predation by bethylid wasps. *Biological Control* **30**, 149–155.
- Powell, W., Walton, M. & Jervis, M.A. (1996) Populations and communities. pp. 223–292 in Jervis, M.A. & Kidd, N.A.C. (Eds) *Insect Natural Enemies. Practical Approaches to Their Study and Evaluations*. Oxford, Great Britain, Chapman and Hall.
- Price, P.W. (1972) Parasitoids utilizing the same host: adaptive nature of differences in size and form. *Ecology* **53**, 190–195.
- Rosenheim, J.A. (1998) Higher-order predators and the regulations of insect herbivore populations. *Annual Review of Entomology* **43**, 421–447.
- Rosenheim, J.A., Kaya, H.K., Elher, L.E., Marois, J.J. & Jaffee, B. A. (1995) Intraguild predation among biological control agents: theory and evidence. *Biological Control* **5**, 303–335.
- Sivinski, J. (1996) The past and potential of biological control of fruit flies. pp. 369–375 in McPherson, B.A. & Steck, G.J. (Eds) *Economic Fruit Flies: A World Assessment of Their Biology and Management*. DelRay Beach, FL, St. Lucie Press.
- Sivinski, J., Smittie, B. & Burns, E. (1991) Effects of irradiating host larvae in the mass-reared braconid *Diachasmimorpha longicaudata* in Abstract of the “5th International Workshop, IOBC Mutuality Control of Mass Reared Arthropods”. Wageningen, The Netherlands, International Agricultural Center.
- Sivinski, J., Calkins, C., Baranowski, R.M., Harris, D., Brambila, J., Diaz, J., Burns, R., 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). *Biological Control* **6**, 177–185.
- Sivinski, J., Aluja, M. & López, M. (1997) Spatial and temporal distributions of braconid parasitoids of Mexican *Anastrepha* spp. in the canopies of host fruit trees. *Annals of the Entomological Society of America* **90**, 604–618.
- Sivinski, J., Vulinec, K., Menezes, E. & Aluja, M. (1998) The bionomics of *Coptera haywardi* (Oglobin) (Hymenoptera: Diapriidae) and other pupal parasitoids of tephritid fruit flies (Diptera). *Biological Control* **11**, 193–202.
- Stiling, P. & Cornelissen, T. (2005) What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. *Biological Control* **34**, 236–246.
- Statgraphics (2008) *Statgraphics Centurion XV, User Manual*. Warrenton, Virginia, USA, Statpoint Technologies, Inc., 287pp.
- Sullivan, D. (1987) Insect hyperparasitism. *Annual Review of Entomology* **32**, 49–70.
- Sullivan, D. & Völkl, W. (1999) Hyperparasitism: Multitrophic ecology and behavior. *Annual Review of Entomology* **44**, 291–315.
- Wang, X.G. & Messing, R.H. (2004) The ectoparasitic pupal parasitoid, *Pachycrepoideus vindemniae* (Hymenoptera: Pteromalidae), attacks other primary tephritid fruit fly

- parasitoids: host expansion and potential non-target impact. *Biological Control* **31**, 227–236.
- 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. *Biological Control* **44**, 312–320.
- White, E., Bernal, J., Gonzáles, D. & Triapitsyn, S.** (1998) Facultative hyperparasitism in *Brachymeria pomonae* (Hymenoptera: Chalcididae). *European Journal of Entomology* **95**, 359–366.
- Wong, T.T.Y. & Ramadan, M.M.** (1987) Parasitization of the Mediterranean and Oriental fruit flies (Diptera: Tephritidae) in the Kula area of Maui, Hawaii. *Journal of Economic Entomology* **80**, 77–80.
- Wong, T.T.Y., Ramadan, M.M., McInnis, D.O., Mochizuki, N.L., Nishimoto, J.A. & Herr, J.C.** (1991) Augmentative releases of *Diachasmimorpha tryoni* (Hymenoptera: Braconidae) to suppress a Mediterranean fruit fly population in Kula Maui, Hawaii. *Biological Control* **1**, 2–7.
- Xu, H.Y., Yang, N.W. & Wan, F.H.** (2013) Competitive interactions between parasitoids provide new insight into host suppression. *PLoS ONE* **8**(11), e82003. <https://doi.org/10.1371/journal.pone.0082003>.