

# Best Host Age of *Anastrepha obliqua* (Diptera: Tephritidae) for Multiplication of Four Native Parasitoids from the Americas

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## Abstract

The success of the mass rearing of parasitoids is directly related to host quality, and it requires selecting the best biological host age to ensure the optimal performance of the parasitoids released into the field. The larval development of the parasitoids *Utetes anastrephae* (Viereck) (Hymenoptera: Braconidae) and *Odontosema anastrephae* Borgmeier (Hymenoptera: Eucilidae) and the pupal development of the parasitoids *Coptera haywardi* (Ogloblin) (Hymenoptera: Diapriidae) and *Dirhinus* sp. (Hymenoptera: Chalcididae) on the native host *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) in different larvae and pupae ages were investigated under laboratory conditions. Not all parasitoid species developed with the same efficiency in immature individuals of *A. obliqua*; *U. anastrephae* and *C. haywardi* showed the higher parasitism rates. The emergence and parasitism of *U. anastrephae* were equal using larvae from 5 to 8 d, while *C. haywardi* reared in 1- to 8-d-old pupae showed higher averages of parasitism. These results suggest that native parasitoids can be used to strengthen the implementation of biological control projects against *A. obliqua*, a pest of economic importance in South America.

**Key words:** host preference, *Utetes anastrephae*, *Odontosema anastrephae*, *Coptera haywardi*, *Dirhinus* sp.

*Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) is an economically fruit fly pest in the Americas (Hernández-Ortiz and Aluja 1993). Its native hosts are fruits of the genus *Spondias* spp. (Fam. Anacardiaceae) (Aluja and Birke 1993). The commercial importance of *A. obliqua* is related mainly to the damage caused to mango crops in Mexico and the tropical Americas (Peña et al. 2009).

Integrated management of this pest has effectively reduced its populations, and biological control can play an important part in this effort (Ovruski et al. 2000). The biological control of fruit flies has been based mainly on the augmentative release of parasitoids for short-term suppression of pest populations, which usually requires mass rearing of the host (Montoya et al. 2000, Ovruski et al. 2000).

Augmentative releases have been conducted with the exotic parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), a species that has acted effectively in the population control of flies of the genus *Anastrepha* (Sivinski et al. 1996, Montoya et al. 2000, Paranhos et al. 2003). However, in the Americas, a wide variety of native parasitoid species parasitoids may play an important role in the population control of *Anastrepha* spp.

In recent years, several studies have reported the use of different *Anastrepha* species as hosts for rearing their native hymenopteran parasitoids. For example, *Coptera haywardi* (Ogloblin) (Hymenoptera: Diapriidae), *Pachycrepoides vindemiae* (Rondani) (Hymenoptera: Pteromalidae) (Guillén et al. 2002), *Doryctobracon crawfordi* (Viereck) (Hymenoptera: Braconidae), *Utetes anastrephae* (Viereck) (Hymenoptera: Braconidae), *Opius hirtus* (Fischer) (Hymenoptera: Braconidae), *Aganaspis pelleranoi* (Brethes) (Hymenoptera: Figitidae), *Odontosema anastrephae* (Borgmeier) (Hymenoptera: Eucilidae), *Eurytoma sivinski* Gates and Grissell (Hymenoptera: Eurytomidae), and *Dirhinus* sp. (Hymenoptera: Chalcididae) have been reared in laboratory conditions using immature *Anastrepha ludens* (Loew) (Diptera: Tephritidae) (Cancino et al. 2009a). *C. haywardi*, *A. pelleranoi*, *Opius bellus* (Gahan) (Hymenoptera: Braconidae), and *D. crawfordi* were studied using *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) as a host (Núñez-Campero et al. 2012, Núñez-Campero et al. 2014, Schliserman et al. 2014, Gonçalves et al. 2016). Finally, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae) was studied as a host for rearing *Doryctobracon areolatus* (Szépligeti) (Hymenoptera:

Braconidae) (Eitam et al. 2003). All mentioned authors concluded that these neotropical parasitoids species listed above could be successfully reared on the evaluated *Anastrepha* species.

