Austral Entomology (2015) ••, ••–••

Is *Anastrepha obliqua* (Diptera: Tephritidae) a natural host of the Neotropical parasitoids *Doryctobracon crawfordi* and *Opius hirtus*?

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Abstract An understanding of the search, selection and host use behaviours of parasitoids that have the potential to be used as biological control agents is becoming increasingly important. We studied under laboratory conditions the host suitability of *Anastrepha obliqua* (Macquart) and *Anastrepha ludens* (Loew) larvae for the parasitoids *Doryctobracon crawfordi* (Viereck) and *Opius hirtus* (Fischer), which are native to the Americas. By counting the oviposition scars on the puparium, we found that both types of larvae were equally attacked; however, the pupa dissections revealed that different numbers of eggs were laid in each type of larvae. The *A. obliqua* larvae were significantly less parasitised than those of *A. ludens*, and immature insect development or adult emergence was not in either parasitoid species. Dissections of the parasitised *A. obliqua* pupae also showed that the immature parasitoids of both species died by encapsulation and melanisation, and there was a high proportion of unemerged adult flies. By contrast, *A. ludens* parasitised pupae contained several viable immature parasitoids that subsequently emerged as adult parasitoids. These results indicated contrasting suitability conditions of *A. ludens* and *A. obliqua* larvae as hosts of *O. hirtus* and *D. crawfordi* parasitoids, which suggest that *A. obliqua* is not a natural host for either parasitoid species. These findings will improve the understanding for the use of these parasitoid species in projects for biological control against economically important fruit flies of the genus *Anastrepha*.

Key words biological control, Braconidae, fruit fly.

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are considered one of the primary pests affecting fruit production worldwide (Aluja 1994; Souza-Filho *et al*. 2003). The fruit fly *Anastrepha obliqua* (Macquart) is an important species among those causing the most damage to fruit crops in the Neotropical region, with a wide distribution from the southern United States (Texas) to northern Argentina. In Mexico, this pest is found in tropical regions, causing damage primarily to mango crops (Peña *et al*. 2009) and to fruits from the genus *Spondias* (Aluja & Birke 1993). In Brazil, this species is the second most important of the genus, after *Anastrepha fraterculus* (Wiedemann) (Zucchi 2008).

The parasitoids *Doryctobracon crawfordi* (Viereck) and *Opius hirtus* (Fischer) (Braconidae: Opiinae) can be an important alternative for the implementation of biological control projects against these types of pests because they coexist in the same habitat as *A. obliqua*, and their colonisation and domestication have been reported by authors such as Aluja *et al*. (2009) and Cancino *et al*. (2009). These parasitoid species have a wide range and distribution of hosts. *Doryctobracon crawfordi* is distributed from the central region of Mexico to Argentina (Ovruski *et al*. 2005) and is mainly associated with *Anastrepha ludens* (Loew) (López *et al*. 1999), although this parasitoid has also been reported as a parasitoid of *A. fraterculus*, *Anastrepha striata* (Schiner), *Anastrepha serpentina* (Wiedemann) and *A. obliqua* larvae in the states of Chiapas and Veracruz in Mexico (Aluja *et al*. 1990; López *et al*. 1999). In Central America, *D. crawfordi* has been recovered from *A. striata* and *Anastrepha distincta* (Greene) larvae (Jiron & Mexzon 1989), and from *Anastrepha anomala* (Stone) and *A. serpentina* (Medianero *et al*. 2006). For South America, the list of countries and genera from which this parasitoid has been recovered is long and includes the following: (1) in Ecuador, from *A. obliqua*, *A. striata* (Arias *et al*. 2003), *A. fraterculus* and *A. distincta* (Tigrero 2007); (2) in Colombia, from *A. fraterculus* and *A. striata* (Bueno *et al*. 2004) pupae; (3) in Bolivia, from *A. fraterculus* pupae (Ovruski *et al*. 2005); (4) in northwestern Argentina, from *A. fraterculus* pupae (Ovruski *et al*. 2005); and (5) in northern Brazil, as parasites of *Anastrepha atrigona* (Hendel) and *A. serpentina* larvae (Silva *et al*. 2011; Zucchi *et al*. 2011).

