

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

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Abstract

In Mexico, both native and exotic fruit fly parasitoids exhibit spatial and temporal overlaps in distribution. To better characterize the spatial component of foraging in the braconid portion of this guild, and to examine the effects of intra- and interspecific competition on resource partitioning, we conducted two field-cage experiments aimed at: (1) assessing the host-finding ability of parasitoids when single- or multiple-species cohorts were confronted with very low host-densities only at canopy level; (2) determining the height level preference (canopy vs. ground) for parasitoid foraging activity when single- or multiple-species cohorts were present and host density was high; (3) identifying candidate species for biological control programs using multiple-species releases. We studied two species exotic to Mexico, *Diachasmimorpha longicaudata* and *D. tryoni*, and four species native to Mexico, *Doryctobracon areolatus*, *D. crawfordi*, *Opius hirtus*, and *Utetes anastrephae* (all Braconidae, Opiinae). Parasitoids were allowed to forage for 8-h as single- or multiple-species cohorts in a room-sized cage containing potted trees with guavas artificially infested with *Anastrepha ludens* larvae and attached to the branches. When parasitoids were released as single-species cohorts into low host-density environments (fruit only at canopy level), *D. longicaudata*, *D. tryoni* and *O. hirtus* clearly distinguished uninfested from infested fruit and exerted the highest rates of parasitism with a significantly female-biased offspring sex ratio. When multiple-species cohorts were released, the same pattern was observed but, *D. crawfordi* and *D. areolatus* did not parasitize any larvae. In the case of the high host-density condition and with fruit at canopy and ground levels, when parasitoids were released in single-species cohorts, only *D. crawfordi* and *D. longicaudata* parasitized larvae at ground level. At canopy level, *D. longicaudata*, *D. tryoni* and *D. crawfordi* achieved the highest parasitism rates. When parasitoids were released as multiple-species cohorts, individuals of none of the species foraged at ground level, and in the canopy foraging activity and parasitism rates dropped dramatically in all species, except *O. hirtus*. Given the performance of *O. hirtus*, it should be considered a potential candidate to complement *D. longicaudata* in low-host density prevalence areas.

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1. Introduction

Understanding the dynamics of coexistence among multiple species has been a goal of many ecologists (Hawkins, 2000; Ritchie, 2002; Amarasekare, 2003) and biological control practitioners and theoreticians (e.g.,

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Pemberton and Willard, 1918; Knipling, 1992). Members of parasitoid guilds foraging for a shared resource offer a unique opportunity to address this issue (e.g., Palacio et al., 1991; Wang and Messing, 2002, 2003; Wang et al., 2003). Parasitoid foraging success depends, among other factors, on efficiency in locating resources, and also on the ability to respond to environmental variability (Bell, 1990; Browne, 1993; Vet, 2001; Lewis et al., 2003). The presence of interspecific competitors and the existence of host refuges (Hawkins et al., 1993) are sources of environmental variability. Heterospecific differences in capacities to exploit resources can change the distribution of suitable hosts in ways that differ from the presence of conspecific competitors alone. Interspecific competitive interactions might be particularly acute when some members of a parasitoid guild are recently introduced and there has been little opportunity for selection to generate niche divergence or create facultative responses to competition (Sivinski et al., 1997; Pedersen and Mills, 2004). The spatial distribution of parasitism is one aspect of foraging that might be predicted to change under interspecific competition. Particular microhabitats may be abandoned to a superior competitor, and movement into an otherwise marginal environment might be a consequence (Bogran et al., 2002; Lewis et al., 2003).

How parasitoids with or without a common selective history forage over space and time in the presence and absence of one another is important to the design of biological control programs, particularly augmentative schemes considering multiple species releases (Murdoch and Briggs, 1996; Pedersen and Mills, 2004; Knipling, 1992). Releasing two or three different parasitoid species simultaneously may result in efficient suppression of a pest population especially when no niche overlap exists (Knipling, 1992). When niche overlap exists, multiple introductions can still be beneficial when members of the artificially released parasitoid guild are more efficient than a naturally occurring primary parasitoid due to a greater combined search ability (Pedersen and Mills, 2004). For example, the solitary, koinobiont fruit fly parasitoid *Opius hirtus* (Fischer) (Hymenoptera: Braconidae) is principally associated in nature with rare *Anastrepha* species (Hernández-Ortiz et al., 1994; López et al., 1999), suggesting it may be a superior searcher that might not interfere with other species adapted to search for hosts present in high densities. While an additional species added to an existing guild may “break a proportional refuge” (i.e., be able to exploit hosts previously sheltered from attack; Pedersen and Mills, 2004), there is increasing evidence of “non-additive effects” where mortalities inflicted by multiple natural enemies are less than the sum of their individual capacities to suppress prey populations (Ehler and Hall, 1982; Ferguson and Stiling, 1996; van Lenteren et al., 2006). Therefore, the avoidance of unsuitable combinations should be a consideration when trying to choose candidate species

for augmentative releases (but see Pedersen and Mills, 2004).

In Veracruz, Mexico, several native and exotic parasitoid-fruit fly species overlap in space and time to various degrees (Sivinski et al., 1996, 2000; López et al., 1999). For example, *Diachasmimorpha longicaudata* (Ashmead), *Doryctobracon crawfordi* (Viereck), *Doryctobracon areolatus* (Szépligeti) and *Utetes anastrephae* (Viereck), all Braconidae with varying ovipositor lengths (Sivinski et al., 2001), have been recovered from a single guava (*Psidium guajava* L.) fruit. On the other hand, there is some niche segregation in the case of *U. anastrephae* and *D. areolatus* attacking *Anastrepha obliqua* (Macquart) larvae in *Spondias mombin* (L.) fruit (Anacardiaceae). *U. anastrephae* is significantly more abundant in the interior parts of the tree canopy, and *D. areolatus* more numerous in the exterior part of the canopy where fruit are bigger (Sivinski et al., 1997). While *D. areolatus* is better able to reach hosts in larger fruit, another reason for this size/spatial separation may be its avoidance of multiparasitism. The eggs and first-instar larvae of *U. anastrephae* are larger than those of *D. areolatus*, and if larvae of both species are present in a single host *U. anastrephae* invariably kills *D. areolatus* (M.A., Sergio Ovruski, Guadalupe Córdova, and J.S., unpub. data). In contrast to the distributional differences between these native species, the exotic *D. longicaudata* and the native *D. crawfordi* apparently have no niche segregation when they both parasitize *A. ludens* (Loew) larvae in citrus (Sivinski et al., 1997). Perhaps the recency of the interaction between these two species (*D. longicaudata* was introduced into the region in 1956; Jiménez, 1956), has not allowed competition to select for niche separation (Miranda, 2002).