Native parasitoids of *Anastrepha* species with the potential for biological control include parasitoids attacking larvae or pupae. We chose for this study two larval parasitoid species and two pupal parasitoid species; thus, they may offer a complementary control once released in the field. Our larval species include *U. anastrephae*, which occurs from Florida (USA) to Argentina (Ovruski et al. 2000), which has a close relationship with *A. obliqua* (Sivinski et al. 2000, Silva et al. 2010); and *O. anastrephae*, which in South America preferentially oviposits in larvae of *Anastrepha striata* Schiner (Diptera: Tephritidae) and *A. fraterculus* by direct contact using holes in fallen fruits (Ovruski 1994, Sivinski et al. 1997, Ovruski 2004). These species are solitary koinobionte, larval-pupal endoparasitoids that oviposit in the host larvae and emerge as adults from the puparium of members of Tephritidae. The pupal parasitoid species include the solitary pupal endoparasitoid *C. haywardi* that is considered as a specific *Anastrepha* pupal parasitoid (Sivinski et al. 1998). It occurs in countries such as Argentina (Loiacono 1981), Mexico (López et al. 1999), Venezuela (García and Montilla 2001), and Brazil (Aguiar-Menezes et al. 2003), parasitizing the pupae of *A. fraterculus*, *A. ludens*, *A. striata*, *Anastrepha schultzi* Blanchard (Diptera: Tephritidae), *Anastrepha serpentina* (Wiedemann) (Diptera: Tephritidae), and *Anastrepha sororcula* Zucchi (Diptera: Tephritidae). We also chose a solitary pupal ectoparasitoid (*Dirhinus* sp.), which was collected from *A. ludens* in Chiapas, Mexico (Figueroa 1998). With the exception of *D. guiffardii* Silvestri (Ovruski et al. 2000, Wang and Messing 2004a), there is no other *Dirhinus* spp. reported as natural enemies of fruit flies, and because it is considered a generalist parasitoid, its potential to be used as a biological control agent for the pupae of *A. obliqua* is unknown.

In view of the growing interest in biological control against *Anastrepha* species of economic importance in Latin America, we evaluated larvae and pupa of *A. obliqua* at different ages for the laboratory rearing of four native parasitoid species described above. We aimed to identify the host age preference of each host stage as well to analyze the host suitability of most promising parasitoid species to be used as an augmentative biocontrol agent against *A. obliqua* in South America. The importance of this study lies to the convenience in mass rearing projects of using older hosts that facilitate the rearing process, then providing a better support to augmentative releases in the field.

## Materials and Methods

### Study Site

The experiments were conducted in the Biological Control Laboratory of the Moscafrut Program, SAGARPA-IICA, located in Metapa de Domínguez, Chiapas, Mexico. The assessments were performed at  $23 \pm 1^\circ\text{C}$ , a relative humidity (RH) of  $70 \pm 10\%$  and a photophase of 12 h.

### Biological Material

The larvae and pupae of *A. obliqua* were provided by the Moscafrut plant, where this species is mass reared at 65 million pupae/wk, as described by Artiaga-López et al. (2004) and Domínguez et al. (2010).

We evaluated the native third-instar larval parasitoids *U. anastrephae* and *O. anastrephae*, and the pupal parasitoids *C. haywardi* and *Dirhinus* sp., which were obtained from the Biological

Control Laboratory of the Moscafrut Program and reared according to Cancino et al. (2009b) and Aluja et al. (2009). The molecular identification of *Dirhinus* sp. was performed through the sequencing of the genes 28S, 18S, and COI. The sequences were deposited in the GenBank with the respective access numbers: KX255639, KX255638, and KX255637. Voucher specimens of *Dirhinus* were deposited in the Entomological Collection of Department of Biological Science of the Federal University of Espírito Santo.

