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Determining the Functional Response and Mutual Interference of *Utetes anastrephae* (Hymenoptera: Braconidae) on *Anastrepha obliqua* (Diptera: Tephritidae) Larvae for Mass Rearing Purposes

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Abstract

Although functional response and mutual interference studies may be of great importance to augmentative biological control projects focussed on pests of economic importance, such studies have traditionally been overlooked in mass rearing projects of natural enemies. In this study, we aimed to explore both interactions in *Utetes anastrephae* (Viereck), a native parasitoid commonly associated with *Anastrepha obliqua* (Macquart), a major fruit fly pest of *Spondias* spp. and *Mangifera indica* L. in the Americas, for mass rearing purposes. The functional response of *U. anastrephae* on *A. obliqua* larvae fit a type II model according to Holling's disc equation, with an instantaneous rate of discovery (a') of 0.470 and a handling time (Th) of 0.099. The effects of mutual interference were more severe at high parasitoid densities due to the higher frequency of interactions. An increasing number of foraging females resulted in an increasing number of oviposition scars and immature parasitoids per pupa without significant differences in the total number of attacked larvae. Superparasitism at high densities of foraging females had a negative effect on adult emergence, was associated with a female-biased sex ratio, and had no effect on survival or flight ability parameters. Strategies for optimizing the mass rearing of this parasitoid species are outlined.

Key words: mass rearing, Braconidae, augmentative biological control, fruit fly, superparasitism

Augmentative biological control (ABC) refers to the rearing and release of a large number of natural enemies to reduce pest populations in the short term (Knipling 1992). In the case of fruit flies, control has been accomplished primarily through the use of exotic braconid parasitoids, such as *Diachasmimorpha tryoni* (Cameron) (Wong et al. 1991, 1992) and *Diachasmimorpha longicaudata* (Ashmead) (Sivinski et al. 1996, Montoya et al. 2000a), for which significant suppression of pest populations has been reported in Hawaii and Florida, USA, and Chiapas, Mexico, respectively. However, several authors (Ovruski et al. 2000, Aluja et al. 2008) have recommended native parasitoid species, because they share an evolutionary history with the target pests in their regions of origin (see Stiling 1987).

There are few species of parasitoids available for release purposes. In addition, the mass rearing of natural enemies is associated with several problems and limitations that need to be addressed. One such limitation is a susceptibility to male-biased sex ratios under artificial rearing conditions, which may be caused by poor host quality and/or high parasitoid:host ratios (Waage et al. 1985). Indeed, the relative abundance of males and females commonly fluctuates when parasitic Hymenoptera are propagated for several generations in closed laboratory systems (Wylie 1979), and knowledge of which factors influence the sex ratio is important for successfully rearing parasitoids (King 1993, Heimpel and Lundgren 2000, Ode and Heinz 2002, Ode and Hardy 2008).

A high parasitoid:host ratio leads to mutual interference, which results from encounters with conspecifics while searching for hosts and usually leads to a reduced search efficiency (Hassell 1978, Visser et al. 1999, Merkel 2014). Hassell and Varley (1969) noted that parasitoid searching efficiency showed an inverse relation to the density of searching parasitoids, which is known as mutual interference (Skovgård and Nachman 2015). This relationship is to be expected because at high parasitoid densities, individual parasitoids will use an increasing proportion of their searching time to

encounter other conspecifics (Fathipour et al. 2006). The effects of mutual interference are more severe at high parasitoid densities, a typical situation under mass rearing conditions.

Parasitoids of many species respond to mutual interference by fleeing the experimental arena (Wajnberg et al. 2004); others defend their host resources (Lawrence 1981, Field and Calbert 1998, Goubault et al. 2005) or increase searching and oviposition behaviors (Montoya et al. 2000b). Intraspecific competition is a potential factor (among others) that can drive density-dependent responses in host–parasitoid systems.

The functional response of a natural enemy describes per capita attack as function of prey or host density (Solomon 1949). Functional response can be used to assess the impact of a natural enemy on a host population (Lester and Harmsen 2002), and to infer whether a natural enemy is able to regulate the density of its host when the response depends on density (Murdoch and Oaten 1975). Three basic types of functional response have been described (Holling 1959) although other authors as Fujii et al. (1986) include a type IV. In the type I, the attack rate describes a linear relation between prey density and the number of prey consumed until satiation is reached (Hassell 1978). Type II response incorporate handling time of hosts and the relationship described is curvilinear since saturation level is reached gradually (Fathipour et al. 2006). Type III shows a (sigmoid) density-dependent relationship which decreases as saturation level is reached; this type of functional response reveals that predator can learn to concentrate in a prey as it becomes abundant (Fujii et al. 1986). The effectiveness of a natural enemy has been associated to a density-dependent functional response (Solomon 1949, Nicholson 1958).

It is recognized that laboratory studies may bear little resemblance to the responses exhibited in nature (Munyaneza and Obrycki 1997). Nonetheless, such studies can be used to infer basic mechanisms underlying enemy–host interactions (Houck and Strauss 1985) and may provide valuable information for biological control programs. For example, comparisons of the attributes of different parasitoid species can be performed and baseline information established for quality control procedures in mass rearing programs (Montoya et al. 2000b).

Under a mass rearing situation, the required high host density facilitates the aggregation of parasitoids, which in turn leads to mutual interference. In this scenario, functional response and mutual interference are closely linked and difficult to disentangle (Merkel 2014). This prompted us to explore the role of both interactions in mass rearing of fruit fly parasitoids to assess the suitability of functional response parameters (i.e., instantaneous rate of discovery [*a'*], handling time [*Th*]), as they can be useful to provide sound recommendations for new protocols based upon their values.

