



Research Paper

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Functional response and mutual interference in the parasitoid *Coptera haywardi* (Oglobin) (Hymenoptera: Diapriidae) attacking *Anastrepha ludens* (Loew) (Diptera: Tephritidae) pupae

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Abstract

Functional response and mutual interference are important attributes of natural enemies that should be analysed in species with the potential to be used as biological control agents in order to increase the predictive power of the possible benefits and/or consequences of their release in the field. Our main objective was to determine the functional response and mutual interference of *Coptera haywardi* (Oglobin), a pupal parasitoid of economically important fruit flies (Diptera: Tephritidae). The functional response of *C. haywardi* on *A. ludens* pupae corresponded to a type II model, with an attack rate of 0.0134 host pupa/h and a handling time of 1.843 h, which reveals a meticulous selection process of pupal hosts. The effect of mutual interference among foraging females was negatively correlated with increased parasitoid density in the experimental arena, showing a gradual decline in attack rate per individual female. The increase in the number of foraging females also had an impact on the number of oviposition scars per pupa and the number of immature parasitoids per dissected pupa, but not on the percentage of adult emergence or the sex ratio. Our results suggest that *C. haywardi* could act as a complementary parasitoid in the control of fruit fly pupae, since the random distribution of these pupae in the soil would decrease the possibility of aggregation and mutual interference between foraging females.

Introduction

Augmentative releases of parasitoids as pest control agents are increasingly relevant in the context of current agriculture worldwide (Wang *et al.*, 2019; Murali-Baskaran *et al.*, 2021). For the use of this control alternative, studies on the functional response and mutual interference of foraging females revealing their behaviour and effectiveness as natural enemies are critical (Ghorbani *et al.*, 2019; DeLong and Uiterwaal, 2022). The functional response of a predator/parasitoid describes the relationship between the number of prey/hosts it consumes per unit of time and the abundance or density of the prey or hosts (Solomon, 1949; Holling, 1959). Studies on the foraging ecology of natural enemies contribute to estimate their attack capacity (Savino *et al.*, 2012; Guleria *et al.*, 2020), and allow inferences about their searching capacity, handling time, and intrinsic attack rate in a given interval of time and space (Fernández-Arhex and Corley, 2004).

Mutual interference between foraging conspecific parasitoid females results from the aggregation behaviour around the location of their hosts (Hirose *et al.*, 1990). Periodic releases of parasitoids can generate a high density of foraging females in the same patch, which would increase intraspecific competition at low host densities (Salas, 2017; Rezaei *et al.*, 2019). Mutual interference between female parasitoid wasps reduces their searching and parasitic efficiency due to a higher probability of antagonistic encounters (Kumar *et al.*, 2019). It can also cause an increase in the occurrence of superparasitism (i.e., more than one immature parasitoid of the same species per host) (Skovgard and Nachman, 2015; Poncio *et al.*, 2016; López *et al.*, 2021), which can cause an imbalance in their offspring sex ratio and emergence rates (Li *et al.*, 2022) because of competition for the same host resource (Skovgard and Nachman, 2015). Functional response and mutual interference studies allow to determine the host or prey density that results in the lowest percentage of antagonistic encounters between conspecifics of natural enemies, which would then result in a more effective control

of the pest (Rezaei *et al.*, 2019; Soni and Kumar, 2021). This information would not only provide an idea of the efficiency that a parasitoid species would be able to achieve but would also allow us to understand the behaviour, benefits, and/or consequences in predator-prey population dynamics (Skovgard and Nachman, 2015; Nakamichi *et al.*, 2020).