### Host-Larva Age Preference by *U. anastrephae* and *O. anastrephae*

Thirty mating couples of sexually mature adults (5-d old) of *U. anastrephae* (Aluja et al. 2009) were placed in individual Hawaii-type cages (wooden structure of  $30 \times 30 \times 30$  cm cover with 0.5-mm mesh with an elliptic hole of  $20 \times 10$  cm covered with organza cloth in the bottom) (Wong and Ramadan 1992). The insects were fed with honey and water. Late-second- and third-instar larvae of *A. obliqua* aged 5, 6, 7, and 8 d were simultaneously exposed during 2 h in a free choice to the females described above. Each host age was exposed in a parasitism unit type Petri dish (base of a Petri dish 10 cm and 0.25-cm deep, containing larvae mixed with diet, and covered with a piece of fine mesh secured with a rubber band). A sample of 30 larvae by age was simultaneously exposed to the parasitoids. For *O. anastrephae*, we used 5-d-old females (Aluja et al. 2009) applying the same procedures but changing the parasitism unit because the larvae mixed with diet were exposed on an open Petri dish lid of 1-cm deep without fine mesh for a period of 2 h. Following exposure, the host larvae from different ages were kept in separated cylindrical plastic containers ( $7 \times 5$  cm) with the diet until they were 9-d old, after which they were washed and placed with vermiculite in the same container. Seventy-two hours after exposure, the mortality rate of the larvae was recorded. The parasitized hosts were remained for 15 d at  $26^\circ\text{C}$ . When the adults emerged, we calculated the percentage of adult emergence (flies + parasitoids  $\times$  100/No. host exposed), parasitism percentage (No. parasitoids  $\times$  100/No. parasitoids + flies), and the sex ratio (No. females/No. males). Ten replications were performed.

### Host-Pupa Age Preference by *C. haywardi* and *Dirhinus* sp.

Fifty mating pairs of sexually mature adults (5-d old) of each parasitoid species (Aluja et al. 2009, Cancino et al. 2009a, López-Arriaga et al. 2014) were placed in alm-frame cages ( $20 \times 20 \times 20$  cm), where they were fed honey and water. Pupae of *A. obliqua* of four age ranges were exposed to the parasitoid (range 1: 1–4 d; range 2: 5–8 d; range 3: 9–11 d; and range 4: pupae older than 12 d). One Petri dish (9 cm and 1.5-cm deep) per age range was used for each parasitoid species. In total, four Petri dishes, each with 25 pupae and vermiculite as the soil substrate, were exposed. The Petri dishes were covered with a layer of black cardboard (10 cm in diameter) to simulate dark conditions (as in Cancino et al. 2012). After a 24-h exposure period, the host pupae were kept in containers with vermiculite at  $26^\circ\text{C}$  for a period of 30 d. As in the previous test, the emergence and parasitism rates as well as the sex ratio were calculated based on the emergence of adults. Ten replications were performed.

### Fitness Tests

Based on the preliminary results (i.e., percentages of parasitism, Tables 1 and 2), *U. anastrephae* and *C. haywardi* were selected as the species with the best potential for use in mass rearing the larvae and pupae of *A. obliqua*, respectively. In the second phase of the study, the

**Table 1.** Mean rates ( $\pm$ SE) of parasitism, sex ratio, emergence, and larval mortality of *U. anastrephae* and *O. anastrephae* from larvae of *A. obliqua* parasitized at 5, 6, 7, and 8 d of development in a choice test, by each parasitoid species

Parameters	Age of host larvae (d)			
	5	6	7	8
<i>U. anastrephae</i>				
Parasitism (%)	81.43 $\pm$ 6.24a	81.53 $\pm$ 5.20a	87.11 $\pm$ 3.95a	72.03 $\pm$ 8.42a
Sex ratio	0.78 $\pm$ 0.03a	0.70 $\pm$ 0.05a	0.65 $\pm$ 0.06a	0.68 $\pm$ 0.07a
Emergence (%)	50.66 $\pm$ 6.79a	49.00 $\pm$ 6.49a	39.00 $\pm$ 3.44a	34.66 $\pm$ 2.81a
Larvae mortality (%)	4.58 $\pm$ 1.34a	2.96 $\pm$ 1.62a	3.03 $\pm$ 1.40a	7.18 $\pm$ 2.23a
<i>O. anastrephae</i>				
Parasitism (%)	29.35 $\pm$ 10.81a	30.25 $\pm$ 9.54a	11.31 $\pm$ 5.57a	11.85 $\pm$ 9.84a
Sex ratio	0.95 $\pm$ 0.02a	0.93 $\pm$ 0.02a	0.94 $\pm$ 0.02a	0.89 $\pm$ 0.05a
Emergence (%)	74.66 $\pm$ 5.37b	83.00 $\pm$ 3.63ab	89.66 $\pm$ 2.78a	90.00 $\pm$ 2.98a
Larvae mortality (%)	5.10 $\pm$ 1.61a	0.96 $\pm$ 0.49a	1.27 $\pm$ 0.69a	1.61 $\pm$ 0.53a

Temperature of  $23 \pm 1^\circ\text{C}$ , RH  $70 \pm 10\%$ , and a photoperiod of 12 h. Means followed by the same lowercase letter in the row not differ significantly. Tukey test ( $P \leq 0.05$ ).

fitness of the emerged parasitoids was assessed through the quality-control parameters of flight ability and survival without water and food, as in Cancino et al. (2009b) and Poncio et al. (2016).