Diachasmimorpha longicaudata is a solitary, late-instar larval-prepupal, koinobiont fruit fly parasitoid that was originally collected in the Indo-Philippine region attacking *Bactrocera* spp. (White and Elson-Harris, 1992) and later introduced throughout much of the tropical and subtropical New World (Ovruski et al., 2000). Females locate infested fruit by responding to volatiles (Greany et al., 1977; Messing and Jang, 1992; Eben et al., 2000) and detect individual larvae through the vibrations and sounds produced by them while feeding within fruits (Lawrence, 1981). It is considered one of the most important biological control agents for augmentative releases worldwide (Clausen et al., 1965; Sivinski, 1996; Montoya et al., 2000). The other exotic parasitoid included, *D. tryoni*, is an Australasian larval-prepupal, koinobiont parasitoid of several tephritid species (Wharton and Gilstrap, 1983), that was originally introduced into Hawaii to combat the Mediterranean fruit fly *Ceratitidis capitata* (Wideman) (Wong et al., 1992) and subsequently released for the same purpose in Guatemala (Sivinski et al., 2000).

Doryctobracon areolatus is a solitary, larval-prepupal endoparasitic koinobiont that attacks hosts in both native and commercial exotic fruits (Aluja et al., 1990, 2003; Hernández-Ortiz et al., 1994; López et al., 1999). It exhibits

diapause (Aluja et al., 1998; Ovruski et al., 2004), which allows it to expand its range into regions with low plant diversity (Eitam et al., 2004). This species is one of the most common and widespread native parasitoids of *Anastrepha* spp. (Ovruski et al., 2000) occurring from Florida to Argentina (Wharton and Marsh, 1978). *D. crawfordi*, another native, solitary, larval pre-pupal, koinobiont parasitoid, is more tropical in distribution compared to other Mexican opiines (Ovruski et al., 2000, 2004). It is relatively abundant at higher elevations (Sivinski et al., 2000) where it encounters moister environments and does not enter diapause (Aluja et al., 1998). It has one of the largest ovipositors of any native *Anastrepha* parasitoid (Sivinski et al., 2001). In Mexico its principal native host is *A. ludens* (Plummer and McPhail, 1941; López et al., 1999). *U. anastrephae* is also a native, solitary, larval pre-pupal, koinobiont parasitoid of *Anastrepha*, found from Florida to Argentina (Ovruski et al., 2000). The ovipositor is short compared to other Mexican opiines, and it forages upon a relatively few species of generally small fruits (Sivinski et al., 1997, 2000). Finally, *O. hirtus* is yet another solitary, larval-prepupal koinobiont parasitoid but with an unusual host range. Among its other hosts, it attacks two rare species, *Anastrepha cordata* (Aldrich) and *A. alveata* (Stone), which occur locally and in low numbers (Hernández-Ortiz et al., 1994; Piedra et al., 1993; Sivinski et al., 2000; Aluja et al., 2003).

To better characterize the niches of the various native and exotic *Anastrepha* spp. parasitoids described above and to test predictions on foraging ability under conditions of low host prevalence, we investigated their foraging patterns in single-species cohorts and in the presence of potential competitors. Specifically we examined: (1) the relative efficacy of parasitoids in single-species and heterospecific cohorts in locating rare (by field standards) hosts in a particular microhabitat (tree canopy); and (2) the spatial component of foraging across two microhabitats (tree canopy and ground under tree), both in single and multiple-species cohorts. The first of these experiments pits intra- and interspecific competitors against each other in a particularly competitive environment. The second looks at the spatial consequences of intra and interspecific competition. The addition of the exotic species, as noted above, allowed us to examine species interactions with both long and short evolutionary histories and which have had various opportunities to resolve competition for hosts (Sivinski et al., 1997). In particular, we wanted to test the prediction that in the presence of superior competitors in one microhabitat, the less competitive species would forage in otherwise less suitable habitats. We also wanted to test the prediction that females of *O. hirtus* would be particularly efficient at finding larvae at low densities given their interaction with rare host species in nature. The information presented below could thus influence the directions of the various classical, augmentative and conservation fruit fly biological control programs contemplated or underway in Latin America.

2. Methods

2.1. Experimental conditions

Experiments were carried out under laboratory conditions in a climate-controlled room at the Instituto de Ecología, A.C., Xalapa, Veracruz, Mexico. Insects were observed in a cylindrical field cage (3 m diam. × 3 m height) similar to those described by Calkins and Webb (1983). In the center of the cage, orange (*Citrus sinensis* L., Rutaceae), mango (*Mangifera indica* L., Anacardiaceae), guava (*Psidium guajava* L., Myrtaceae), sapodilla (*Manilkara zapota* L., Sapotaceae), tropical plum (*Spondias mombin* L., Anacardiaceae) and rose-apple (*Syzygium jambos* L., Myrtaceae) trees, were arranged so as to simulate a patch of mixed *Anastrepha* host fruits (Fig. 1). Environmental conditions were 25 ± 2 °C, 70% RH and 200–400 lux of light intensity.

2.2. Parasitoid species

Female parasitoids were obtained from laboratory cultures maintained at the Instituto de Ecología A.C., in Xalapa, Veracruz, Mexico at 26 ± 1 °C, 70% RH and a photoperiod of L12:D12. All colonies were maintained using 7- to 9-d old *Anastrepha ludens* (Loew) larvae as hosts.