Opius hirtus has a more restricted distribution (Mexico, Costa Rica and the Dominican Republic) (Ovruski *et al*. 2000)

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doi:10.1111/aen.12146

and is apparently a more specialised parasitoid, with most of the specimens recovered in Mexico taken from *Anastrepha cordata* Aldrich, *Anastrepha alveata* Stone (Hernández-Ortiz *et al*. 1994; López *et al*. 1999) and the larvae of *A. obliqua* (Hernández-Ortiz *et al*. 1994; Sivinski *et al*. 2000). Moreover, *O. hirtus* has been recovered from *Toxotrypana curvicauda* Gerstaecker and from *Rhagoletis* spp. (Hernández-Ortiz 1993).

Contrary to the range of hosts previously described for both parasitoid species, preliminary results indicated that parasitoid adults did not emerge when either of these two species parasitised *A. obliqua* larvae under laboratory conditions (personal information). However, a different situation, with emergence of parasitoid adults, occurred when the attacked host was *A. ludens* (see Aluja *et al*. 2009; Cancino *et al*. 2009). These findings highlight the necessity of performing more detailed studies on the parasitoid–host relationship that each of these parasitoid species has established with the fruit fly species with which it coexists under natural conditions.

Similar results have been published by different authors, but with different species involved. Ramadan *et al*. (1994) observed that the parasitoid *Diachasmimorpha tryoni* (Cameron) could only emerge from *Bactrocera dorsalis* (Hendel) larvae when *Diachasmimorpha longicaudata* (Ashmead) attacked under multiparasitic conditions; Messing and Ramadan (2000) reported that *Diachasmimorpha kraussii* (Fullaway) did not develop in *B. dorsalis* and *Bactrocera cucurbitae* (Coquillett) larvae, and Ero *et al*. (2010) reported that this parasitoid did not develop in *Bactrocera cacuminata* (Hering) and *Bactrocera cucumis* (French) larvae. Behavioural ecology studies have become more relevant because they can reveal the mechanisms involved in parasitoid search and selection behaviour and the methods by which parasitoids use their hosts (Hassan 1994; Vinson 1998; Duan *et al*. 2000; Eben *et al*. 2000), especially for parasitoid species with the potential to be used as biological control agents.

Therefore, in this study we aimed to determine the host suitability of *A. obliqua* and *A. ludens* larvae for the development of native parasitoids *O. hirtus* and *D. crawfordi* because all these species occur sympatrically in the Neotropical region. We also aimed to elucidate if these parasitoid species show any host preference between the two types of larvae.

MATERIALS AND METHODS

Study site

The experiments were performed in the Biological Control Laboratory of the Moscafrut Program, SAGARPA-IICA (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación-Instituto Interamercano de Cooperación para la Agricultura), located on Metapa de Domínguez, Chiapas, Mexico, under controlled conditions with a temperature of 23 ± 1°C, a relative humidity of 70 ± 10% and a photoperiod of 12 h.

Biological material

The *A. obliqua* and *A. ludens* larvae were provided by the Moscafrut production facility where these species are mass produced at a rate of 65 and 130 million pupae every week, respectively, following the procedures reported by Artiaga-López *et al*. (2004) and Domínguez *et al*. (2010). The native parasitoids *D. crawfordi* and *O. hirtus* were provided by the Biological Control Laboratory, where they are reared on *A. ludens* larvae for more than 100 generations following the protocols reported by Cancino *et al*. (2009).