**Flight ability of *U. anastrephae*:** Six samples of 50 pupae aged 14 d, obtained from each age of *A. obliqua* larvae tested previously, were placed each one in a black PVC tube (10-cm internal diameter and 10-cm high), which were then placed in a cage (1.20  $\times$  0.4  $\times$  0.5 m) covered with tulle mesh. The inner walls of the tubes were covered with neutral talc to prevent adults from escaping by climbing (as in FAO/IAEA/USDA 2014). Five days after emergence, any adults remaining in the tube were considered nonflying, and the number and percentage of flying adults were obtained by assessing the proportion of nonflying to total adults. Each sample was considered a replica of this test. In the case of *C. haywardi*, the same procedure was followed. Given the long immature development of this species, we used pupae 28 d after exposure, close to adult emergence, and adults in the tube were considered as nonflying 5 d later.

**Survival of parasitoids:** Random samples of 50 newly emerged parasitoids of each sex from each treatment were taken and placed in a Hawaii-type cage (30  $\times$  30  $\times$  30 cm) without water or food. The dead males and females were counted each day. The assessment period continued until all parasitoids were dead.

### Statistical Analysis

First, the data were analyzed for normality using the Shapiro–Wilk test, and to analyze unequal variances, the Bartlett test was applied.

Due problems with data normal distribution, we used a generalized lineal mixed model, considering the multichoice option design (Pimentel-Gomes 2009). The proportion of females and index of sex ratio were analyzed by nonparametric Wilcoxon test. Curves for adult survival were calculated by the Kaplan–Meier method. Pairwise tests were performed with the log-rank test (Francis et al. 1993), and an overall  $\alpha = 0.05$  was maintained applying the modified Bonferroni procedure.

## Results

### Host Preference for Age of Larvae

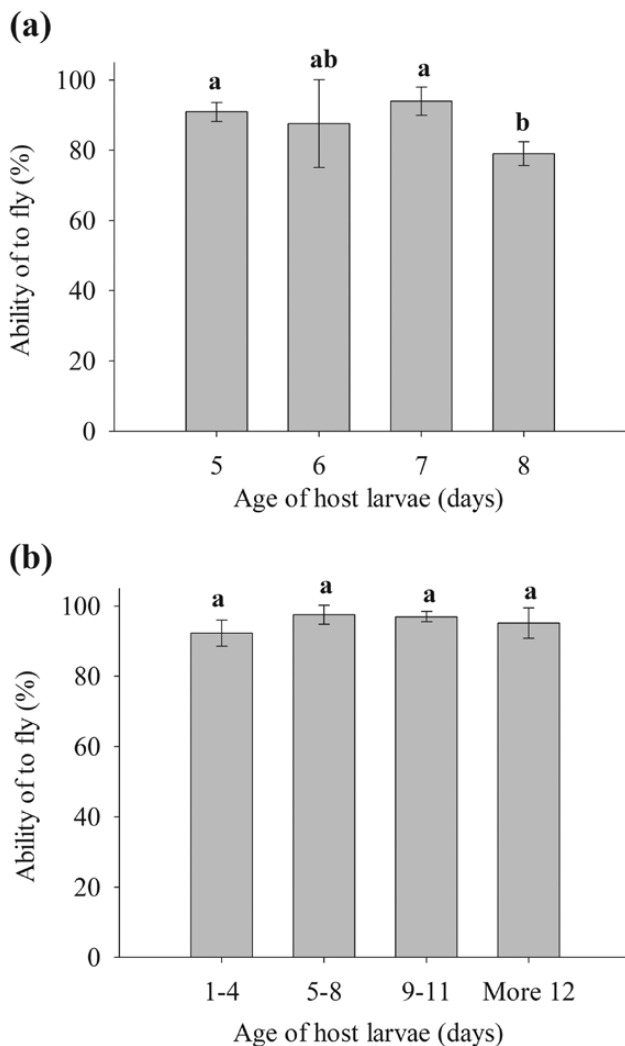
The parasitism rate for *U. anastrephae* did not differ among the ages of offered *A. obliqua* larvae ( $\chi^2 = 3.27$ ,  $df = 3$ ,  $P = 0.35$ ; Table 1). The sex ratio of *U. anastrephae* did not change with the different host ages ( $\chi^2 = 3.02$ ,  $df = 3$ ,  $P = 0.38$ ; Table 1). Similarly, there was no difference in the emergence of adults (flies + parasitoids) from larvae of different ages ( $\chi^2 = 6.77$ ,  $df = 3$ ,  $P = 0.07$ ; Table 1). The mortality rate of the larvae showed no difference among the different ages of larvae ( $\chi^2 = 4.30$ ,  $df = 3$ ,  $P = 0.23$ ; Table 1).