Fig. 1. View of field cage showing guavas hanging from tree canopies and laying on floor.

In the case of the two exotic species, larvae were offered to the females in naked form (i.e., without diet), whereas in the case of all the native parasitoids, larvae were mixed with diet when exposed to females in the rearing cages (for further details on parasitoid rearing methods see Aluja et al., 2007). After emergence, female parasitoids were offered honey, allowed to mate but host-deprived, which stimulates orientation towards habitats with suitable hosts for oviposition (Messing et al., 1997). Female parasitoids were used in experiments when they were 5- to 10-d old.

2.3. Oviposition units

Oviposition units consisted of artificially infested guavas (35–55 g ripe fruit) that were hollowed by removing the mesocarp and endocarp (pulp). Guavas were cut open transversally along the peduncle, about 1/4 down the length of the fruit (measured from the proximal end). The proximal quarter sections functioned as “lids” for the filled fruits and the remainder of the fruit served as “bases” for filling (Aluja et al., 2007). For the experiment on searching ability under extremely low host density conditions (Experiment 1), the cavities were filled with either 12 g of artificial diet and 2 *A. ludens* larvae or diet alone (i.e., no fly larvae). For Experiment 2 (details follow), we filled the cavities with 15 *A. ludens* larvae and about 12 g of artificial diet (Burns, 1995). Once guavas were filled, “lids” and “bases” were joined with 1.5 × 10 cm strips of parafilm (Parafilm “belts”) (Parafilm[®] Laboratory Film, American National Can Tm, Chicago, IL). Also, holes were pricked into the fruit with a 1 mm metal needle to allow for aeration.

2.4. Experiment 1. Parasitoid ability to find and parasitize hosts at very low densities

Twenty guavas were hung from the roof of the cage and distributed so as to form two circles in the canopy (i.e., 10 guavas per circle, central and peripheral) at a height of 180 cm above ground level (Fig. 1). Individual fruit were suspended from the ceiling by means of a cotton string tied to a plastic paper clip which in turn was inserted into the Parafilm “belt” described in the preceding section. Of the 20 guavas, five were randomly chosen and artificially infested with two *A. ludens* larvae following the same procedure used to prepare the oviposition units. The 15 remaining fruit contained only artificial diet. Adult parasitoids were released in (1) single- or (2) multiple-species cohorts and allowed to forage freely for 8 h starting at 10:00 h. In the case of single-species cohort releases, 30 females per species were released and in the case of multiple-species cohorts releases, 5 females per species were released (i.e., 30 females in total). At the beginning of every hour, we counted the number of females that were inserting their ovipositor into fruit and resting on guavas. After the eight-hour period was over (18:00 h), guavas were retrieved from the cage and placed individually into plastic cups (8 cm diam. × 7 cm height) which were in turn placed inside

another plastic container (11 cm diam. × 7.5 cm height) with fine Vermiculite[®] in the bottom part as pupation substrate. The outer plastic containers were sealed with their lids, which had a ~7 cm diam. perforation to allow for ventilation. Samples were placed in a room at 26 ± 2 °C, 60–70% RH and a L12:D12 light regimen. After six days, pupae were rinsed with water and held until an adult fly or parasitoid emerged. Pupae were moistened every four days to avoid desiccation. Following Aluja et al. (1990), Sivinski et al. (2000) and Ovruski et al. (2004), we calculated parasitism rate as the total number of emerged adult parasitoids divided by the sum of the number of parasitoids and flies that emerged. Observations were replicated seven times with different parasitoid cohorts.

2.5. Experiment 2. Preferred height for foraging activity (canopy vs. ground) under high host density conditions

Two microhabitats were identified within the cage: tree canopy and the ground directly beneath the canopy. Hosts within the canopy were exposed at 180 cm above ground level and were suspended from the cage ceiling as described under “Experiment 1”. Hosts on the ground mimicking fallen fruit were placed on the cage floor. In each microhabitat, 20 artificially infested guavas (15 third-instar, *A. ludens* larvae in each fruit) were distributed forming two circles (i.e., 10 fruits forming a central circle and 10 fruits forming a peripheral circle). As was the case in the searching ability experiment, adult parasitoids were released in single- or multiple-species cohorts and allowed to forage freely for 8 h starting at 10:00 h. After parasitoid releases, observation procedures and sample manipulation were as described under searching ability experiment. This experiment was replicated five times, and as was the case with searching ability experiment. For each replicate a new cohort was released into the cage.

2.6. Statistical analyzes

Since neither the number of visits, ovipositions or parasitism rates in both experiments were normally distributed, we rank-transformed the data prior to the analysis (Conover and Iman, 1981; Potvin and Roff, 1993). Data on the number of females that landed on fruit (visits) and ovipositor insertions into fruit (purported ovipositions) in the single-species experiments were analyzed by means of a split-plot MANOVA. Following a significant overall MANOVA, ANOVAs and Least Square Mean *t*-tests were run on individual responses. In the case of the low-host-density experiment, since a different proportion of infested/uninfested fruit were used, a rate of visits and ovipositor insertions per fruit was employed. Because in the multiple-species experiments no foraging activity at ground level was recorded, data were analyzed by means of a one-way MANOVA. Parasitism was analyzed by means of one-way ANOVAs except in the case of the canopy vs. ground comparison in the single-species, high host density

experiment, in which case data was compared by using a split-plot ANOVA (Zar, 1998). Sex ratios were calculated in all the cases in which a fruit yielded parasitoids but were not formally analyzed given the low N values.