To achieve a better understanding of the dynamics of a parasitoid–host system, suitable evaluations should be performed in order to gain knowledge about how the system works, which may provide better tools to increase the effectiveness of biological control programs when a beneficial parasitoid is released against a pest species (Skovgård and Nachman 2015). Thus, we studied the rearing of *Utetes anastrephae* (Viereck) (Hymenoptera: Braconidae), a native parasitoid that attacks larva of fruit flies in the genus *Anastrepha* (Schiner) (Aluja et al. 2003). This parasitoid is naturally distributed from Florida, USA, to northern Argentina (Sivinski et al. 1997) and is commonly associated with *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae), a major pest of mango (*Mangifera indica* L.) and hog plums (*Spondias* spp.) (Hernández-Ortiz and Aluja 1993). The objectives of this study were to determine: 1) the functional response of *U. anastrephae* attacking *A. obliqua* larvae, 2) the

influence of foraging females on the per female rate of parasitism and on offspring fitness, and 3) the frequency of superparasitism and its effect (if any) on sex ratio and fitness parameters. Our results may suggest ways to optimize mass rearing protocols and reduce production costs.

Materials and Methods

Workstation
The experiments were performed in the Laboratory of Biological Control of the Moscafrut SAGARPA-SENASICA program located in Metapa Dominguez, Chiapas, Mexico. Environment was controlled with a temperature of $23 \pm 1°C$, relative humidity of $70 \pm 10\%$, and a photoperiod of 12:12 (L:D) h.

Biological Materials
The *A. obliqua* second-instar larvae (6–7 d old) used as hosts were provided by the Moscafrut plant, a facility where this species is produced at a massive level using procedures described by Artiaga-López et al. (2004). Adult *U. anastrephae* were produced by the Biological Control Laboratory of the Moscafrut Program, SAGARPA-SENASICA, following the guidelines of Cancino et al. (2009).

Functional Response Bioassays
Following the procedures of Montoya et al. (2000b), individual mated females of *U. anastrephae* (5–6 d old) were placed with 6- to 7-d-old fly larvae in glass cages (10 by 10 by 10 cm) for 3 h, a time period sufficient for foraging females at high host densities to become satiated and stop searching for additional hosts. Evaluated female:larva ratios were 1:1, 1:5, 1:10, 1:15, 1:20, 1:30, and 1:40. *Anastrepha* larvae in 8.5 ml of diet were exposed to parasitoids in Petri dish lids (5.5 cm diameter [$= 23.8 cm^2$] and 0.3 cm depth) covered by thin fabric (= oviposition dishes). The attacked larvae were not replaced, because they were concealed within the oviposition dishes, and discriminating between attacked and nonattacked larvae is time consuming and disturbs the system.

Two days after exposure, the number of attacked larvae and number of oviposition scars per larva were determined using a stereomicroscope at 20× (Discovery V8, Carl Zeiss, Oberkochen, Germany). To corroborate the presence of immature parasitoids, 20% of the superparasitized larvae (i.e., larvae with more than one oviposition scar) were dissected. Remaining larvae were kept in plastic containers with vermiculite at 26°C until adult parasitoid emergence. The assay was repeated 10 times for each female:larvae ratio.

Mutual Interference Bioassays
We exposed two sets of 20 second-instar larvae (6–7 d old) *A. obliqua* to variable numbers of mated female (1, 2, 4, 8, 10, and 20) in individual aluminium-frame cages (20 by 20 by 20 cm³) using the oviposition dish described earlier. One set was used for dissections and the other to determine percentages of adult parasitoid emergence and sex ratio. Exposure times were 3 h, and 10 repetitions were performed per treatment.

Two days after exposure, the number of attacked larvae and number of oviposition scars per larva were determined using a stereomicroscope. One set of larvae for each treatment was dissected as follow: 5 larvae 2 d after exposure, 5 pupae 2, 4, and 8–10 d after pupariation to count the number of live immature parasitoids. Using the other set of larvae, the percent parasitoid emergence (i.e., percentage of parasitism) and sex ratio were determined for each

treatment. Pupae were placed individually in cells of 1 cm diameter × depth, filled with 1 g of damp vermiculite from a 24-cell clear plastic chamber (8.5 wide by 12.5 cm long), until parasitoid emergence.

Fitness Tests on Parasitoids Emerging From Superparasitized Hosts

The effect of superparasitism on adults emerging from superparasitized pupae was studied through the formation of groups of pupae with different numbers of oviposition scars: 1, 2–4, 5–7, and > 8 (as in Gonzalez et al. 2007 and Ayala et al. 2014). Parasitoid adults from pupae with only one oviposition scar were used as controls. The pupae with different number of scars were selected from 10 additional repetitions of the mutual interference bioassays. The parameters evaluated were as follows:

1) Percent adult emergence. Ten fly pupae were placed inside a 250-ml plastic container with humid vermiculite until parasitoid emergence. The percentage adult emergence for each scar range was calculated as the total number of parasitoids emerged divided by the number of fly pupae.

2) Flight ability. A black PVC tube (10 cm diameter by 10 cm high) with 10 pupae was placed inside a wire cage covered with knit cloth (1.20 by 0.4 by 0.5 m). The internal tube walls were coated with talc to prevent parasitoids from escaping by walking. Five days after emergence, parasitoids still inside the tubes were considered flightless (Cancino et al. 2009). For both test four repetitions were performed.

3) Survival without water or food. Using an aspirator, 50 male–female pairs of newly emerged adults were placed inside a wood frame cage (30 by 30 by 30 cm^3; Wong and Ramadan 1992) and deprived of water and food. Dead parasitoids were sexed and counted each day (Cancino et al. 2009).

Data Analyses

Functional Response

We fitted the number of attacked larvae (Na) and the number of exposed larvae (No) to a logistic quadratic regression, where a negative linear parameter (p1) is evidence of a type II response. If the linear parameter (p1) is positive and the quadratic term (p2) is negative, a type III response is inferred (Trexler et al. 1988, Juliano 1993, Okuyama 2013). The expected numbers of attacked larvae were obtained by using the disk equation introduced by Holling (1959):

$$Na = a'TtNo/(1 + a'ThNo)$$

where a' is the instantaneous rate of host discovery, Tt is the total time of host exposure, and Th is the handling time. The parameters a' and Th were previously estimated by fitting the observed Na and No to a nonlinear least-square regression.