Determination of host suitability

This experiment was performed separately for each parasitoid species using Hawaii-type cages (27 × 27 × 27 cm; Wong & Ramadan 1992) in which 30 adult, sexually mature (5 days old) parasitoid couples were placed and fed with honey and water. Different numbers and proportions of *Anastrepha* larvae (*A. obliqua* at 8 days and *A. ludens* at 9 days of age) mixed with yeast and corn diet (see Domínguez *et al*. 2010) were exposed to the caged parasitoids after the larvae were placed in Petri dish lids (5.5 cm diameter and a depth of 0.9 cm for the *D. crawfordi* and 0.3 cm for the *O. hirtus* parasitoids) covered with a thin fabric. The exposure duration was 3 h. The treatments were as follows: (1) the exposure of 90 *A. obliqua* larvae and 90 *A. ludens* larvae in separated cages; (2) the exposure of 45 *A. obliqua* larvae and 45 *A. ludens* larvae inside the same cage but in different Petri dishes, which were rotated to avoid any positional bias; and (3) the exposure of 45 *A. obliqua* larvae and 45 *A. ludens* larvae mixed inside the same cage and in the same Petri dish. For each *Anastrepha* species, the control consisted of 90 larvae that were not exposed to parasitoids but were maintained under the same experimental conditions as the larvae in the treatments. Ten replicates were performed for each treatment.

To determine which larvae species experienced greater parasitism activity by the female parasitoids within the same experimental arena (treatment 2), 10 *Anastrepha* pupae of each species per replicate were collected 72 h after larval exposure. Using a stereoscopic microscope (Discovery V8, Carl Zeiss, Oberkochen, Germany), we obtained the number of oviposition scars (a dark and melanised point on the puparium) per pupa following the methods of Montoya *et al*. (2000, 2011), and dissect the pupae with fine point tweezers and entomological need to quantify the number of immature parasitoids (eggs and/or first instar larvae) inside the pupae.

The following parameters were determined: percentage of emerged and unemerged parasitoids, sexual proportion of adult parasitoids, percentage of dead *Anastrepha* larvae (72 h after exposure), percentage of 'dry' pupa (i.e. not containing a fly or parasitoid), and percentage of flies that were emerged and unemerged.

Statistical analysis

The effects of the treatments on the percentages of larvae that died, flies that emerged and pupae that dried up were analysed

using analysis of variance (ANOVA), and the means were compared using Tukey's test ($P \leq 0.05$). The relationship between the number of oviposition scars per pupa and the number of immature parasitoids per pupa was determined with a simple linear regression. The analyses were performed using the statistical software Statgraphics Centurion XV (Statgraphics 2008).

RESULTS

Host suitability

Opius hirtus

In all the treatments, adult parasitoids emerged from the *A. ludens* but not from the *A. obliqua* insects exposed to the parasites as larvae (Table 1).The sexual proportion favoured female parasitoids in all the treatments: 0.72 when the larvae were exposed in different cages (treatment 1), 0.63 when the larvae were exposed in different Petri dishes but in the same cage (treatment 2) and 0.79 when the larvae of both species were exposed in the same Petri dish (treatment 3). The percentages of unemerged formed flies in the dissected pupae were significantly higher for *A. obliqua* than for *A. ludens* in all the treatments (Table 1), with the following statistics for treatments 1, 2 and 3, respectively: $F_{1,18} = 12.38$, $P = 0.002$; $F_{1,18} = 24.22$, $P = 0.0001$; and $F_{1,18} = 9.74$, $P = 0.0059$. The percentages of emerged flies were also significantly higher for *A. obliqua* in all the treatments (Table 1): $F_{1,18} = 11.76$, $P = 0.003$; $F_{1,18} = 22.92$, $P = 0.0001$; and $F_{1,18} = 15.03$, $P = 0.081$ for treatments 1, 2 and 3, respectively. The percentages of dried up parasitoid pupae did not show any significant difference between the *Anastrepha* species for treatments 1 ($F_{1,18} = 1.181$, $P = 0.194$) and 2 ($F_{1,18} = 3.14$, $P = 0.081$) but did differ significantly for treatment 3 ($F_{1,18} = 18.44$, $P = 0.0004$) (Table 1).