The diapiiid wasp *Coptera haywardi* (Oglobin) is a solitary idiobiont endoparasitoid of tephritid fruit fly pupae, native to and with a wide distribution in the Neotropical region, from Mexico to Argentina (Nuñez-Campero *et al.*, 2012). The females of this species can parasitise fruit fly pupae buried in soils that are difficult for other parasitoids to access (Baeza-Larios *et al.*, 2002; Guillén *et al.*, 2002). In addition, this species has a high ability to discriminate against pupae previously parasitised by conspecific and heterospecific parasitoids such as *Diachasmimorpha longicaudata* (Ashmead) (Cancino *et al.*, 2012; 2019). It has been documented that *C. haywardi* develops successfully in pupae of various species of the genus *Anastrepha*, such as *A. serpentina*, *A. striata*, and *A. obliqua* (García and Montilla, 2001), and that it can develop in wild and irradiated strains of *Ceratitis capitata* pupae (Wied) (Sivinski *et al.*, 1998; Nuñez-Campero *et al.*, 2020). In addition, it has been suggested that *C. haywardi* can be used together with other biological control agents against both *Anastrepha* and *Ceratitis* flies due to its great discriminatory capacity (Cancino *et al.*, 2014; Van Nieuwenhove *et al.*, 2016; Martínez-Barrera *et al.*, 2021). However, the use of *C. haywardi* (or any other species of parasitoid) in augmentative biological control programmes must be carefully planned and analysed in advance to avoid failure and/or harmful effects of their interaction (Montoya *et al.*, 2019; Rezaei *et al.*, 2019). Our purpose here was to characterise the functional response and mutual interference of *C. haywardi* females foraging on *A. ludens* pupae under laboratory conditions. These findings will reinforce existing knowledge on the biology and potential of *C. haywardi* as a natural enemy of tephritid fruit flies and may contribute to improving the rearing methods and field release strategies for the control of these important pests.

Materials and methods

Study area

The functional response experiment was carried out under laboratory conditions at El Colegio de la Frontera Sur, Tapachula Unit, Chiapas, Mexico, while the mutual interference experiment was carried out in the Biological Control laboratory of the Moscafrut Program (SADER-SENASICA) located in Metapa de Domínguez, Chiapas, Mexico. The experiments were conducted out according to the bioassay criteria established by Montoya *et al.* (2000, 2019) and Poncio *et al.* (2016).

Biological material

The Moscafrut facility, (SADER-SENASICA) located in Metapa, Chiapas, Mexico, provided 3–4-day old *Anastrepha ludens* pupae. The 7–8-day old *C. haywardi* females were provided by the Biological Control laboratory of the Fruit Fly Program located in the same locality. The laboratory strain was started in 1996 with specimens collected as described by Aluja *et al.* (2009). This colony has been maintained using *A. ludens* pupae produced in the Moscafrut facility, with an average of nine generations per year (approximately 234 generations under laboratory rearing).

Functional response experiment

Seven host densities (1, 5, 10, 20, 30, 40, and 50 pupae) were used as treatments, which were placed in a Petri dish bottom (60 × 15 mm) with 2 g of humid and sterile vermiculite to simulate soil. Each treatment was placed separately in 3.7 L plastic containers (25 cm high and 12 × 14 cm diameter) with a wide mouth. Petri dishes inside plastic containers were covered with a black cardboard roof to obtain an illuminance of ~20 lux (Cancino *et al.*, 2012). Each pupal density was exposed to a 7-day-old mature *C. haywardi* female with previous oviposition experience and previously provided with water and food consisting of bee honey mixed with soft paper *ad libitum* (Montoya *et al.*, 2012). The pupae were exposed to each female for 24 h with a 12:12 L: D photoperiod at 25 ± 1°C. After exposure, the pupae remained under the same environmental conditions for 3–4-days to later determine the number of pupae attacked, the number of oviposition scars per pupa, and the number of parasitoid larvae per pupa obtained by dissection using a stereomicroscope (Carl Zeiss®, Model Stemi 2000C). Sixteen replicates per host density were made with different females in each replicate.

Mutual interference experiment

The experiment was carried out using the same type of containers as in the previous experiment, which were conditioned with water and food *ad libitum*. The bottom of a Petri dish (60 × 15 mm) with 2 g of moist vermiculite to simulate soil was also placed in the containers. Ten unparasitised 3–4-day old *A. ludens* pupae were placed in each Petri dish, which were exposed to six different parasitoid densities: 1, 2, 4, 8, 12, and 16 mated *C. haywardi* females with previous oviposition experience, which were free in the above described container. The Petri dishes with pupae were covered with a black cardboard cutout to obtain a lighting level of ~20 lux to stimulate the searching and oviposition behaviour of the females. The exposure time of the pupae was 24 h with a photoperiod of 12:12 L: D at 25 ± 1°C. Each treatment was repeated ten times in duplicate to obtain two groups of host pupae (groups 1 and 2) for further analysis. All host pupae utilised were from the same production batch.