Effect of Mutual Interference

To determine mutual interference, we used the model proposed by Skovgård and Nachman (2015):

$$N_a = No\left(1 - e^{-(EP/No)t}\right)$$

where No is the number of hosts available, N_a the number of hosts attacked at least once during time t, P is the number of parasitoids, and E is the rate of encounters between a parasitoid individual and hosts eliciting attacks. The per capita instantaneous rate of attack (E) was calculated as a function of parasitoid density as $E = Emax\ f(P)$, where $Emax$ is the maximum rate of attack achieved if all

factors are optimal. We assume that $f(P)$ declines monotonically with the number of parasitoids, so that $f(P) = 1$ when $P = 1$. The model used (Skovgård and Nachman 2015) to describe the relationship between density and rate attack has two parameters (r and q), as follows:

$$E = f(P) = e^{-r((P-1)/A)^q}$$

Where r is the product of the encountering rate with conspecifics and the time wasted per encounter (parasitoids^{-1}), and A is the area to which hosts and parasitoids are confined. Values of r > 0 indicate the effect of parasitoid density, and values of $q > 1$ indicate the level of interference as parasitoid density increase. The difference between the expected data from the model and the observed attacked larvae illustrates the effect of mutual interference.

The total number of attacked larvae, the number of larvae attacked per female, the number of scars per pupa, and the number of parasitoid larvae per pupa were analysed by one-way ANOVA followed by Turkey's multiple range tests; data on the last three parameters were log $x + 1$ transformed for analysis. The data for the percentage of emerged adults and the sex ratio were arcsine square-root transformed before the ANOVA. The relationship between the number of oviposition scars per pupa and the number of parasitoid larvae inside the pupae, as well as the relationship between the percentage of adult emergence and the number of oviposition scars per pupa, was fitted using simple linear regression. The relationship between the number of oviposition scars and the sex ratio was fitted using logistic regression.

Fitness Tests

Differences among the ranges of oviposition scars on percent emergence and flight ability were analysed by one-way ANOVA followed by Tukey tests, with previous data being arcsine square-root transformed (Pimentel-Gomes 2009). Adult survival curves were compared using the log-rank test (Francis et al. 1993). All analyses were performed using the JMP statistical package (version 5.0.1; Sas Institute 2003). Data were transformed back to original units for presentation in tables and figures.

Results

Functional Response

Functional response and percent parasitism curves are depicted in Fig. 1. The estimate of the linear coefficient ($p1 = -0.076$, $P < 0.001$) and quadratic coefficient ($p2 = 0.002$, $P = 0.005$) of the logistic regression were significant, suggesting a type II response of attacked larvae that decreased with increasing host density. The values of the instantaneous rate of discovery (a') and handling time (Th) were 0.470 h^{-1} and 0.099 h, respectively.

The greatest number of attacked larvae (20.1 ± 1.73 [mean ± SE]) was observed at a density of 40 larvae (= 1.7 larvae per cm^2), and the average number of scars per pupa decreased with an increasing number of available larvae per female. The highest average number of scars per pupa was 4.4 (± 1.91) at the lowest parasitoid:larval ratio (1:1). The highest percentage of pupae with more than one scar was observed at a density of 10 larvae per female, though the average number of immature parasitoids within a dissected pupa was always ≈ 1 (Table 1).

Mutual Interference

The maximum rate of attack (Emax) was 26.688 ± 1.803 h^{-1}. The parameter r was 0.560 ± 0.085 parasitoid^{-1}, significantly different
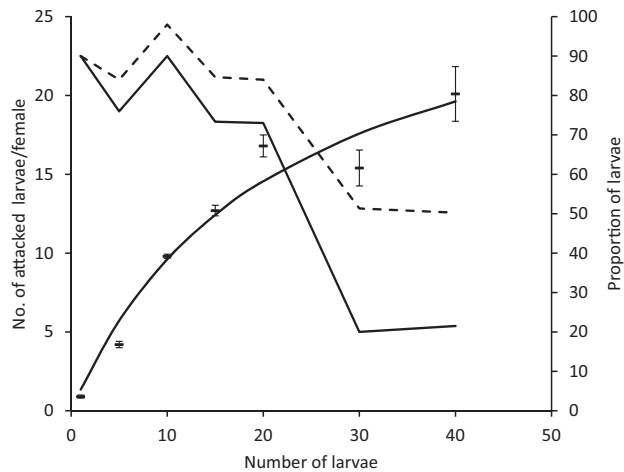
Fig. 1. Functional response of *U. anastrephae* on *A. obliqua* larvae (left axis). Proportion of attacked larvae (---); proportion of parasitized larvae (—).

Table 1. Performance of individual *U. anastrephae* females attacking variable numbers of *A. obliqua* larvae

Treatment female:host	No. scars per pupa	% Pupae with >1 scar	No. parasitoid larvae per attacked pupa
1:1	4.4 ± 1.91	50	1.0 ± 0.00
1:5	3.1 ± 0.43	62	1.1 ± 0.15
1:10	3.4 ± 0.31	74	1.0 ± 0.05
1:15	2.6 ± 0.20	58	0.9 ± 0.05
1:20	2.3 ± 0.16	54.5	0.9 ± 0.04
1:30	0.9 ± 0.06	23	0.5 ± 0.06
1:40	0.9 ± 0.05	20.6	0.6 ± 0.05

Data parameters are mean ± SE ($n = 10$).

from zero ($t = 6.6$, df = 4, $P = 0.003$), and q was $0.567 ± 0.058$ different significantly from 1 ($t = 7.4$, df = 4, $P = 0.002$), indicating an increase of interference as a function of parasitoid density. In Fig. 2 are shown the expected values of attacked larvae if mutual interference was absent and the expected values of attacked larvae if mutual interference was incorporated. The difference between the curves reflects the magnitude of mutual interference.