Doryctobracon crawfordi

In all the treatments, the adult parasitoids emerged from the *A. ludens* but not the *A. obliqua* insects that were exposed to the parasites as larvae (Table 2). The sexual proportion slightly favoured male parasitoids in all the treatments: 0.47 for the larvae exposed in different cages (treatment 1), 0.41 for the larvae exposed in different Petri dishes but in the same cage (treatment 2) and 0.49 when the larvae of both species were exposed in the same Petri dish (treatment 3). The percentages of formed unemerged flies in the dissected pupae were significantly higher for *A. obliqua* than for *A. ludens* in all the treatments (Table 2), with the following statistics for treatments 1, 2 and 3, respectively: $F_{1,18} = 357.84$, $P < 0.0001$; $F_{1,18} = 29.01$, $P < 0.0001$; and $F_{1,18} = 26.51$, $P < 0.0001$. The percentages of emerged flies did not differ significantly between the *Anastrepha* species for treatment 1 ($F_{1,18} = 0.08$, $P = 0.7727$) but were significantly higher for *A. obliqua* in the other two treatments ($F_{1,18} = 4.44$, $P = 0.0493$ for treatment 2 and $F_{1,18} = 16.86$, $P = 0.0007$ for treatment 3) (Table 2). The percentages of dried up parasitoid pupae were significantly different between the *Anastrepha* species in treatment 1 ($F_{1,18} = 9.24$, $P = 0.0070$) and were similar in treatments 2 ($F_{1,18} = 0.05$, $P = 0.8134$) and 3 ($F_{1,18} = 0.16$, $P = 0.6913$) (Table 2). The percentages of dead larvae were similar for both species in all the treatments (Table 2).

Oviposition scars and immature parasitoids per pupa

Opius hirtus

A significant relationship was observed between the number of parasite oviposition scars on the *A. ludens* pupae and the number of immature parasitoids in the pupae ($P < 0.001$, $R^2 = 30.58$). However, the relationship between oviposition scar numbers and the number of immature parasitoids was not statistically significant for the *A. obliqua* pupae ($P = 0.94$,

Table 1 Average (±SD) percentages of emerged parasitoids, emerged flies and dead larvae; unemerged parasitoids, unemerged flies and dried parasitoid pupae of *Anastrepha obliqua* and *A. ludens* exposed as larvae to *Opius hirtus* parasitoids

Treatments	Emergence (%)		Dead larvae (%)	Pupae without emergence (%)		
	Parasitoids	Flies		Formed parasitoids	Formed flies	Dried up pupae (%)
Separate exposure						
A. obliqua	–	63.66 ± 2.74a	7.66 ± 1.31a	–	18.77 ± 4.18a	9.88 ± 1.94a
A. ludens	28.00 ± 3.99	43.00 ± 5.36b	9.00 ± 2.18a	2.44 ± 0.67	3.11 ± 1.51b	14.44 ± 2.76a
Different dishes in the same cage						
A. obliqua	–	63.23 ± 6.69a	8.53 ± 3.82a	–	24.31 ± 4.69a	3.91 ± 2.76a
A. ludens	50.09 ± 5.75	25.47 ± 4.16b	6.11 ± 2.74a	6.94 ± 0.94	1.09 ± 0.44b	10.22 ± 2.02a
Mixed inside the same dish						
A. obliqua	–	68.66 ± 7.03a	12.66 ± 3.64a	–	18.66 ± 5.23a	0.00 ± 0.00a
A. ludens	43.33 ± 6.30	30.66 ± 6.82b	5.33 ± 2.77a	7.33 ± 3.05	2.00 ± 1.01b	11.33 ± 2.53b
Control						
A. obliqua	–	91.33 ± 2.92	0.44 ± 0.18	–	7.11 ± 2.57	1.11 ± 0.52
A. ludens	–	95.57 ± 1.16	0.00 ± 0.00	–	4.42 ± 1.16	1.35 ± 0.23

Means within columns and larval exposure treatments followed by different letters are statistically different by analysis of variance (ANOVA) and Tukey tests ($P \leq 0.05$).