Four days after exposure, the pupae of the first group were observed to determine the number of pupae attacked, the number of oviposition scars per pupa, and the level of superparasitism by determining the number of parasitoid larvae per dissected pupa using a stereoscopic microscope (Carl Zeiss®, Model Stemi 2000C). The pupae of the second group were used to wait for the emergence of adults (~ 34–35 days) and then determine the survival and sex ratio in each replicate and treatment. Interference behaviour among foraging *C. haywardi* females was randomly filmed between treatments using a Motorola smartphone (Mod.G60). In each recording, the black cardboard roof was removed for 30 min.

Statistical analysis

Functional response data were analysed following the models and parameters proposed by Trexler *et al.* (1988) and Juliano (1993). Mutual interference data were analysed according to the model proposed by Skovgard and Nachman (2015). The data from both experiments were analysed using 'R Commander' (Version 4.2.0) (Fox, 2005, 2017; Fox and Bouchet-Valat, 2019).

Functional response

The functional response analysis was performed by fitting a logistic regression model to the proportion of pupae attacked in relation to the density of pupae offered. The values of the linear $P1$ and quadratic $P2$ parameters were used to differentiate between type II and type III functional responses (Holling, 1959). If the linear parameter $P1$ was negative, it would indicate a type II functional response, whereas if the $P1$ parameter was positive and the square coefficient was negative ($P2$), then it would indicate a type III functional response (Poncio et al., 2016). Attack rate (a') and handling time (t) were estimated by fitting the number of hosts attacked (Na) against the number of hosts offered (No) to a non-linear regression of least squares. The expected number of pupae attacked was calculated using Holling's (1959) 'disc equation':

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

where Na is the expected number of hosts attacked, a' is the constant attack rate, Tt is the exposure time of the host, No is the number of hosts available, and Th is the handling time.

Mutual interference. The mutual interference analysis was performed by modelling the functional response of a single female parasitoid according to the following formula:

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

where Na is the number of hosts attacked at least once during the exposure time t , No is the number of exposed hosts, E is the rate of encounters between a parasitoid and the hosts that caused attacks, and P is the number of parasitoids. E was calculated based on parasitoid density as $E = Emax f(P)$; if all the experimental factors are optimal, $Emax$ will represent the maximum attack rate achieved. It is assumed that the effect of parasitoid density ($f(P)$) decreases monotonically with the number of parasitoids, so that $f(P) = 1$ when $P = 1$. The model used by Skovgard and Nachman (2015) has two parameters (r and q) used in the description of the relationship between parasitoid density and parasitoid attack rate, as follows:

$$E = F(P) = e^{-r\left(\frac{P-1}{A}\right)q}$$

Parameter r is the result obtained from the rate of encounters with conspecifics and the wasted time per encounter ($P-1$), A is the size of the area where the hosts and parasitoids are confined. The r values reflect the effect of parasitoid density, while the q values denote the degree of interference as the parasitoid density increases. The effect of mutual interference is evidenced by the difference between the number of attacked pupae observed and the number of attacked pupae expected by the model.

The total number of pupae attacked, the number of pupae attacked per female, the number of oviposition scars per pupa, and the number of immature parasitoids per pupa were analysed with a one-way ANOVA followed by a Tukey's multiple range test. The values of the last three parameters were log-transformed [$x + 1$] for the analysis. Adult emergence percentage and sex ratio were arcsine square-root-transformed prior to the ANOVA. The relationship between the number of oviposition scars per pupa and the number of parasitoid larvae was analysed using the Pearson correlation method. The correlation between the number of oviposition scars per pupa and the percentage of adult emergence was evaluated by means of a simple linear regression. In addition,

a logistic regression was used to analyse the relationship between the number of oviposition scars and sex ratio.

Results

Functional response experiment

The values of the linear and quadratic coefficients of the logistic regression ($P1 = -2.4736$, $P < 0.00379$; $P2 = -0.61860$, $P > 0.3456$) suggest that the proportion of pupae attacked decreases as host density increases, and thus female *C. haywardi* exhibit a type II functional response. The attack rate (a') and handling time (Th) of *C. haywardi* were 0.0134 host pupa/h and 1.8429 h, respectively. The functional response and the parasitism average curves are shown in fig. 1, where it is possible to observe that the highest of attacked pupae and the highest number of parasitised pupae occurred in the treatment of (1:50) female parasitoid: host pupae. The highest average of parasitised pupae was observed at 10 host pupae per female.