3. Results

3.1. Experiment 1. Parasitoid abilities to find and parasitize hosts at very low densities

3.1.1. Single-species treatment

According to our split-plot MANOVA analysis, when single-species cohorts were released, highly significant differences were observed among parasitoid species with respect to visits and ovipositor insertions (Pillai trace, $F_{10,60} = 5.4$, $P < 0.0001$). Both exotic species exhibited the highest levels of activity (Fig. 2a and b). Also, highly significant differences were observed between infested and uninfested fruit, with the former receiving the most visits and ovipositor insertions (Pillai trace, $F_{2,35} = 121.8$, $P < 0.0001$). The interaction between parasitoid species

and fruit condition was also highly significant, indicating that foraging behavior differed among species (Pillai trace, $F_{10,72} = 3.9$, $P < 0.0003$).

Females of some parasitoid species visited and inserted their ovipositor significantly more often than others (visits $F_{5,36} = 27.4$, $P < 0.0001$; ovipositor insertions $F_{5,36} = 21.9$, $P < 0.0001$) (Fig. 2a and b). Differences with respect to visits and ovipositor insertions in infested vs. uninfested fruit were also highly significant (visits $F_{1,36} = 240.4$, $P < 0.0001$; ovipositor insertions $F_{5,36} = 201.3$, $P < 0.0001$). The interaction of species by fruit condition was significant with respect to fruit visits but not ovipositor insertions (visits $F_{5,36} = 3.1$, $P < 0.01$; ovipositions $F_{5,36} = 2.3$, $P < 0.06$) (Fig. 2a and b).

Percent parasitism was highest in the two exotic species (*D. longicaudata* [91.42%] and *D. tryoni* [85.71%]). Among the native species, *O. hirtus* exhibited the highest (42.85%) and *D. areolatus* the lowest parasitism rate (12.85%) ($F_{5,36} = 48.3$; $P < 0.0001$) (Fig. 3a). Sex ratio was 1:1 in the case of *D. areolatus* and *U. anastrephae* while in all other species we detected a strong female bias (Table 1).

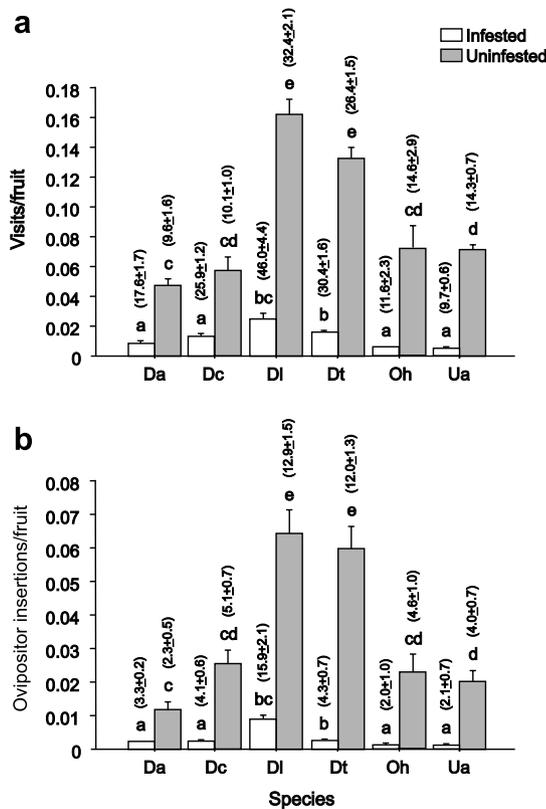


Fig. 2. (a) Mean (\pm SE) fruit visitation rate (visits/fruit) and (b) mean (\pm SE) number of ovipositor insertions/fruit when female parasitoids foraged in the presence of conspecifics (i.e., *single-species cohorts*) under very low host-density condition (five of 20 fruit infested with only two larvae per fruit). All fruit was placed in canopy. Numbers (mean \pm SE) above bars represent the total number of fruit visits (a) and ovipositor insertions (b) averaged over seven replicates. Different letters indicate significant differences among parasitoid species. *Doryctobracon areolatus* (D.a.), *D. crawfordi* (D.c.), *Diachasmimorpha longicaudata* (D.l.), *D. tryoni* (D.t.), *Opius hirtus* (O.h.) and *Utetes anastrephae* (U.a.).

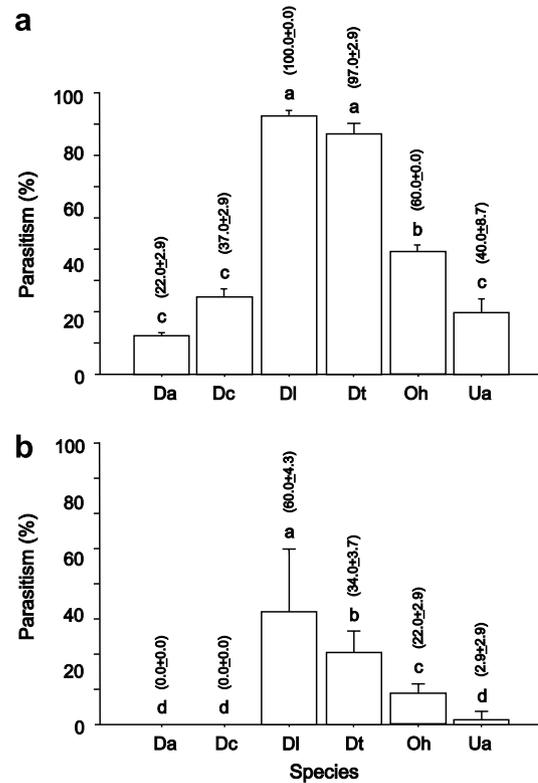


Fig. 3. (a) Percent parasitism (mean \pm SE) in a field cage in which parasitoids were released as *single-species cohorts* under the very low host-density condition (five of 20 fruit infested with only two larvae per fruit). All fruit was placed in canopy. (b) Percent parasitism (mean \pm SE) in same cage (fruit placed only in canopy) but when females were released in *multiple-species cohorts* under same very low host-density condition. Different letters indicate significant differences among parasitism rates. Numbers in parenthesis above bars indicate the mean (\pm SE) proportion of fruit with parasitized larvae. *Doryctobracon areolatus* (D.a.), *D. crawfordi* (D.c.), *Diachasmimorpha longicaudata* (D.l.), *D. tryoni* (D.t.), *Opius hirtus* (O.h.), and *Utetes anastrephae* (U.a.).