*O. anastrephae* also showed no differences in parasitism rates ( $\chi^2 = 4.30$ ,  $df = 3$ ,  $P = 0.23$ ) and sex ratio ( $\chi^2 = 0.76$ ,  $df = 3$ ,  $P = 0.85$ ) for the different ages of host larvae (Table 1). The adult emergence (flies + parasitoids) was higher for older (7 and 8 d) larvae ( $\chi^2 = 10.32$ ,  $df = 3$ ,  $P = 0.01$ ; Table 1). The larval mortality rate was significantly different among ages ( $\chi^2 = 11.77$ ,  $df = 3$ ,  $P = 0.01$ ; Table 1).

**Table 2.** Mean rates ( $\pm$ SE) of parasitism, sex ratio, and emergence of *C. haywardi* and *Dirhinus* sp. from pupae of *A. obliqua* parasitized in the age ranges of 1–4, 5–8, 9–11, and older than 12 d in a choice test, by each parasitoid species

Parameters	Age of host pupae (d)			
	1–4	5–8	9–11	+12
<i>C. haywardi</i>				
Parasitism (%)	67.12 $\pm$ 8.58a	63.43 $\pm$ 8.20a	63.56 $\pm$ 8.60a	31.74 $\pm$ 6.25b
Sex ratio	0.52 $\pm$ 0.07b	0.53 $\pm$ 0.07ab	0.58 $\pm$ 0.07ab	0.79 $\pm$ 0.05a
Emergence (%)	84.40 $\pm$ 5.91a	83.60 $\pm$ 5.76a	81.60 $\pm$ 4.30a	68.00 $\pm$ 7.27a
<i>Dirhinus</i> sp.				
Parasitism (%)	20.38 $\pm$ 4.69a	31.09 $\pm$ 6.41a	22.93 $\pm$ 7.03a	31.52 $\pm$ 7.78a
Sex ratio	0.57 $\pm$ 0.10a	0.53 $\pm$ 0.07a	0.59 $\pm$ 0.07a	0.62 $\pm$ 0.08a
Emergence (%)	87.60 $\pm$ 3.06ab	94.40 $\pm$ 1.06a	91.20 $\pm$ 2.58ab	80.40 $\pm$ 3.44b

Temperature of  $23 \pm 1^\circ\text{C}$ , RH  $70 \pm 10\%$  and a photoperiod of 12 h. Means followed by the same lowercase letter in the row not differ significantly. Tukey test ( $P \leq 0.05$ ).



**Fig. 1.** Flying ability of *U. anastrephae* (a) and *C. haywardi* (b) in hosts of different ages. Temperature of  $23 \pm 1^\circ\text{C}$ , RH  $70 \pm 10\%$ , and a photoperiod of 12 h. Different letters above the bars indicate significant difference among treatments, following a GLMM.

The average parasitism on hosts of all ages showed a high difference between both parasitoid species ( $F_{1,78} = 103.32$ ,  $P < 0.0001$ ; Table 1).

### Host Preference for Pupae Age

*C. haywardi* showed differences in parasitism rate among different host-pupae ages ( $\chi^2 = 12.28$ ,  $df = 3$ ,  $P = 0.01$ ); the oldest pupae (+12 d) had lower parasitism rates (Table 2). The female ratio was also higher in parasitoids emerging from pupae older than 12 d ( $\chi^2 = 8.81$ ,  $df = 3$ ,  $P = 0.03$ ; Table 2). The emergence rate (flies + parasitoids) was not different among pupae of different ages ( $\chi^2 = 5.29$ ,  $df = 3$ ,  $P = 0.15$ ;  $F_{3,36} = 1.79$ ,  $P = 0.16$ ; Table 2).