Per capita parasitism was significantly affected by parasitoid density ($F = 461.2$; df = 5, 54; $P < 0.001$), being the lowest values for treatments with 8 to 20 foraging females. The number of oviposition scars per pupa ($F = 78.9$; df = 5, 54; $P < 0.001$) and the number of parasitoid larvae inside pupae ($F = 44.2$; df = 5, 54; $P < 0.001$) increased with increasing numbers of foraging females. In most treatments the sex ratio was biased toward females, and the percent parasitoid emergence was highest in the treatment with four foraging females (Table 2).

A positive correlation was observed between the number of oviposition scars per pupa and the number of immature parasitoids ($r^2 = 0.88$; $F = 51.5$; df = 1, 153; $P < 0.0001$). The probability of an emerging parasitoid being a female was positively associated with the number of scars per pupa ($\chi^2 = 4.963$; df = 1; $P = 0.0259$; Fig. 3), with a female-biased sex ratio (0.63).

Fly larvae dissected two days after exposure hosted a variable number of first-instar parasitoids (Table 2), which was evidence of superparasitism. However, when 2-, 4-, and 8-d-old pupae were dissected, only one live immature parasitoid (2nd and 3rd instar and prepupa, respectively) per host was observed. Remaining larvae
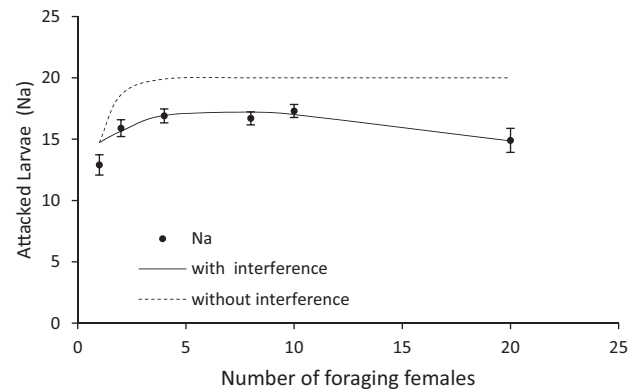

Fig. 2. Relationship between the number of *U. anastrephae* females and the number of attacked larvae of *A. obliqua* during 3 h of exposure. Experiments were conducted with 20 hosts on an area of 23.8 cm^2. (•) Observed values of attacked larvae (±SE); (--) predicted number of attacked larvae without mutual interference; (—) predicted number of attacked larvae if mutual interference is present.

were dead; 22.5% of which had wounds indicating physical combat. When dissecting 10-d-old pupae, we found a formed adult with a white body and brown eyes.

Fitness of Adults Emerging from Superparasitic Conditions

The percent adult emergence was statistically different within groups of pupae with different number of scars ($F = 10.45$; df = 3, 76; $P < 0.001$; Table 3), but no significant differences were observed in flight ability ($F = 0.48$; df = 3, 23; $P = 0.69$). The curves of adult survival ($\chi^2 = 11.84$; df = 3; $P = 0.008$ for males; $\chi^2 = 10.39$; df = 3; $P = 0.016$ for females) varied relative to the number of oviposition scars per host (Fig. 4).

Discussion

Information about the functional response and the mutual interference process may be important to better support biological control projects (Fathipour et al. 2006, Merkel 2014), since they may increase our understanding about the role of natural enemies as biocontrol agents. However, investigation of these concepts, as well as their possible implications, has not been applied to mass rearing protocols of biocontrol agents. This study is one of the first attempts to provide insight on the use of these concepts in mass rearing and release projects of natural enemies.

Functional Response

Although studies of functional response can provide valuable tools for describing population dynamics, at the present time they have shortcomings when the aim is to apply them in biological control programs (Fernández-Arhex and Corley 2003, Fathipour et al. 2006), since data from field conditions are elusive. This fact is more notorious under the augmentative biological control strategy involving mass rearing and mass releases of natural enemies in which the densities of natural enemies can be easily manipulated, but still having limitations to predict or estimate the effects of such releases. Studies on the interaction between natural enemies and prey or host populations can give us important clues about the role of the insects on the population dynamics of their hosts, and the structure of the insect communities in which they exist (Jervis and Kidd 1996). This kind of studies should be a necessary prerequisite for the selection of biological control agents and for the evaluation of their performance

Table 2. Effects of mutual interference (mean ± SE) of different number of *U. anastrephae* females attacking *A. obliqua* larvae

Treatment females:hosts	No. larvae attacked	No. of attacked larvae/♀	No. scars per pupa	No. parasitoid larvae per attacked pupa	% Emergence of parasitoid	Sex ratio ♀/♂
1:20	12.9 ± 0.83b	12.9 ± 0.83a	1.4 ± 0.10e	0.3 ± 0.04e	30.0 ± 8.66b	0.22 ± 09b
2:20	15.9 ± 0.69ab	7.9 ± 0.35b	2.7 ± 0.20d	0.7 ± 0.04d	40.0 ± 8.66ab	0.48 ± 08ab
4:20	16.9 ± 0.57a	4.2 ± 0.14c	3.9 ± 0.34c	1.0 ± 0.07cd	61.3 ± 3.14a	0.64 ± 07a
8:20	16.7 ± 0.53a	2.1 ± 0.07d	8.5 ± 0.61ab	1.3 ± 0.14bc	32.5 ± 10.30b	0.72 ± 08a
10:20	17.3 ± 0.53a	1.8 ± 0.05d	7.4 ± 0.54b	1.6 ± 0.16b	55.0 ± 14.57a	0.75 ± 06a
20:20	14.9 ± 0.98ab	0.8 ± 0.05d	10.8 ± 1.12a	2.5 ± 0.27a	46.3 ± 10.48ab	0.79 ± 07a

Values with different letters in the same column are significantly different (Tukey α = 0.05).
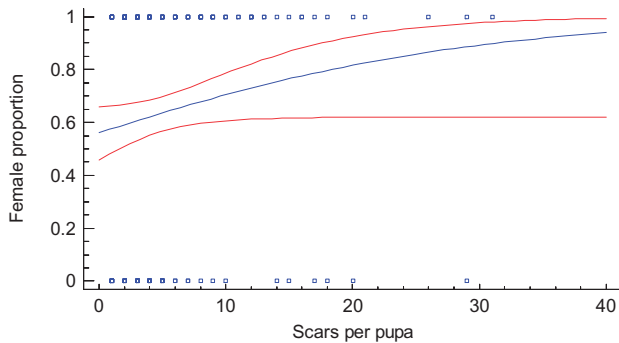
Fig. 3. Logistic regression between the sex ratio (proportion of females) and number of scars per pupa in *U. anastrephae*. Data are binary (1 = females, 0 = males; *n* = 406).