Mutual interference experiment

The highest attack rate ($Emax$) was $0.153 \pm 0.055 \text{ h}^{-1}$. The r value was 2.265 ± 0.83 $P-1$, which differs from 0 ($t = 2.699$, $P = 0.00914$, $df = 57$), while the q value of 0.356 ± 0.229 was significantly different from 1 ($t = 2.812$, $P = 0.00673$, $df = 57$), which indicates the existence of a mutual interference effect dependent on the density of *C. haywardi* females. The expected average values of pupae attacked without mutual interference and the average of pupae attacked with the presence of mutual interference are shown in fig. 2. The difference between the curves reflects the magnitude of the mutual interference.

The number of parasitised pupae per female was negatively affected by parasitoid density ($F = 4.52$, $df = 5, 54$, $P < 0.0016$), where treatments with 12 and 16 foraging females showed the lowest values. The number of oviposition scars per pupa ($F = 4.652$, $df = 5, 54$, $P < 0.0013$) and the number of parasitoid larvae per pupa ($F = 4.059$, $df = 5, 54$, $P < 0.0033$) increased as the number of foraging females increased (Table 1). A positive Pearson correlation was observed between the number of oviposition scars per pupa and the number of immature parasitoids ($F = 8.414$, $df = 1, 49$, $P = 0.00556$) (fig. 3). There was no significant difference in the adult emergence percentage between treatments ($F = 0.6764$, $df = 5, 54$, $P > 0.6432$) or in the sex ratio of adults emerging from the different treatments ($F = 1.4682$, $df = 5, 54$, $P > 0.2155$) (Table 1).

Discussion

Several authors have stated (e.g., Merkel, 2014; Sereno et al., 2016; Francati, 2018; Stucchi et al., 2019) that functional response and mutual interference are important attributes of natural enemies because they provide valuable information about parasitoid-host interactions, which can be used to infer the impact they may have on the regulation of target pests, and even assist to improve their laboratory rearing (Poncio et al., 2016; López et al., 2021).

Our results show that *C. haywardi* exhibits a type II functional response, where the number of pupae attacked increased as host density increased. A type II functional response has also been determined in other pupal parasitoids such as *Dirhinus giffardii* (Hymenoptera: Chalcididae) attacking *Bactrocera zonata* and *B. cucurbitae* (Khan et al., 2020), as well as in the parasitoid

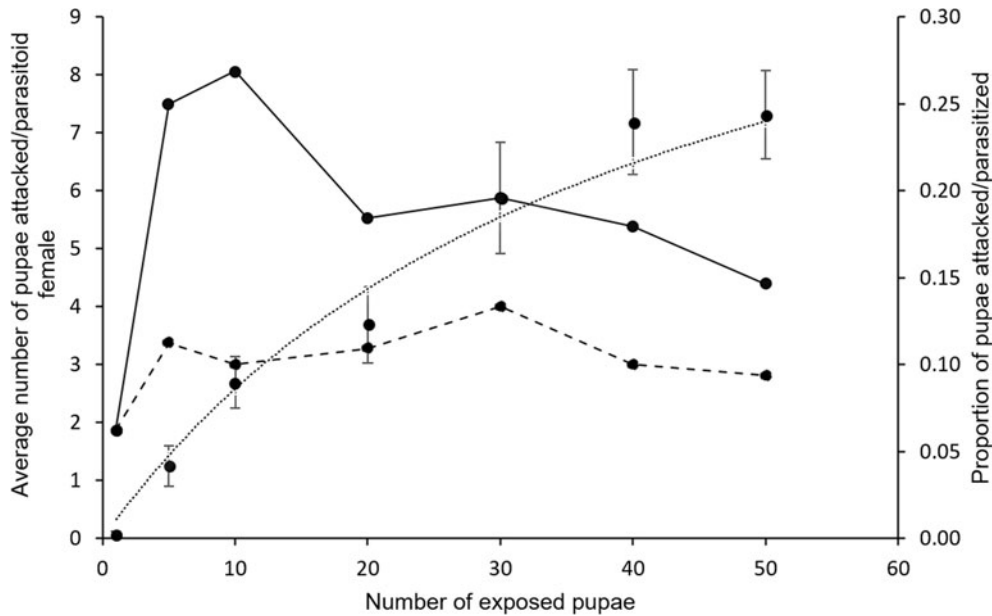


Figure 1. Functional response of *C. haywardi* attacking *A. ludens* pupae (left axis). (.....) Fitted model, (---●) pupae parasitized per female, (—●) average of pupae attacked per female.