The parasitism rate of *Dirhinus* sp. ( $\chi^2 = 2.40$ ,  $df = 3$ ,  $P = 0.49$ ) and the sex ratio ( $\chi^2 = 0.59$ ,  $df = 3$ ,  $P = 0.89$ ) were not affected by the pupae age (Table 2). Among the different pupa ages, the emergence rate was different ( $\chi^2 = 13.85$ ,  $df = 3$ ,  $P = 0.01$ ); older pupae (+12 d) had a lower emergence rate (Table 2). The average parasitism of both pupal parasitoid species on all host ages showed a highly significant difference ( $F_{1,78} = 27.74$ ,  $P < 0.0001$ ; Table 2).

### Fitness Analyses

However, the ability of *U. anastrephae* to fly was high; this attribute was affected by the age at which the host larvae was parasitized ( $\chi^2 = 13.83$ ,  $df = 3$ ,  $P = 0.003$ ; Fig. 1a). The lowest percentage of parasitoids emerging from larvae parasitized at 8-d old was able to fly (= 79.03%). In the case of *C. haywardi*, the flight ability was not affected by the age of parasitized pupae ( $\chi^2 = 9.52$ ,  $df = 3$ ,  $P = 0.05$ ; Fig. 1b). All treatments produced a high proportion of adults able to fly (>90%).

Survival of *U. anastrephae* adults: There was a difference in the survival rates of the females and males that emerged from larvae parasitized at different ages. Males emerging from 6-d-old parasitized larvae showed the highest survival ( $\chi^2 = 75.74$ ,  $df = 3$ ,  $P \leq 0.0001$ ; Fig. 2a), but in the case of females the highest survival was obtained from larvae parasitized at ages of 6 and 7 d ( $\chi^2 = 203.31$ ,  $df = 3$ ,  $P \leq 0.0001$ ; Fig. 2b).

Survival of *C. haywardi* adults: There was a difference in the survival rates of the females ( $\chi^2 = 167.69$ ,  $df = 3$ ,  $P \leq 0.0001$ ) and males ( $\chi^2 = 149.84$ ,  $df = 3$ ,  $P \leq 0.0001$ ) that emerged from pupae parasitized at different ages. Males emerged from older pupae were significantly less long lived (Fig. 3a). Females emerging from 2-d-old pupae had the highest survival rate (Fig. 3b), but there were not statistical differences in the survival of males emerging from 2-, 6-, and 9-d-old pupa.

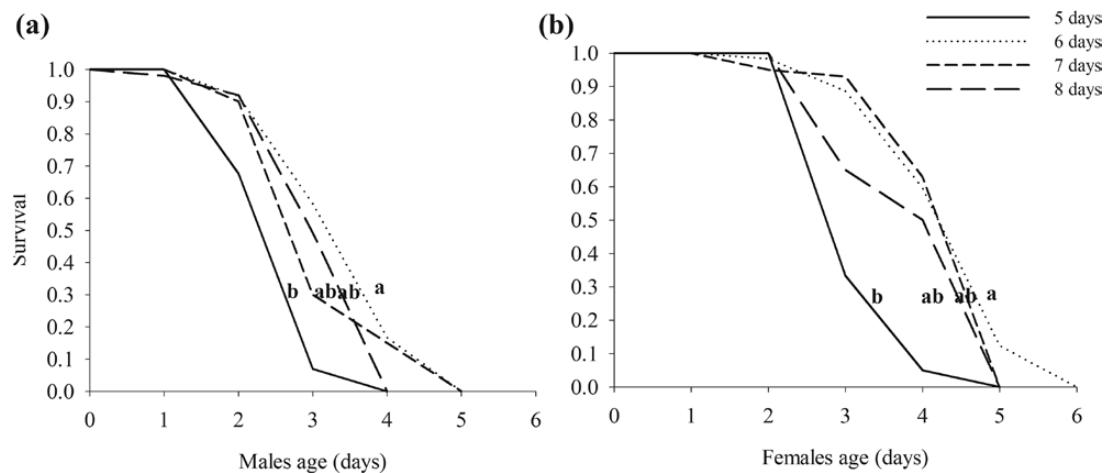
### Discussion

Not all species of parasitoids evaluated developed efficiently in immature *A. obliqua*; *U. anastrephae*, and *C. haywardi* showed the greatest potential to be reared in the larvae and pupae of *A. obliqua*, respectively. The low parasitism percentages by *O. odontosema* and *Dirhinus* sp., attacking *A. obliqua* hosts, may be attributed to the more generalist nature of these parasitoid species (Copeland et al. 2010, Wang and Messing 2004b).