Table 1

Mean sex ratio (\pm SE) (proportion of individuals that are male) for six braconids wasps under two host-availability conditions and under single- and multiple-species releases in a field cage (N, total number of fruit from which parasitoids emerged)

Parasitoid species and fruit location	High host-density				Low host-density			
	Single-species		Multiple-species		Single-species		Multiple-species	
	Sex ratio	N	Sex ratio	N	Sex ratio	N	Sex ratio	N
Da, Canopy	0.65 \pm 0.03	75	0.89 \pm 0.11	9	0.5 \pm 0.19	8		
Dc, Canopy	0.46 \pm 0.01	83	0.34 \pm 0.11	9	0.04 \pm 0.04	13		
Dc, Ground	0.40 \pm 0.02	11						
DI, Canopy	0.29 \pm 0.01	100	0.25 \pm 0.02	35	0.11 \pm 0.04	35	0.05 \pm 0.05	21
DI, Ground	0.28 \pm 0.02	20						
Dt, Canopy	0.31 \pm 0.01	100	0.24 \pm 0.04	14	0.10 \pm 0.03	34	0 \pm 0	12
Oh, Canopy	0.43 \pm 0.14	14	0.39 \pm 0.08	11	0.24 \pm 0.07	21	0.25 \pm 0.16	8
Ua, Canopy	0.45 \pm 0.06	44	0.56 \pm 0.17	8	0.5 \pm 0.14	14	1	1

3.1.2. Multiple species treatment

As was the case with the single-species experiment, the MANOVA indicates that there were highly significant differences in overall activity patterns (i.e., independent of fruit condition) among parasitoid species (Pillai trace, $F_{10,144} = 0.64$, $P < 0.0001$). Notably, among the native species, *O. hirtus* exhibited the highest activity levels (Fig. 4a

and b). Furthermore, the few infested fruit ($N = 5$) received significantly more visits and ovipositor insertions than uninfested fruit ($N = 15$) (Pillai trace, $F_{2,71} = 0.60$, $P < 0.0001$). Finally, we detected a significant interaction between species and fruit condition (Pillai trace, $F_{10,144} = 0.61$, $P < 0.0001$).

There were highly statistically significant differences with respect to visits and ovipositor insertions among parasitoid species (visits $F_{5,72} = 16.3$, $P < 0.0001$; ovipositions $F_{5,72} = 11.1$, $P < 0.0001$) with *D. longicaudata* females exhibiting the most activity. Similar to what we found in the single-species experiment, when comparing activity in infested vs. uninfested fruit, significantly more infested fruit were visited and probed (i.e., ovipositor insertion observed) than uninfested ones (visits $F_{1,72} = 89.9$, $P < 0.0001$; ovipositions $F_{1,72} = 40.3$, $P < 0.0001$). Finally the species by fruit treatment (i.e., infested vs. uninfested) interaction was also highly significant for both response variables (visits $F_{5,72} = 12.8.1$, $P < 0.01$; ovipositions $F_{5,72} = 10.4$, $P < 0.0001$) (Fig. 4a and b).

Notably, when multiple-species cohorts were released, parasitism levels dropped in all the species. Nevertheless, significant differences were detected among them (ANOVA; $F_{5,36} = 121.2$, $P < 0.0001$) (Fig. 3b). As was the case with single-species cohorts, the exotic species *D. longicaudata* and *D. tryoni* achieved the highest percentages of parasitism (40.0 and 25.71%, respectively). Among native species, *O. hirtus* again exhibited the highest parasitism levels (11.42%). Importantly, when foraging in the presence of individuals of other species, *D. areolatus* and *D. crawfordi* did not parasitize any larvae inside guavas. The sex ratio was female biased for *D. longicaudata*, *D. tryoni* and *O. hirtus* (Table 1).

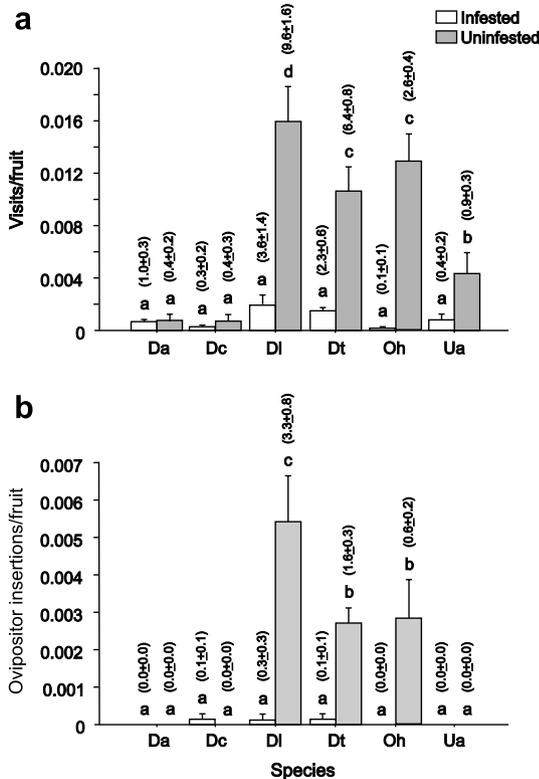


Fig. 4. (a) Mean (\pm SE) fruit visitation rate (visits/fruit) and (b) mean (\pm SE) number of ovipositor insertions/fruit, when parasitoids were released as multiple-species cohorts and females foraged under very low host-density condition (five of 20 fruit infested with only two larvae per fruit). All fruit was placed in canopy. Numbers (mean \pm SE) above bars represent the total number of fruit visits (a) and ovipositor insertions (b) averaged over seven replicates. Different letters indicate significant differences among parasitoid species. *Doryctobracon areolatus* (D.a.), *D. crawfordi* (D.c.), *Diachasmimorpha longicaudata* (D.l.), *D. tryoni* (D.t.), *Opius hirtus* (O.h.) and *Uetes anastrephae* (U.a.).