Table 3. Average (±SE) of fitness parameters of *U. anastrephae* adults emerging from pupae with different numbers of scars

No. of scars per pupa	Percent emergence	Flight ability (%)
1	53.6 ± 3.61b	100 ± 0.00a
2–4	75.1 ± 2.12a	98.9 ± 1.11a
5–7	71.5 ± 2.85ab	97.1 ± 2.85a
>8	64.6 ± 2.87b	96.3 ± 2.84a

Values with different letters in the same column are significantly different (Tukey α = 0.05).

in the field (Luck 1990, Skovgård and Nachman 2015), which involves the mass releases of natural enemies.

The type II functional response (i.e., a curvilinear relationship between host density and number of host attacked where the saturation level is reached gradually) exhibited by *U. anastrephae* is characteristic of many braconid parasites (Aragón et al. 2007, Tahriri et al. 2007), but there are also reports of type III functional response (a density-dependent relationship) in braconids, as in the case of *Diachasmimorpha longicaudata* (Ashmead), a larval parasitoid of fruit flies (Montoya et al. 2000b). Several authors have tried to explain why a type II response is more common than a type III (van lenteren and Baker 1976, Collins et al. 1981, Hosfang and Hagvar 1983). They argue that in laboratory tests parasitoids are forced to remain in the patch, whereas under field conditions they would leave because of the low host density. Various other potential factors (see De Clercq et al. 2000, Montoya et al. 2000b, Mohaghegh et al. 2001, Moezipour et al. 2008, Merkel 2014) can drive a density-dependent response in host–parasitoid systems.

There are different criteria to determine key parameters (i.e., searching rate a' and handling time Th) in functional response models (see Fan and Petit 1994, Williams and Juliano 1996, Okoyuma 2013). In this study, we felt justified in using the model proposed by Holling (1959), even though it does not include host depletion (Roger 1972), because in our study larvae were concealed within the oviposition dishes (as in mass rearing conditions), and replacing attacked larvae during the test disturbs the system.

Our data show that *U. anastrephae* possesses a good searching capacity and an efficient rate of parasitization. In the treatment with the lowest host density (1 larva per arena), females attacked 90 % of the exposed hosts (Table 1), which was unexpected because according to Murdoch (1994) and Hochberg and Hawkins (1994), at low host densities hunting efficiency may be reduced and the importance

of refuge increases. In treatments with a high host densities (15 and 20 larvae per female), a solitary female attacked >70% of the available hosts in three hours. The handling time of *U. anastrephae* (Th = 0.099) was lower than that reported for another braconid parasitoid (*D. longicaudata*, Th = 0.189, Montoya et al. 2000b) under similar experimental conditions, which is favourable according to Menon et al. (2002), because parasitoids with short handling times have a greater amount of time available for searching and thus an increased likelihood of finding more hosts. The treatments with low host densities showed the highest numbers of scars per pupa, but superparasitism (i.e., two or more immature parasitoids inside a pupa) was not observed. This observation reflects that *U. anastrephae* females avoid self-superparasitizing their hosts (see Mackauer 1990), a situation reported for *D. longicaudata* by Lawrence et al. (1978), Lawrence (1981), and Montoya et al. (2000b), even at high host densities.

Mutual Interference

The effects of mutual interference among *U. anastrephae* females were more severe at high parasitoid densities. We observed that an increasing number of foraging females resulted in a continuous increase in the number of oviposition scars and immature parasitoids per pupa, without significant differences in the total number of larvae attacked (see Table 2). The last reflects that conspecific females attacked more times the concealed larvae but did not oviposit inside the same proportion, maybe due to a high rate of conspecific encounters while foraging, thereby disturbing host-finding behavior and interrupting oviposition. These females may behave in different ways: under laboratory conditions, some species increase their tendency to emigrate from the experimental arena (Hassell 1978), whereas others have been shown to defend the remaining host resources against conspecifics (Lawrence 1981, Field and Calbert 1998, Goubault et al. 2005, Humphries et al. 2006). Both situations will lead to a reduced parasitoid efficiency.

This experiment subjected females to crowded conditions with conspecifics, which frequently leads to conspecific-superparasitism
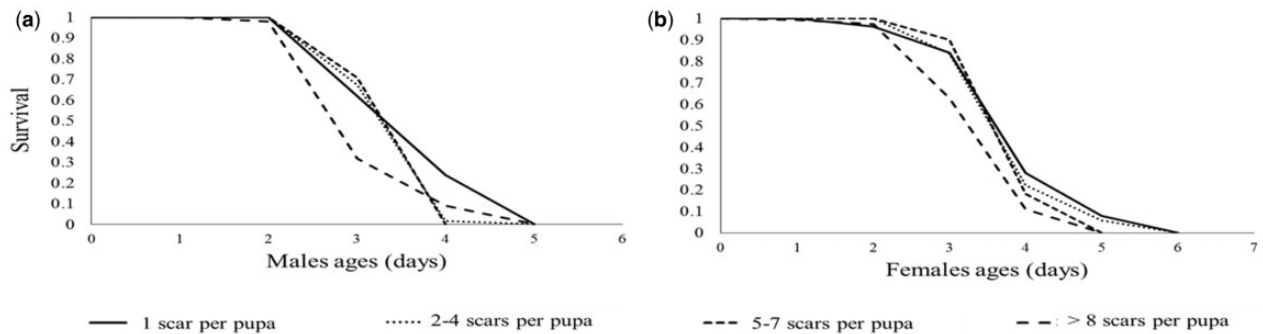
Fig. 4. Survival of *U. anastrephae* males (**a**) and females (**b**) emerging from pupae of *A. obliqua* with 1, 2–4, 5–7, or >8 scars per pupa.