SD, standard deviation.

Table 2 Average (±SD) percentages of emerged parasitoids, emerged flies and dead larvae; unemerged parasitoids, unemerged flies and dried parasitoid pupae of *Anastrepha obliqua* and *A. ludens* exposed as larvae to *Doryctobracon crawfordi* parasitoids

Treatment	Emergence (%)		Dead larvae (%)	Pupae without emergence (%)		
	Parasitoids	Flies		Formed parasitoids	Formed flies	Dried up pupae (%)
Separate exposure						
A. obliqua	–	14.88 ± 2.05a	2.22 ± 1.49a	–	55.88 ± 2.78a	27.00 ± 3.05a
A. ludens	57.11 ± 5.23	16.00 ± 3.18a	2.44 ± 1.14a	6.00 ± 1.44	1.77 ± 0.64b	16.66 ± 1.48b
Different dishes in the same cage						
A. obliqua	–	23.32 ± 6.19a	7.88 ± 4.61a	–	52.09 ± 9.18a	16.69 ± 7.07a
A. ludens	58.89 ± 3.94	9.20 ± 2.55b	0.85 ± 0.43a	13.93 ± 3.97	2.27 ± 1.11b	14.89 ± 2.57a
Mixed inside the same dish						
A. obliqua	–	35.33 ± 4.77a	8.66 ± 4.10a	–	42.00 ± 7.63a	14.00 ± 9.29a
A. ludens	59.33 ± 7.27	8.00 ± 4.64b	2.00 ± 1.42a	10.66 ± 2.66	2.00 ± 1.42b	18.00 ± 3.44a
Control						
A. obliqua	–	91.33 ± 2.92	0.44 ± 0.18	–	7.11 ± 2.57	1.11 ± 0.52
A. ludens	–	95.57 ± 1.16	0.00 ± 0.00	–	4.42 ± 1.16	1.35 ± 0.23

Means within columns and larval exposure treatments followed by different letters are statistically different by analysis of variance (ANOVA) and Tukey tests ($P \leq 0.05$).

SD, standard deviation.

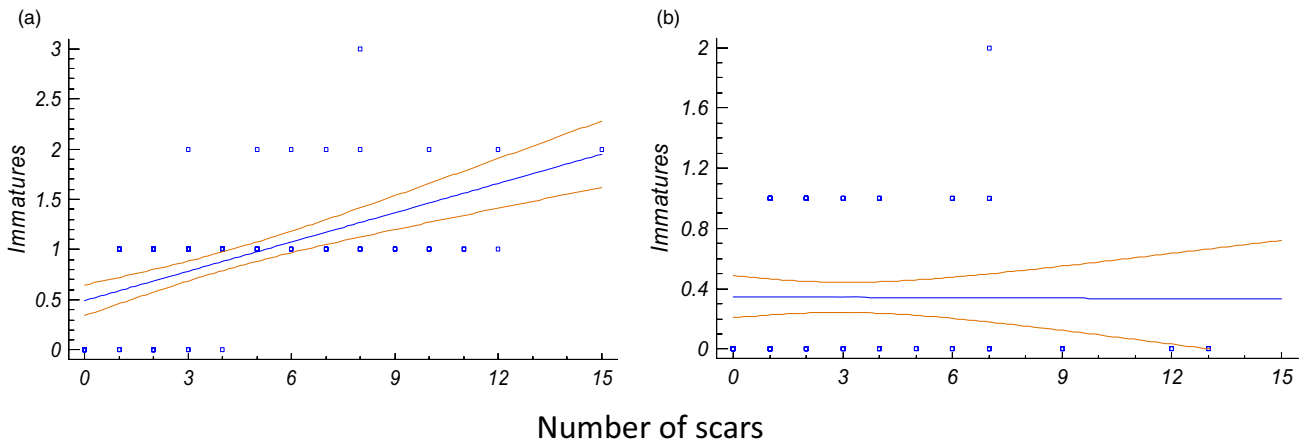

Fig. 1. Relationship between the number of scars and the number of immature *Opius hirtus* parasitoids per pupa of (a) *A. ludens* and (b) *A. obliqua* exposed to the parasitoids as larvae in different Petri dishes within the same cage.