3.2. Experiment 2. Preferred height for foraging activity (canopy vs. ground) under high host density conditions

3.2.1. Single-species treatment

Under high host density conditions (i.e., all twenty fruit in canopy and ground containing 15 larvae each), patterns of parasitoid activity were quite similar to those observed under the low host condition, with *D. longicaudata* females

exhibiting the highest levels of activity (MANOVA, Pillai trace, $F_{10,40} = 1.24$, $P < 0.0001$). When single-species cohorts were released, activity was significantly different when comparing activity patterns at canopy and ground levels for all species (Pillai trace, $F_{2,23} = 0.96$, $P < 0.0001$). Furthermore, a highly significant interaction between species and foraging stratum (i.e., canopy vs. ground) was detected (Pillai trace, $F_{10,48} = 1.01$, $P < 0.0001$).

In accordance with the above, the ANOVAs exposed highly significant differences with respect to fruit visits and ovipositor insertions among parasitoid species (ANOVA; visits $F_{5,24} = 69.3$, $P < 0.0001$; ovipositions $F_{5,24} = 56.7$, $P < 0.0001$). *Diachasmimorpha longicaudata* females were the most active. Fruit position (canopy vs. ground) exerted a highly significant effect on foraging activity as fruit in the canopy were more visited and probed than fruit on the ground (visits $F_{1,24} = 430.1$, $P < 0.0001$; ovipositions $F_{1,24} = 351.5$, $P < 0.0001$). The species by fruit position interaction also highly significant for both

response variables (visits $F_{5,24} = 12.5$, $P < 0.01$; ovipositions $F_{5,24} = 17.9$, $P < 0.0001$) (Fig. 5a and b).

With respect to the level of parasitism, *D. longicaudata* achieved the highest parasitism rate among all species (91.61%) (ANOVA; $F_{10,24} = 1517.13$, $P < 0.0001$). Individuals of all six species exhibited a significant preference to forage in the tree canopy (ANOVA; $F_{1,24} = 6999.05$, $P < 0.0001$). Notably, only *D. longicaudata* and *D. crawfordi* parasitized hosts at ground level despite the fact that females of some of the other species were also seen landing on such type of fruit (ANOVA; $F_{5,24} = 863.06$, $P < 0.0001$) (Fig. 6a). With respect to sex ratios, *D. areolatus* exhibited a male-biased sex ratio while *D. crawfordi* and *U. anastrephae* tended to exhibit 1:1 ratio. In contrast, *D. longicaudata* and *D. tryoni* exhibited a female-biased sex ratio (Table 1).

3.2.2. Multiple-species treatment

An interesting pattern was observed when multiple-species cohorts were released because no significant differences in activity among species were observed (Pillai Trace,

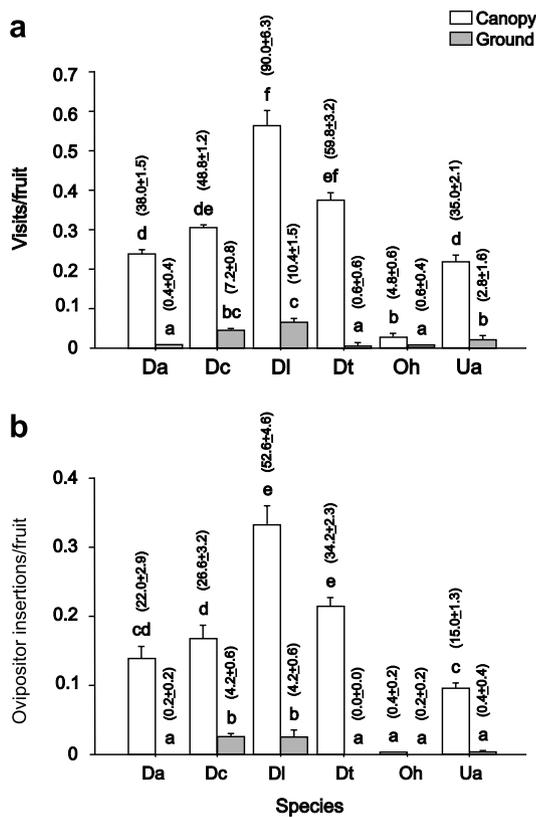


Fig. 5. (a) Mean (\pm SE) fruit visitation rate (visits/fruit) and (b) mean (\pm SE) number of ovipositor insertions/fruit when female parasitoids foraged in the presence of conspecifics (i.e., single-species cohorts) under the high host density condition (40 fruit each containing 20 larvae). Guavas were placed in canopy ($N = 20$) and on ground ($N = 20$). Numbers (mean \pm SE) above bars represent the total number of fruit visits (a) and ovipositor insertions (b) averaged over five replicates. Different letters indicate significant differences among parasitoid species. *Doryctobracon areolatus* (D.a.), *D. crawfordi* (D.c.), *Diachasmimorpha longicaudata* (D.l.), *D. tryoni* (D.t.), *Opius hirtus* (O.h.) and *Utetes anastrephae* (U.a.).