(Makauer 1990). Based on our data, we deduce that superparasitism is not a common strategy in *U. anastrephae*, as this strategy was observed only under very crowded conditions (i.e., > 8 females:20 larvae). From data contained in Table 2, we found that 53.9% of the sampled pupae had more than one oviposition scar but that only 21.7% were superparasitized, which suggests that in nearly 60% of events, females avoided laying more eggs in hosts previously parasitized by conspecifics. However, discrimination ability has not been properly studied in this species.

Increasing numbers of immature parasitoids inside pupae resulted in lower adult emergence, possibly due to intrinsic competition. When 4-d-old pupae with high numbers of oviposition scars were dissected, we frequently observed that only one larva was alive and others were dead, some of with wounds caused by physical combat. Lawrence (1988) and Montoya et al. (2000b) also reported evidence of physical combat between immature *D. longicaudata*, though both concluded that the main cause of larval mortality under superparasitism conditions involved physiological mechanisms of suppression.

We found a positive correlation between the number of scars per pupa and the emergence of females in *U. anastrephae*, most likely due to differential mortality of the sexes, as proposed by Darrouzet et al. (2003, 2008) for *Eupelmus vuilleti* (Hym.: Eupelmidae) and by Montoya et al. (2011) for *D. longicaudata*. In the case of *D. longicaudata*, González et al. (2007) suggested that superparasitism could represent an adaptive strategy because it was related to a female-biased sex ratio without detrimental effects on other fitness parameters such as longevity and flight ability. In mass rearing projects, it is important to determine which factors influence sex ratio (King 1993, Ode and Heinz 2002, Ode and Hardy 2008), because females lead to increased population growth rates and because males do not contribute to pest mortality (Heimpel and Lundgren 2000).

We also observed that flight ability and survival without water and food were not different in adult *U. anastrephae* emerging from pupae with various levels of superparasitism, suggesting that this condition does not extensively impact the fitness of individuals. According to van Alphen and Visser (1990), superparasitism might be positive if it helps to overcome host defence mechanisms and thus supports immature development.

These findings serve to increase our knowledge of an important parasitoid of fruit fly pests and could be relevant in mass rearing projects aimed at applying biological control by augmentation, because the results suggest ways to optimize the use of biological material and strategies to reduce production costs. For instance, the short handling time (*Th*) exhibited by *U. anastrephae* suggests that the time of host exposure in a mass rearing scenario should be shorter than that reported for *D. longicaudata* by Montoya et al. (2011). The negative effect of mutual interference among foraging females suggests that the female:host ratio should be no greater than 1:5, which is markedly more efficient than the 1:2 ratio used for *D. longicaudata* and represents an opportunity to optimize the number of active females in a lab colony. Although superparasitism appears to have a negligible impact on *U. anastrephae* fitness, it should be minimized in mass rearing scenarios.

Acknowledgments

References

Aluja, M., J. Rull, J. Sivinski, A. L. Norrbom, R. A. Wharton, R. Macías-Ordóñez, F. Díaz-Fleischer, and M. López. 2003. Fruit Flies of the Genus *Anastrepha* (Diptera: Tephritidae) and Associated Native Parasitoids (Hymenoptera) in the Tropical Rainforest Biosphere Reserve of Montes Azules, Chiapas, Mexico. Environ. Entomol. 32: 1377–1385.

Aluja, M., P. Montoya, J. Cancino, L. Guillén, and R. Ramírez-Romero. 2008. Control biológico de moscas de la fruta, pp. 292–222. *In* H. Arredondo and L.A. Rodríguez del Bosque (eds.), Control Biológico en México: Una Revisión de Casos. Mundi-Prensa, Ciudad de México, México.

Aragón, S., F. Cantor, J. Cure, and D. Rodriguez. 2007. Capacidad parasítica de *Praon* pos. *occidentale* (Hymenoptera: Braconidae) sobre *Macrosiphum euphorbiae* (Hemiptera: Aphididae) en condiciones de laboratorio. Agron. Colomb. 25: 142–148.

Artiaga-López, T., E. Hernández, J. Domínguez-Gordillo, D. S. Moreno, and D. Orozco-Dávila. 2004. Mass-production of *Anastrepha obliqua* at the Moscafrut Fruit Fly Facility, pp. 389–392. *In* B. E. Barnes (ed.), Proceedings of the 6th. International Symposium on Fruit Flies of Economic Importance. Isteg Scientific Publications Irene, South Africa.

Ayala, A., A. M. Martínez, I. Figueroa, S. Pineda, M. Miranda, P. Liedo, and P. Montoya. 2014. Superparasitism strategies by a native and an exotic parasitoid species attacking the Mexican fruit fly, *Anastrepha ludens* (Diptera: Tephritidae). Biocontrol Sci. Technol. 24: 925–935.

Cancino, J., L. Ruiz, J. Hendrichs, and K. Bloem. 2009. Evaluation of sequential exposure of irradiated hosts to maximize the mass rearing of fruit fly parasitoids. BioControl Sci. Technol. 19: 95–109.

Collins, M. D., S. D. Ward, and F. G. Dixon. 1981. Handling time and the functional response of *Aphelinus tompsoni*, a predator and parasite of the aphid *Drepanosiphum platanoidis*. J. Anim. Ecol. 50: 479–487.

Darrouzet, E., E. Imbert, and C. Chevier. 2003. Self-superparasitism consequences for offspring sex ratio in the solitary ectoparasitoid *Eupelmus vuilleti*. Entomol. Exp. Appl. 109: 167–171.

Darrouzet, E., G. Boivin, and C. Chevrier. 2008. Sex allocation decision under superparasitism by the parasitoid wasp *Eupelmus vuilleti*. J. Insect. Behav. 21: 181–191.