$R^2 = 0.004$) (Fig. 1). Of all the *A. ludens* pupae examined, 92% showed scars, with a mean of 4.18 scars per pupa, and 80% carried first-instar larvae (98% viable and 2% not viable). For the *A. obliqua* pupae, 81% showed scars, with a mean of 3.11 scars per pupa, but only 34% carried immature parasitoids (68% encapsulated eggs and 32% melanised first-instar larvae) (Table 3).

Doryctobracon crawfordi

A significant relationship was observed between the number of parasite oviposition scars on the *A. ludens* pupae and the number of immature parasitoids inside the pupae ($P < 0.001$, $R^2 = 16.27$), but a similar relationship in *A. obliqua* pupae was not significant ($P = 0.08$, $R^2 = 2.95$) (Fig. 2). For the *A. ludens* pupae, 94% showed scars with a mean of 4.77 scars per pupa, and 83% carried first-instar larvae (98% viable and 2% not viable). In *A. obliqua*, 95% showed scars, with a mean of 4.85

Table 3 Average (±SD) percentage of *Anastrepha* pupae with scars, immature parasitoids and number of scars per pupa after exposure of *Anastrepha obliqua* and *A. ludens* larvae to adult parasitoids of *Opius hirtus* or *Doryctobracon crawfordi*

	A. obliqua	*A. ludens*
Opius hirtus		
Pupae with scars (%)	81.00 ± 6.22a	92.00 ± 2.19a
Pupae with immature stages (%)	34.00 ± 6.35a	80.00 ± 5.16b
Scars per pupae (≈)	3.11 ± 0.66a	4.18 ± 0.48a
Doryctobracon crawfordi		
Pupae with scars (%)	95.0 ± 2.23a	94.00 ± 2.21a
Pupae with immature stages (%)	35.00 ± 9.45a	83.00 ± 4.98b
Scars per pupae (≈)	4.85 ± 0.61a	4.77 ± 0.49a

Means within rows followed by different letters are significantly different by Tukey's test ($P \leq 0.05$).

SD, standard deviation.