Several factors, as optimal age and host size, have high influence on the quantity and quality of viable adults obtained under mass rearing conditions (Lawrence et al. 1976, López et al. 2009, Ueno 2015), and host quality (represented by the size and age of the host) is one of the most important (Cancino et al. 2009b, Poncio et al. 2016). Ravensberg (1992) stated that the ultimate objective of all rearing systems of natural enemies is to produce the largest number of organisms with an optimal quality level.

Although it is known that host preference by parasitoid species is defined genetically (Singer et al. 1992), under a mass rearing situation, different intraspecific interactions (i.e., extrinsic and intrinsic competition) may occur and influence the results and quality of the produced parasitoids (Canale and Benelli 2012, Queiroz et al. 2017). For this reason, under this scenario the host preference by parasitoid species must be properly analyzed, looking for those ages that better render higher levels of parasitism, adult emergence, and a female biased sex ratio (Li et al. 2006, Iranipour et al. 2010).

*U. anastrephae* appears not to be affected by host age because no significant differences in the studied parameters among treatments were found (see Table 1). However, some authors have noted that in young hosts, the defence mechanisms may still be incomplete (Gerlin and Rejouan 2004), making them more vulnerable. For example, Lawrence et al. (1976) found that younger larvae of *A. suspensa* were better for *D. longicaudata* rearing. In further studies, Lawrence and Akin (1990) found that the hormonal profile in older hosts was antagonistic to the development of *D. longicaudata*. The wide



**Fig. 2.** Survival of *U. anastrepha* males (a) and females (b) emerging from pupae that had its *A. obliqua* larvae parasitized at 5, 6, 7, and 8 d of development. Temperature of  $23 \pm 1^\circ\text{C}$ , RH  $70 \pm 10\%$ , and a photoperiod of 12 h.

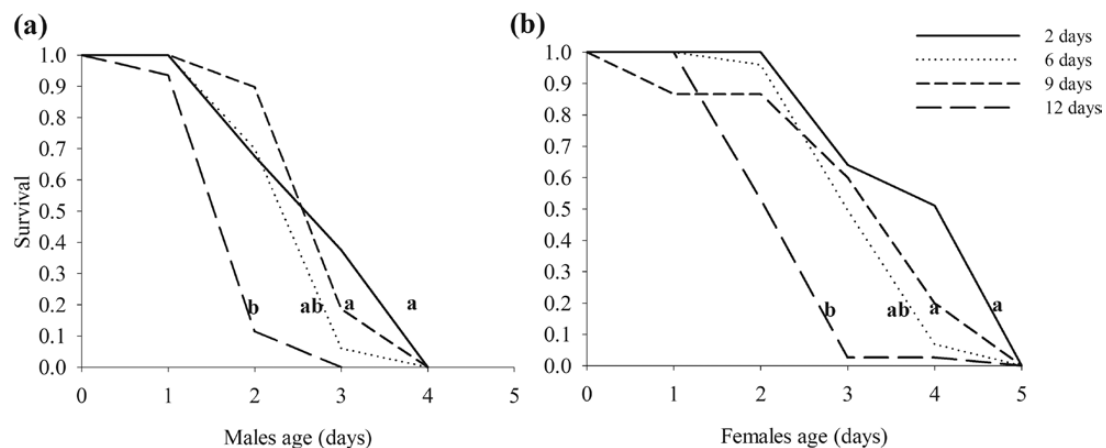
period of *A. obliqua* larvae preference as host by *U. anastrepha* could be consequence of one strong native association between both species (Vinson and Iwantsch 1980, Le Ralec et al. 2011), mainly when *A. obliqua* infests *Spondias* spp. where is highly parasitized by *U. anastrepha* (Serra et al. 2011, Jesus-Barro et al. 2012).

In *C. haywardi*, the age range of the host that enabled rearing in laboratory was wider because the percentage of parasitism was negatively affected only in older pupae (12-d or older). The reduced preference of older hosts may be due to physiological unsuitability of the host as the fly adults approach emergence (Aluja et al. 2009, López et al. 2014). A possible reason for the wide age range of the pupae susceptible to attack may be the more generalist nature of pupal parasitoids, which can attack hosts with more varying physiological conditions (Dressner 1954, Wang and Messing 2004c). Our results diverge from those presented by Aluja et al. (2009), who reported that the best development of *C. haywardi* occurs in younger pupae (0–2 d) of *A. ludens*. Meanwhile, our data suggest that the rearing of *C. haywardi* in the pupae of *A. obliqua* is probably more successful, because it does not depend on a limited age range of pupae.