De Clercq, P., J. Mohaghegh, and L. Tirry. 2000. Effect of host plant on the functional response of the predator *Podisus nigrispinus* (Heteroptera: Pentatomidae). Biol. Control. 18: 6–70.

Fan, Y., and F. L. Petit. 1994. Parameter estimation of the functional response. Environ. Entomol. 23: 785–794.

Fathipour, Y., A. Hosseini, A. A. Talebi, and S. Moharramipour. 2006. Functional response and mutual interference of *Diaeretiella rapae* (Hymenoptera: Aphidiidae) on *Brevicoryne brassicae* (Homoptera: Aphididae).Entomol. Fennica. 17: 90–97.

Fernández-Arhex, V., and J. C. Corley. 2003. The functional response of parasitoids and its implications for biological control. Biocontrol Sci. Technol. 13: 403–413.

Field, S. A., and G. Calbert. 1998. Patch defence in the parasitoid wasp *Trissolcus basalis*: when to begin fighting? Behaviour 135: 629–642.

Fujii, K., C. S. Holling, and P. M. Mace. 1986. A simple generalized model attack by predators and parasites. Ecol. Res. 1: 141–156.

Francis, B., M. Green, and C. Payne. 1993. Statistical system for generalized linear interactive modelling. Clarendon Press, Oxford, United Kingdom.

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

Goubault, M., Y. Outreman, D. Poinsot, and A. M. Cortesero. 2005. Patch exploitation strategies of parasitic wasps under intraspecific competition. Behav. Ecol. 16: 693–701.

Hassell, P. M. 1978. The dynamics of arthropod predator-prey systems. Princeton University Press, NJ.

Hassell, M. P., and G. C. Varley. 1969. New inductive population model for insect parasites and its bearing on biological control. Nature 223: 1133–1137.

Heimpel, G. E., and J. G. Lundgren. 2000. Sex ratios of commercially reared biological control agents. Biol. Control 19: 77–93.

Hernández-Ortiz, V., and M. Aluja. 1993. Listado de especies del género Neotropical *Anastrepha* (Diptera: Tephritidae) con notas sobre su distribución y plantas hospederas. Fol. Entomol. Mexicana 88: 89–105.

Hochberg, M. E., and B. A. Hawkins. 1994. The implications of population dinamics theory to parasitoid diversity and biological control, pp. 451–471. *In* B. A. Hawkins and W. Sheenan (eds.), parasitoid community ecology. Oxford University Press, Oxford.

Holling, C. S. 1959. Some characteristics of simple types of predation and parasitism. Can. Entomol. 91: 385–398.

Hosfang, T., and E.B. Hagvar. 1983. Functional responses to prey density of *Ephedrus cerasicola* (Hym.: Aphidiidae), an aphidid parasitoid of *Mizuz persicae* (Hom.: Aphidae). Entomophaga 28: 317–324.

Houck, M. A., and R. E. Strauss. 1985. The comparative study of functional responses: experimental design and statistical interpretation. Can. Entomol. 115: 617–629.

Humphries, E. L., A. J. Hebblethwaite, T. P. Batchelor, and I. C. W. Hardy. 2006. The importance of valuing resources: host weight and contender age as determinants of parasitoid wasp contest outcomes. Anim. Behav. 72: 891–898.

Jervis, M., and N. Kidd 1996. Insect Natural Enemies. Practical Approaches to their Study and Evaluation. Chapman & Hall, London, p. 491.

Juliano, S. A. 1993. Nonlinear curve fitting: Predation and functional response curves, pp. 159–182. *In* S. M. Scheiner and J. Gurevitch (eds.), Design and analysis of ecological experiments. Chapman & Hall, Nueva York.

King, B. H. 1993. Sex ratio manipulation by parasitoid wasps, pp. 418–441. *In* D.L. Wrensch, and M. Ebberte (eds.), Evolution and diversity of sex ratio in insects and mites. Chapman and Hall, New York.

Knipling, E. F. 1992. Principles of Insect Parasitism Analyzed from New Perspectives. Agriculture Handbook No. 693. ARS-USDA. Washington, DC.

Lawrence, P. O. 1981. Interference competition and optimal host selection in the parasitic wasp, *Biosteres longicaudatus*. Ann. Entomol. Soc. Am. 74: 540–544.

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

Lawrence, P. O., P. D. Greany, J. L. Nation, and R. M. Baranowski. 1978. Oviposition behavior of *Biosteres longicaudatus,* a parasitoid of the Caribbean Fruit Fly, *Anastrepha suspensa*. Ann. Entomol. Soc. Am. 71: 253–256.

Lester, P.J., and R. Harmsen. 2002. Functional and numerical responses do not always indicate the most effective predator for biological control: An analysis of two predator in a two-prey system, J. Appl. Ecol. 39: 455–468.

Luck, R. F. 1990. Evaluation of natural enemies for biological control: a behavioral approach. Trends Ecol. Evol. 5: 196–202.

Mackauer, M. 1990. Host discrimination and larval competition in solitary endoparasitoids, pp. 41–62. *In* M. Mackauer, L.E. Ehler, and J. Roland (eds.), Critical Issues in Biological Control. Intercept/VHC Publishers, Great Britain.

Menon, A., P. W. Flinn, and B. A. Dover. 2002. Influence of temperature on the functional response of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae), a parasitoid of *Rhyzopertha dominica* (Coleoptera: Bostrichidae). J. Stored. Prod. Res. 27: 463–469.

Merkel, K. 2014. Patch exploitation behaviour of the tephritid parasitoid *Fopius arisanus*, a candidate for the biological control of mango flies. PhD Dissertation Universität Bremen, Germany.

Moezipour, M., M. Kafil, and H. Allahyari. 2008. Functional response of *Trichogramma brassicae* at different temperatures and relative humidities. Bull. Insectol. 61: 245–250.