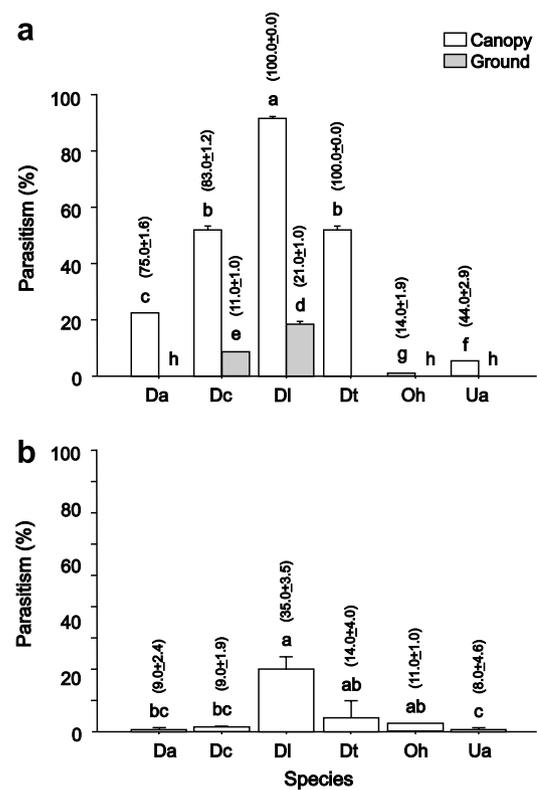


Fig. 6. (a) Percent parasitism (mean \pm SE) in a field cage in which parasitoids were released as single-species cohorts under the high host-density condition (40 fruit each containing 20 larvae). Guavas were placed in canopy ($N = 20$) and on ground ($N = 20$). (b) Percent parasitism (mean \pm SE) when females were released in multiple-species cohorts under same high host-density condition (40 fruit each containing 20 larvae). Guavas were also placed in canopy ($N = 20$) and on ground ($N = 20$). Different letters indicate significant differences among parasitism rates. Numbers in parenthesis above bars indicate the mean (\pm SE) proportion of fruit with parasitized larvae. *Doryctobracon areolatus* (D.a.), *D. crawfordi* (D.c.), *Diachasmimorpha longicaudata* (D.l.), *D. tryoni* (D.t.), *Opius hirtus* (O.h.), and *Utetes anastrephae* (U.a.).

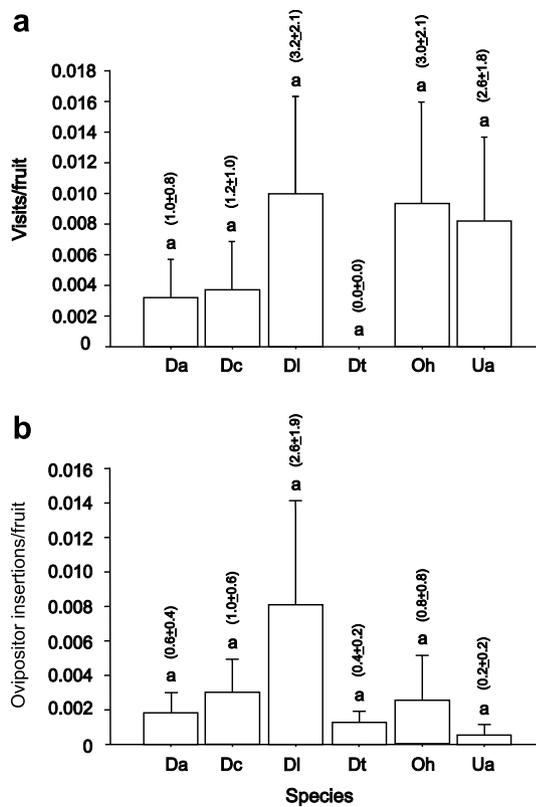


Fig. 7. (a) Mean (\pm SE) fruit visitation rate (visits/fruit) and (b) mean (\pm SE) number of ovipositor insertions/fruit when parasitoids were released as *multiple-species cohorts* and females foraged under the high host density condition (40 fruit each containing 20 larvae). Guavas were placed in canopy ($N = 20$) and on ground ($N = 20$) but foraging activity was only observed in canopy (i.e., no activity observed at ground level). Numbers (mean \pm SE) above bars represent the total number of fruit visits (a) and ovipositor insertions (b) averaged over five replicates. Different letters indicate significant differences among parasitoid species. *Doryctobracon areolatus* (D.a.), *D. crawfordi* (D.c.), *Diachasmimorpha longicaudata* (D.l.), *D. tryoni* (D.t.), *Opius hirtus* (O.h.) and *Utetes anastrephae* (U.a.).

$F_{10,48} = 0.38$, $P = 0.36$). Notably and in sharp contrast to what was observed in single-species experiments, no activity and no parasitism were recorded at ground level for any of the six species (Fig. 7a and b).

With respect to parasitism at canopy level, *D. longicaudata* was the species that reached the highest levels (ANOVA; $F_{5,24} = 11.0$, $P < 0.0001$) (Fig. 6b). Sex ratio was male biased in the case of *D. areolatus*, 1:1 in the case of *U. anastrephae*, while all other species exhibited a female biased ratio (Table 1).

4. Discussion

When parasitoids were released as single-species cohorts into low host-density environments with fruit only at canopy level, *D. longicaudata*, *D. tryoni* and *O. hirtus* clearly distinguished uninfested from infested fruit and exerted the highest rates of parasitism (91, 86 and 43%, respectively) with a significantly female-biased offspring sex ratio. In the case of *D. crawfordi*, *D. areolatus* and *U. anastrephae*,

hae, parasitism levels were quite low (27, 21, and 13%, respectively). When multiple-species cohorts were released, overall parasitism levels dropped considerably but the same three species exerted the highest rates of parasitism (40, 26 and 11% for *D. longicaudata*, *D. tryoni* and *O. hirtus*, respectively). Under these conditions, *D. crawfordi* and *D. areolatus* did not parasitize any larvae and parasitism rate in the case of *U. anastrephae* dropped to 1%. In the case of the high host-density condition and with fruit at canopy and ground levels, when parasitoids were released in single-species cohorts, only *D. crawfordi* and *D. longicaudata* parasitized larvae at ground level. At canopy level, *D. longicaudata*, *D. tryoni* and *D. crawfordi* achieved the highest parasitism rates. Notably under these high-density host conditions, when parasitoids were released as multiple-species cohorts, individuals of none of the species foraged at ground level, and in the canopy, foraging activity and parasitism rates dropped dramatically in all species, except *O. hirtus*. So, contrary to the prediction that competition would force individuals to forage in marginal habitats, interspecific competition did not increase niche breadth, but in fact appeared to narrow the range of microhabitats searched in two species (i.e., *D. longicaudata* and *D. crawfordi*).