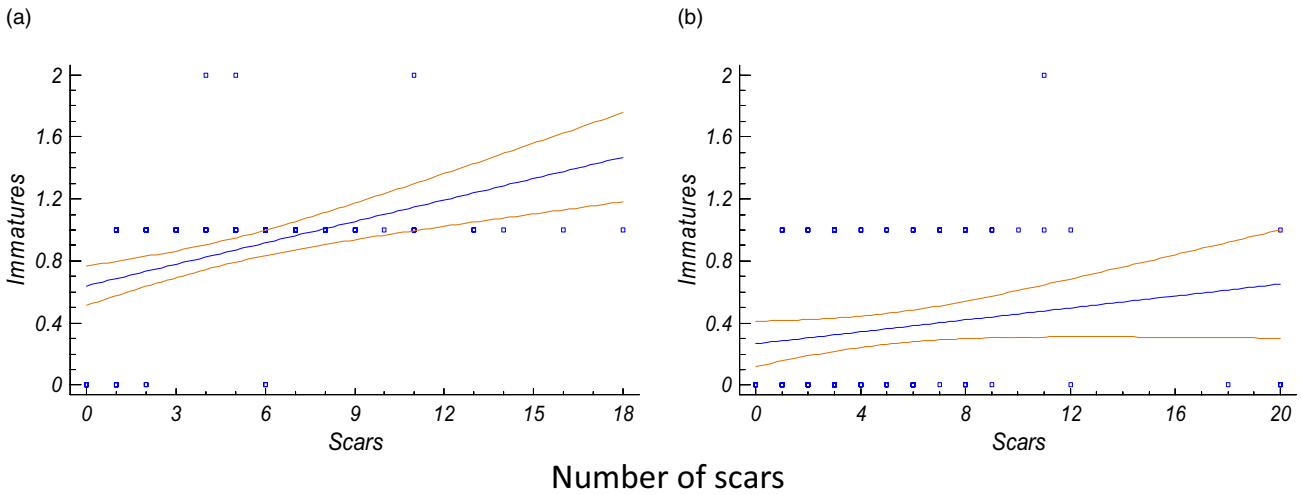

Fig. 2. Relationship between the number of scars and the number of immature *D. crawfordi* parasitoids per pupa of (a) *A. ludens* and (b) *A. obliqua* exposed to the parasitoids as larvae in different Petri dishes within the same cage.

scars per pupa, but only 35% had immature parasitoids (91% melanised first-instar larvae and 9% encapsulated eggs) (Table 3).

DISCUSSION

The results of this study indicate that the progeny of *O. hirtus* and *D. crawfordi* could not complete their development during the larva–pupa period of *A. obliqua*. Nevertheless, the female parasitoids of these species laid their eggs in the *A. obliqua* larvae at reduced rate compared with the eggs laid in the larvae of *A. ludens*. Several studies (e.g. van Alphen & Janssen 1982; van Alphen & Vet 1986; Mohamed *et al*. 2003) have indicate that braconid females only lay their eggs in suitable hosts and reject inappropriate ones, whereas other studies (e.g. Ramadan *et al*. 1994; Messing & Ramadan 2000; Ero *et al*. 2010) have shown that certain braconids also oviposited in unsuitable hosts.

When dissecting the *A. obliqua* pupa, we observed a high percentage of encapsulated and melanised eggs (68% for *O. hirtus* and 9% for *D. crawfordi*) and first-instar larvae that had hatched but died (32% for *O. hirtus* and 91% for *D. crawfordi*), which revealed the poor host suitability of this fruit fly species. Encapsulation is a typical immunological reaction of host insects in response to parasitoid attacks (Mohamed *et al*. 2003; Bokonon-Ganta *et al*. 2005). In this process, eggs are suffocated by the formation of a capsule from plasmocytes around foreign bodies, causing a hardened capsule (Bernal 2007; Carton *et al*. 2008). According to Ramadan *et al*. (1994), the eggs suffer encapsulation because the host is not a natural one, and therefore, an immune response is triggered in the presence of a component foreign to the host. We considered eggs with dark, thick walls to be encapsulated, as described by Ero *et al*. (2010), and dead first-instar larvae were occasionally readily visible inside these capsules.

The encapsulation and melanisation of the immature parasitoids could also be responsible for the large number of unemerged *A. obliqua* adults (or half-emerged) because these mechanisms require considerable energy to surround the invading organism with multiple layers of plasmocytes (Strand & Pech 1995). This process affects the normal development of metamorphosis and manifests as failure of those adults to emerge. Ramadan *et al*. (1994) also reported a significant number of unemerged flies because of the parasitism of *B. dorsalis* larvae by *D. tryoni*. In contrast, for *A. ludens*, in more than 80% of the pupae parasitised by either parasitoid species, 98% of the first-instar larvae were alive, and just 2% had died from unknown causes. According to Pemberton and Willard (1918), most eggs of fruit fly parasites hatch in suitable hosts, as occurred in the present study in the parasitised *A. ludens* larvae.