Mohaghegh, J., P. De Clercq, and L. Tirry. 2001. Functional response of the predators *Podisus maculiventris* (Say) and *Podisus nigrispinus* (Dallas) (Het., Pentatomidae) to the beet armyworm, *Spodoptera exigua* (Hübner) (Lep., Noctuidae): Effect of temperature. J. Appl. Entomol. 125: 131–134.

Montoya, P., P. Liedo, B. Benrey, J. F. Barrera, J. Cancino, J. Sivinski, and M. Aluja. 2000a. Biological control of *Anastrepha* spp. (Diptera: Tephritidae) in mango orchards through augmentative releases of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Biol. Control 18: 216–224.

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

Montoya, P., J. Cancino, G. Perez-Lachaud, and P. Liedo. 2011. Host size, superparasitism and sex ratio in mass-reared *Diachasmimorpha longicaudata*, a fruit fly parasitoid. BioControl 56: 11–17.

Munyaneza, J., and J. J. Obrycki. 1997. Functional response of *Coleomegilla maculata* (Coleoptera: Coccinellidae) to Colorado potato beetle eggs (Coleoptera: Chrysomellidae). Biol. Control 8: 215–224.

Murdoch, W. W. 1994. Population regulation in theory and practice. Ecology 75: 271–277.

Murdoch, W.W., and A. Oaten. 1975. Predation and Population Stability, Adv. Ecol. Res, 9: 2–131.

Ode, P. J., and I.W.C. Hardy. 2008. Parasitoid sex ratios and biological control, pp. 253–291 *In* E. Wajnberg, C. Bernstein, and J.J.M van Alphen (eds.), Behavioural ecology of insect parasitoids: From theoretical approaches to field applications. Blackwell Publishing, Oxford.

Ode, P. J., and K. M. Heinz. 2002. Host-size dependent sex ratio theory and improving mass reared parasitoid sex ratios. Biol. Control 24: 31–41.

Okoyuma, T. 2013. On selection of functional response models: Holling's models and more. BioControl 58: 293–298.

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

Roger, D. 1972. Random search and insect population models. J. Anim. Ecol. 41: 369–383.

Skovgård, H., and G. Nachman. 2015. Effect of Mutual Interference on the Ability of *Spalangia cameroni* (Hymenoptera: Pteromalidae) to Attack and

Parasitize Pupae of *Stomoxys calcitrans* (Diptera: Muscidae). Environ. Entomol. 44: 1076–1084.

Sivinski, J. M., C. O. Calkins, R. Baranowsky, D. Harris, J. Brambila, J. Diaz, R. E. Burns, T. Holler, and D. Dodson. 1996. Suppression of Caribbean fruit fly (*Anastrepha suspensa* (Loew) Diptera: Tephritidae) population through augmented releases of the parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Biol. Control 6: 177–185.

Sivinski, J., M. Aluja, and M. López. 1997. Spatial and temporal distributions of parasitoids of Mexican *Anastrepha* species (Diptera: Tephritidae) within the canopies of fruit trees. Ann. Entomol. Soc. Am. 90: 604–618.

Solomon, M. E. 1949. The natural control f animal populations. J. An. Ecol. 18: 1–35.

Stiling, P. D. 1987. The frequency of density dependence in insect host-parasitoid systems. Ecology 68: 844–856.

Tahriri, S., A. A. Talebi, Y. Fathipour, and A. A. Zamani. 2007. Host stage preference, functional response and mutual interference of *Aphidius matricariae* (Hym.: Braconidae: Aphidiinae) on *Aphis fabae* (Hom.: Aphididae). Entomol. Sci. 10: 323–331.

Trexler, J. C., C. C. Mccullogh, and J, Travis. 1988. How can the functional response best be determined? Oecologia 76: 206–214.

van Alphen, J.J.M., and M. E. Visser. 1990. Superparasitism as an adaptive strategy for insect parasitoids. Annu. Rev. Entomol. 35: 59–79.

van Lenteren, J. C., and K. Bakker. 1976. Functional responses in invertebrates. Neth. J. Zool. 26: 567–572.

Visser, M. E., T. H. Jones, and G. Driessen. 1999. Interference among insect parasitoids: A multi-patch experiment. J. An. Ecol. 68: 108–120.

Waage, J. K., K. P. Carl, N. J. Mills, and D. J. Greathead. 1985. Rearing entomophagous insects, pp. 45–66. *In* P. Singh and R. F. Moore (eds.), Handbook of insect rearing, vol 1. Elsevier, Amsterdam.

Wajnberg, E., C. Curty, and S. Colazza. 2004. Genetic variation in the mechanisms of direct mutual interference in a parasitic wasp: consequences in terms of patch time allocation. J. An. Ecol. 73: 1179–1189.

Williams, F. M., and S. A. Juliano. 1996. Functional response revisited. Environ. Entomol. 25: 549–550.

Wong, T.T.Y., and M. M. Ramadan. 1992. Mass rearing biology of larval parasitoids (Hymenoptera: Braconidae: Opiinae) of Tephritidae flies (Diptera: Tephritidae) in Hawaii, pp. 405–426. *In* T. E. Anderson and N. C. Leppla (eds.), Advances in insect rearing for research and pest management. Boulder, Westview Press.

Wong, T. T. Y., M. M. Ramadan, D. O. McInnis, N. Mochizuki, J. J. Nishimoto, and J. C. Herr. 1991. Augmentative releases of *Diachasmimorpha tryoni* (Hymenoptera: Braconidae) to suppress a Mediterranean fruit fly (Diptera: Tephritidae) population in Kula, Maui, Hawaii. Biol. Control 1: 2–7.

Wong, T.T.Y., M. M. Ramadan, J. C. Herr, and D. O. McInnis. 1992. Suppression of a Mediterranean fruit fly (Diptera: Tephritidae) population with concurrent parasitoid and sterile fly releases in Kula, Maui, Hawaii. J. Econ. Entomol. 85: 1671–1681.

Wylie, H. G. 1979. Sex ratio variability of *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae). Can. Entomol. 111: 105–109.