When parasitoids foraged under very low host-density conditions (five of 20 fruit infested with only two larvae per fruit), females of all species tested located and visited infested fruit more frequently than uninfested ones. Also, females of all species performed better when foraging among conspecifics than when doing so with individuals of the other five species. We would like to highlight the fact that very few females were observed visiting and inserting their ovipositor into fruit (Fig. 2). This pattern could be explained by the fact that only a few females were active at the same time. Nevertheless, high rates of parasitism were recorded in the case of species such as the exotics *D. longicaudata* and *D. tryoni* and the native *O. hirtus*. This indicates that the few females exhibiting foraging activity were very effective at finding their hosts and suggests that high parasitism levels *can be* (but are not always) achieved with relatively low visitation rates and/or relatively little time spent on fruit. It is also consistent with our prediction that females of *O. hirtus* would be particularly efficient at finding larvae at low densities given their interaction with rare host species in nature such as *A. cordata* (Hernández-Ortiz et al., 1994).

Cumulative parasitism rates were similar in multispecies and single species cohorts, and the relative rank successes of the various species were similar as well. Again, both *Diachasmimorpha* spp. inflicted higher mortalities than the native species. Interestingly, the two *Doryctobracon* species were unable to parasitize any larvae in the presence of interspecific competitors. However, they were more successful in the subsequent niche-breadth experiment where host density was higher, suggesting density-dependent foraging. *D. areolatus*, in particular, is a common parasitoid of the sporadically abundant *A. obliqua* (e.g., Sivinski

et al., 1997), and as such might be selected to forage under high host density conditions.

Various fruit fly parasitoids forage over different ranges of microhabitats (e.g., Sivinski et al., 1997). Unlike the other species examined, *D. longicaudata* and *D. crawfordi* attack larvae in fallen fruit upon the ground (Purcell et al., 1994; Miranda, 2002;). In the case of *D. tryoni*, such a behavior has also been reported (Vargas et al., 1991), but we did not record it in our experimental study. It is not clear why other species do not, since appropriate sizes and ages of hosts are commonly present in such fruit. It may be that the risks of adult-parasitoid predation are particularly high or that less-than-completely mature larvae attacked under such conditions are more likely to be consumed by frugivores/predators such as pigs, birds and ants (Hodgson et al., 1998; Aluja et al., 2005).

Competition is one of many potential determinants of niche breadth (Lawton and Hassell, 1984; Hawkins, 2000; Holmgren and Getz, 2000; Pedersen and Mills, 2004). In the presence of superior competitors in one microhabitat, it might be predicted that the less competitive species begins to forage in otherwise less suitable habitats (Lawton and Hassell, 1984; Driessen and Visser, 1993; Holt and Lawton, 1994). Thus, it was hypothesized that native species such as *D. areolatus*, *U. anastrephae* and *O. hirtus* that are restricted to host-tree canopies in single-species cohorts might be found at ground level when confronted with superior competitors such as *Diachasmimorpha* spp. This was not the case. In fact, the microhabitat ranges of *D. longicaudata* and *D. crawfordi* contracted in the presence of interspecific competition and the two species were no longer recovered from fruit on the ground. One explanation is that ground fruit are marginal microhabitats for *D. longicaudata* and *D. crawfordi* as well, and that intraspecific competition drives individual females to exploit larvae in fallen fruit. If this is the case, the presence of less-efficient interspecific competitors represents a less competitive environment, and highly competitive females are not as likely to be driven to less attractive portions of the patch. If so, niche breadth in *D. longicaudata* and *D. crawfordi* should be positively dependent on conspecific density relative to hosts.

The foraging ecology of tephritid parasitoids has implications for biological control. Parasitoid augmentations have significantly suppressed *Anastrepha* populations (Sivinski et al., 1996; Montoya et al., 2000), and may be particularly effective when combined with the Sterile Insect Technique (Wong et al., 1992; Rendón et al., 2006). Low-density foragers, such as *Diachasmimorpha* spp. and *O. hirtus*, might be particularly good candidates for the later stages of such releases since they would be able to continue to inflict mortality as host densities fall (Force, 1974; Sivinski and Aluja, 2003). The failure of *D. areolatus* to attack larvae in the presence of interspecific competitors cautions against multispecies releases without a clear notion of the desired consequences (Pedersen and Mills, 2004). The present evidence of the competitiveness of *D. longicaudata* rel-

ative to *D. areolatus* helps explain their patterns of distribution in Florida where they were introduced sequentially. The once abundant *D. areolatus* virtually disappeared from the southern portion of its host's range following the introduction of *D. longicaudata* (Baranowski et al., 1993). But it has managed to survive in the northern part perhaps because of its diapause capacity and a superior ability to survive widely-spaced host population fluctuations (Eitam et al., 2004).

In conclusion, and addressing the question posed by Hawkins (2000), our results appear to indicate that competition does indeed matter in shaping the dynamics of resource partitioning and species coexistence in fruit fly parasitoid communities. The fact that we discovered that parasitism by two (i.e., *D. areolatus* and *D. crawfordi*) of the six species studied was totally halted when foraging in the presence of individuals of other species under low host density conditions, and that the microhabitat ranges of *D. longicaudata* and *D. crawfordi* contracted in the presence of interspecific competition, warrants further investigation. In particular, we believe that testing various species combinations (e.g., pair-wise or in triplets as Bogran et al. (2002) did), will allow us to determine which of all the six species studied exerts the greatest influence over the other species sharing the resource. Furthermore, manipulating host and adult parasitoid densities as well as looking into the interactions of parasitoid larvae inside host larvae (i.e., fruit fly larvae) when super and hyperparasitism occurs (likely scenario in the cases of *D. areolatus* and *U. anastrephae*), would help us gain deeper insight into the mechanisms shaping the interactions at play in this unique system. Such an approach has been successfully followed in the case of the guild of parasitoids attacking fruit fly eggs and larvae in Hawaii (Wang and Messing, 2002, 2003; Wang et al., 2003; Bokonon-Ganta et al., 2005).

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