One of the questions we sought to answer was whether *O. hirtus* and *D. crawfordi* females showed a preference for or were capable of differentiating between *A. obliqua* and *A. ludens* larvae during egg laying because these species co-occur in the same habitat (Aluja *et al*. 1990; Montoya *et al*. 2000). In the present study, significant differences were not observed in the level of attack (i.e. the percentage of pupae with oviposition scars and the average number of scars per pupa) experienced by the larvae of the two species. However, in the case of the parasitised pupae (i.e. pupae with immature parasitoids inside), the situation was different, with a much lower percentage of *A. obliqua* than *A. ludens* parasitised. This result also suggests that *A. obliqua* larvae are not a suitable host for *O. hirtus* and *D. crawfordi* parasitoids, which was indicated by the high percentage of females of these parasitoids species who did not lay their eggs in most of the *A. obliqua* larvae (≈60%) into which they inserted their ovipositor. These results differ from those reported by Messing and Ramadan (2000) and Ero *et al*. (2010), who reported that *D. tryoni* and *D. kraussii* females, respectively, do not have the capacity to discriminate between suitable and unsuitable hosts

for the development of their progeny, finding extremely high percentages of egg encapsulation in unsuitable hosts.

Our results suggest that parasitoid females also showed good ability to discriminate among conspecifics when introducing their ovipositor into previously parasitised larvae. When attacking *A. ludens* larvae, only 9% of the larvae parasitised by *O. hirtus* and 3% of the larvae parasitised by *D. crawfordi* contained more than one parasitoid larva, even though these pupae showed more than one or two oviposition scar on their surface. These findings indicate that females did not choice to lay eggs in most already parasitised larvae by conspecifics. The above pattern was stronger in the case of *A. obliqua*. These observations are consistent with those reported for *D. crawfordi* by Ayala *et al*. (2014), who concluded that superparasitism is not an adaptive strategy in this species. The present observations are also partially consistent with those reported by Montoya *et al*. (2003), who observed an intermediate capacity of the braconid *D. longicaudata* to discriminate hosts previously attacked by conspecifics.

Our findings are inconsistent with those of a large number of field surveys (Aluja *et al*. 1990; Hernández-Ortiz *et al*. 1994; López *et al*. 1999; Sivinski *et al*. 2000; Arias *et al*. 2003) that reported the emergence of *O. hirtus* and *D. crawfordi* from *A. obliqua* pupae. Certain factors may explain this inconsistency. First, a previous or simultaneous attack on *A. obliqua* larvae by another parasitoid such as *D. longicaudata*, which has been reported to carry an entomopoxvirus that damages the immune system of the host, may promote the development of *O. hirtus* and *D. crawfordi*, as reported by Ramadan *et al*. (1994) for the emergence of *D. tryoni* from *B. dorsalis*. A second potential explanatory factor is a possible confusion regarding the host from which these parasitoids emerged because *A. ludens* and *A. obliqua* can simultaneously attack host fruits such as mango and emerge as adults from them (see Montoya *et al*. 2000; Díaz-Fleischer & Aluja 2003). To validate these potential explanatory factors, it is necessary to generate the appropriate empirical evidence because there are multiple factors in the field associated with the tritrophic relationship among host, herbivore and entomophage, creating relations that are not easy to elucidate (e.g. Vet & Dicke 1992).

Based on our results, we can conclude that the larvae of the studied fruit fly species showed contrasting suitability conditions for the development of *O. hirtus* and *D. crawfordi* immature stages, and that *A. obliqua* larvae do not represent a natural host for either parasitoid species. These findings may improve the use of these natural enemies in the development of biological control programs against economically important fruit flies of the *Anastrepha* genus.

ACKNOWLEDGEMENTS

We thank the Moscafrut production facility (SAGARPA-IICA) for providing the biological material for these studies. We also thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Coordination for the Improvement of Higher Education Personnel) (CAPES Brazil) for the Sandwich doctoral scholarship granted to S. P., and Conselho Nacional de Desenvolvimento Científico e Tecnológico (National Council for Scientific and Technological Development) (CNPq) for the research productivity grants to D. E. N.

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Accepted for publication 16 January 2015.