The relationship between the host age and development of immature individuals is less clear for *Dirhinus* sp., where the parasitism rate remained homogeneous at all ages of pupae evaluated; however, it had lower parasitism rates than observed for *C. haywardi*. The

ectoparasitic nature of the genus *Dirhinus* may account for the lower requirement for a more specific age range for parasitism (Dressner 1954, Sivinski et al. 1998). Although *A. obliqua* can be considered as a low suitable host for *Dirhinus*, there is also evidence that *A. obliqua* exerts a high level of antagonism for the immature parasitoid development of other species (Silva et al. 2002, Poncio et al. 2015). Higher percent of *Dirhinus* parasitism (>30%) have been obtained with *A. ludens* as host (Cancino et al. 2009a,b).

The flying ability of *U. anastrepha* adults was also affected by host age. Fewer *U. anastrepha* adults emerging from 8-d-old larvae were able to fly, possibly to poorer host suitability due to increasing immunity condition in older *A. obliqua* larvae (Silva et al. 2002). The ability of an insect to fly is closely related to its acquisition of nutrients during growth, which in the case of parasitoids are provided exclusively by their host (Visser and Ellers 2008, Fischbein et al. 2011). Silva et al. (2002) reported significant changes in the composition of larvae of *A. obliqua* by age. Dissected pupae coming from old parasitized larvae showed higher concentrations of melamine, which is characterized as an immune antagonist reaction to the presence of immature parasitoids. These factors are probably related to the decrease in the ability of *U. anastrepha* that emerged from 8-d-old larvae to fly. Similar impairment has been observed in other parasitoid species, for example, parasitoids of the genus *Trichogramma* (Hymenoptera:



**Fig. 3.** Survival of *Coptera haywardi* males (a) and females (b) emerging from *Anastrepha obliqua* pupae parasitized at 1–4, 5–8, 9–11, and >12 d of development. Temperature of  $23 \pm 1^\circ\text{C}$ , RH  $70 \pm 10\%$ , and a photoperiod of 12 h.

Trichogrammatidae), which do not develop properly when older eggs are used as hosts (Vinson 1994, 1997).

Another parameter affected by host age was the survival of adults. Males and females of *U. anastrephae* that emerged from 5-d-old larvae lived a shorter period; similar results were reported for *D. longicaudata* with young larvae of *A. suspensa* (Lawrence et al. 1976). These results can be related to the quantity and quality of food resources provide by the young larvae (López et al. 2009, Cicero et al. 2012), which could affect the adult survival of this species. In the case of *C. haywardi*, adult survival was reduced in individuals emerging from 12-d-old pupa, which is congruent with that reported by López-Arriaga et al. (2014) using *A. ludens* pupae as host. According to Cancino et al. (2006), survival is directly related to the quality of the host, which must offer adequate nutritional conditions.

In the mass rearing of parasitoids, the main parameters requiring evaluation are the adult emergence and sex ratio, which reflect the development conditions of immature individuals (Cancino et al. 2006). High parasitism rates can be harmful to young hosts, and they are reflected in lower adult recovery. The low defence capacity of young hosts is an important reason for preferring them in mass rearing projects (Godfray 1994). However, it is always advisable to determine the most appropriate balance between host age and susceptibility to be parasitized to reduce host mortality and ensure high emergence rates.

Our findings showed that 1) the parasitoids *U. anastrephae* and *C. haywardi* have a potential for mass rearing in the larvae and pupae of *A. obliqua*, respectively; 2) *U. anastrephae* appears to have a wider host age preference, but survival and flight ability were higher in adults emerging from 6- to 8-d-old hosts; 3) according to adult survival, 1- to 8-d-old pupae of *A. obliqua* seem to be the most suitable hosts for rearing *C. haywardi*. This basic biological information is an important initial step for the establishment of the mass rearing of the selected parasitoid species with highest rearing potential, as determined in this study. This information will strengthen the prospects of using biological control against the fruit fly genus *Anastrepha*, especially *A. obliqua*, a major economic pest in South America.

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