Spalangia endius (Hymenoptera: Pteromalidae) attacking *Bactrocera dorsalis* and *Musca domestica* pupae (Zheng *et al.*, 2021). However, unlike *D. giffardii* and *S. endius*, our results with *C. haywardi* show a longer handling time that is reflected in a lower attack rate. This low attack rate is a consequence of a long searching time that results in a slow increase in attacked pupae as the number of offered pupae increases (Fernández-Arhex and Corley, 2004).

According to Martínez-Barrera *et al.* (2021), *C. haywardi* females have an average oviposition time of 24.6 min and an average refractory period of 10.6 min. These results agree with our observations, since we recorded times fluctuating between 25 and 45 min per oviposition. This amount of time invested by females in oviposition is considered a characteristic of the species (Cancino *et al.*, 2012), which is much longer compared to other

pupal parasitoids (e.g., *Dirhinus giffardii*, Khan *et al.*, 2020). Cancino *et al.* (2012) mention that *C. haywardi* females carefully examine their hosts before laying eggs in them, which is also consistent with our observations.

According to Holling (1959), a type II functional response is representative of invertebrates including parasitoids, whereas a type III functional response is more representative of vertebrate predators, where learning and switching from one prey to another is more frequent. However, type III functional responses have also been determined in some braconid species (e.g., Montoya *et al.*, 2000, Yazdani and Keller, 2016), where density-dependent mortality at low prey densities has been observed (Fernández-Arhex and Corley, 2004). In our results with *C. haywardi*, we also observed density-dependent mortality at low host densities (fig. 1), but unlike the results with the braconid *D. longicaudata*,

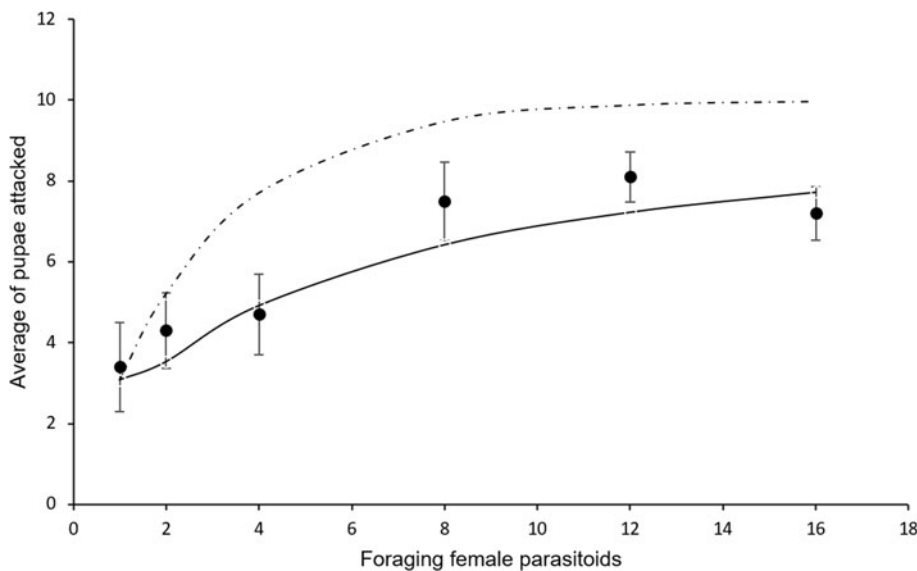


Figure 2. Average of pupae attacked when ten *A. ludens* pupae were exposed to different densities of foraging *C. haywardi* females for 24 h in a 60 × 15 mm area. (●) observed number of pupae attacked (± SE), (—) expected values with mutual interference, (---) expected values without mutual interference.

Table 1. Effect of mutual interference under different foraging parasitoid densities: Mean (\pm SE) number of pupae attacked, percentage of pupae attacked, number of pupae attacked per female, number of scars and parasitoid larvae per pupa attacked, parasitoid emergence percentage, and sex ratio of *Coptera haywardi* females attacking *Anastrepha ludens* pupae, ($n = 10$ replicates per parasitoid density).

Foraging females/10 host pupae	Mean number of attacked pupae	% of attacked pupae	Mean number of attacked pupae /♀	Mean number of oviposition scars per pupa	Mean number of parasitoid larvae per pupa	% of parasitoid adult emergence	Sex ratio ♀/♂
1	3.40 \pm 1.10a	34	3.40 \pm 1.11ab	1.27 \pm 0.51a		29.0 \pm 7.14a	0.89 \pm 0.24a
2	4.30 \pm 0.93ab	43	2.15 \pm 0.47a	2.24 \pm 0.49ab	1.22 \pm 0.27ab	32.2 \pm 7.09a	1.06 \pm 0.31a
4	4.70 \pm 1.00abc	47	1.18 \pm 0.25ab	3.41 \pm 1.44ab	1.15 \pm 0.22ab	28.4 \pm 6.47a	0.96 \pm 0.27a
8	7.50 \pm 0.96abc	75	0.94 \pm 0.12ab	5.30 \pm 1.24b	1.83 \pm 0.30ab	26.9 \pm 8.95a	0.48 \pm 0.17a
12	8.10 \pm 0.62c	81	0.68 \pm 0.05ab	4.20 \pm 0.72b	2.01 \pm 0.15b	43.9 \pm 7.46a	2.26 \pm 0.62a
16	7.20 \pm 0.66bc	72	0.45 \pm 0.04b	4.68 \pm 0.96b	2.04 \pm 0.36b	34.6 \pm 8.18a	1.42 \pm 0.60a

Values followed by the same letter in each column indicate that differences are not significant (one-way Anova, Tukey's test, $P > 0.05$).

the fit of the quadratic term was not significant in our results, and thus the prediction of our model indicated a type II functional response. In addition, due to the long foraging time and low attack rate by *C. haywardi*, we consider that a type II functional response might become more evident under field conditions where parasitoids are free to choose the foraging patch. De Pedro *et al.* (2016) observed that *Aganaspis daci* (Hymenoptera: Figitidae) exhibited a type III functional response when *Ceratitidis capitata* larvae were supplied with fruit and an artificial diet under laboratory conditions, but exhibited a type II functional response when the flies were under greenhouse conditions (more similar to natural conditions). Changes in the functional response type have also been shown in *D. longicaudata* allowed to freely choose among different host density patches under laboratory conditions (Núñez-Campero *et al.*, 2016).

Suitable host pupae available for *C. haywardi* females under natural conditions may not be abundant, since both the larval and pupal stages of fruit flies are attacked by multiple competitors (Aluja *et al.*, 2005), and most host pupae in the soil may have been previously parasitised. We believe that this condition may lead to the type of foraging and attack rate shown by *C. haywardi* in our study, since the distribution of suitable pupae in the ground would tend to be random, reducing thus the possibilities of interference between foraging females. However, it must be considered that the type of soil and its characteristics, together with the abiotic conditions, are factors that can also influence the search time and parasitism success (Aluja *et al.*, 2005; Okuyama, 2012). Guillen *et al.* (2002) found that *C. haywardi* females performed better in clayey soils, where foraging and host parasitism were not affected by moisture or clay compaction. This may be related to the characteristics of *C. haywardi*, such as its hypognathous head and great physical resistance (Sivinski *et al.*, 1998).

The results of the mutual interference experiment show that the attack efficiency of *C. haywardi* females is negatively affected by an increase in the density of foraging females, which became evident in the reduction in the number of pupae attacked by female. The effect of mutual interference between foraging females under laboratory conditions may be caused by high parasitoid densities or low host densities (Yang *et al.*, 2015; Rezaei *et al.*, 2019), as well as by the small dimensions of the

experimental arena (Ridout, 1981). These conditions reduce the searching capacity of the parasitoid (indirect interference) and may force it to migrate to new foraging areas under natural conditions (Visser and Driessen, 1991). However, in some cases, parasitoid females can physically fight (direct interference) for control of the host resource (Mi *et al.*, 2021). We did not detect such direct interference in our observations, and thus our results may rather be due to a low tolerance to the presence of other foraging females in the experimental arena.

Our data also show that the number of attacked pupae, number of oviposition scars, and number of immature parasitoids per pupa increased as the number of foraging females increased. This behaviour of superparasitism in experiments of mutual interference under laboratory conditions has also been observed in other parasitoids of dipteran larvae and pupae (Skovgard and Nachman, 2015; Poncio *et al.*, 2016; López *et al.*, 2021). These authors have found that, at high parasitoid densities in the presence of a low number of hosts, superparasitism increased exponentially, which is consistent with the positive correlation between the number of oviposition scars and the number of immature parasitoids per pupa obtained in our study.

Cancino *et al.* (2012) observed that *C. haywardi* females are capable of discriminating *A. ludens* pupae previously attacked by conspecific and heterospecific parasitoid species under laboratory conditions. We believe that the observed superparasitism may be caused by the competitive pressure among foraging females, the low host densities, and the ovarian loads of *C. haywardi* females (as in Montoya *et al.*, 2019), but it has been shown that *C. haywardi* females also superparasitised *A. fraterculus* pupae in field cages with a 5:1 host-parasitoid ratio (Van Nieuwenhove *et al.*, 2016). It has been reported that superparasitism has a negative effect on the emergence of adults (Tunca and Kilincer, 2009), which may be related to competition between parasitoid larvae for the host resource (Luo *et al.*, 2014). However, in the cases of *D. longicaudata* and *U. anastrephae*, moderate superparasitism has been associated with a favourable, biased female sex ratio (González *et al.*, 2007; Poncio *et al.*, 2016). Unlike the above cases, the emergence and sex ratio of *C. haywardi* were not significantly affected by superparasitism. Interestingly, sex ratio was male biased when *C. haywardi* attacked

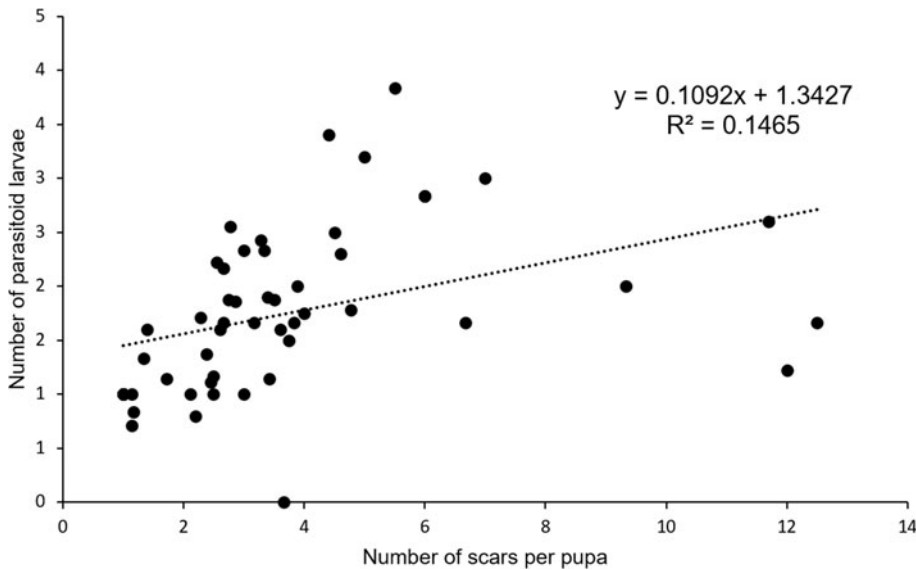


Figure 3. Correlation between the number of oviposition scars per pupa of *A. ludens* and the number of immature parasitoids of *C. haywardi* per host pupa observed in the mutual interference experiment.

host previously parasitised by *D. longicaudata* (Van Nieuwenhove *et al.*, 2016) while in the present study sex ratio was in general female biased. It is unknown if adults of this species emerging from superparasitised hosts present limitations in parameters such as flight ability, longevity, or fecundity. However, Montoya *et al.* (2019) reported that *C. haywardi* adults emerging as hyperparasitoids from *A. ludens* pupae that had been previously parasitised by the braconid *D. longicaudata* do not differ in size, fecundity, or longevity when compared to those that emerge as primary parasitoids.

Our results show that *C. haywardi* exhibits a type II functional response, and that mutual interference between foraging females decreases the attack efficiency of individual females even at low densities. They also suggest that this species could act as a complementary parasitoid against fruit fly pests, since the random distribution of fruit fly pupae in the soil would facilitate their location while reducing the possibility of aggregation and mutual interference between foraging females. This would render a better performance of *C. haywardi* as biocontrol agent.

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