

Foraging behaviour of the parasitoid *Eretmocerus eremicus* under intraguild predation risk by *Macrolophus pygmaeus*

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Abstract

BACKGROUND: Intraguild predation (IGP), predation between species that use a common resource, can affect the populations of a pest, of the pest's natural enemy (IG prey) and of the predator of the pest's natural enemy (IG predator). In this study, we determined whether the parasitoid *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) (IG prey), modifies its foraging behaviour under the risk of IGP by *Macrolophus pygmaeus* (Hemiptera: Miridae) (IG predator). Parasitoid behaviour was analysed using two bioassays (choice and no-choice) with the following treatments: (i) control, tomato leaf infested with whitefly nymphs; and (ii) PEP, tomato leaf infested with whitefly nymphs and previously exposed to the IG predator; and (iii) PP, tomato leaf infested with whitefly nymphs, with both, the IG predator and the IG prey present.

RESULTS: In both bioassays, we found that *E. eremicus* did not significantly modify the number of ovipositions, time of residence, duration of oviposition or behavioural sequence. However, in the no-choice bioassay, the number of attacks was higher and their duration shorter in the PEP treatment than in the control.

CONCLUSION: Our results indicate that the parasitoid may detect IGP risk to a certain extent, but it did not significantly modify its foraging behaviour, suggesting that simultaneous release of the two natural enemies can be successfully employed.

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Keywords: behavioural ecology; biocontrol; *Bemisia tabaci*; generalist predator

1 INTRODUCTION

The whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) is an agricultural pest of great importance worldwide.^{1,2} It can cause crop damage both directly, owing to sap extraction, and indirectly, as a vector of plant viruses.^{3–6} In addition, its impact has been augmented by its resistance to insecticides.^{7–9} In this context, the utilisation of natural enemies represents an alternative method of control. However, the use of natural enemies faces the challenge of understanding these organisms and their interactions. In environments where more than one natural enemy is present, interactions can be developed and can improve or diminish the effectiveness of control of the pest species.^{10–17}

One such interaction, intraguild predation (IGP), is predation among natural enemies that utilise the same resource.^{15,18} This type of interaction can modify the distribution, abundance and biological control efficacy of the interacting species.^{19,20} In an ecological system that includes a general predator and a parasitoid, the general predator often acts as an intraguild predator (IG predator), while the parasitoid usually represents the intraguild prey (IG prey).¹⁹ This is particularly true when coincidental IGP occurs, that is, when the IG prey develops inside the shared pest prey (herbivore) and the IG predator consumes both the shared prey and the IG prey developing inside the shared prey.¹⁹ However, IG prey species have been encountered that are able to avoid IGP

through behavioural changes in response to the presence of the IG predator.^{19,21} For example, some studies have demonstrated that some IG prey can modify their behaviour and stop foraging on patches with IGP risk.^{21–26} Thus, the behaviour of the parasitoid (IG prey) in evading IGP can play a crucial role in the survival and efficacy of all of the species involved,²⁷ with possible consequences for pest biological control.²⁸

B. tabaci is a polyphagous pest species. The females oviposit around 250 eggs over the course of their reproductive lives,²⁹ which develop into adulthood through four nymph stages.³ At present, various species of natural enemies are used for the control

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of *B. tabaci*. In Europe, for example, the predator *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) and the parasitoid *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae) are used.^{30–32}

M. pygmaeus is a predator native to the Mediterranean region³³ with demonstrated ability to control *B. tabaci* efficiently.^{33,34} It is a generalist predator, and, among other exploitable prey, it feeds on all stages of whiteflies, from eggs through adults.^{31,35} Depending on the feeding and thermal regimes, *M. pygmaeus* females can oviposit up to 150 eggs and live for 40 days.³⁶ In addition, this predator species can exert IGP over parasitoids (e.g. *E. mundus*) that share the same prey/host.^{27,37–39}

The parasitoid *E. eremicus* is a species originating in North America that is used for the control of *B. tabaci* through inundative releases.^{30,40,41} *E. eremicus* is an ecto–endo oligophagous parasitoid specialist for whiteflies.⁴² It parasitises all juvenile stages of *B. tabaci*, but second and third instars are preferred.⁴³ Females of this parasitoid lay an average of 23 eggs and require a preoviposition period of approximately 15 h. There are three larval stages before pupation, and the adults live for 14 days on average.⁴² In addition, it has been shown that *E. eremicus* can suffer from IGP; for example, by the generalist predator, *Geocoris punctipes* (Say) (Hemiptera: Lygaeidae).⁴⁴

M. pygmaeus and *E. eremicus* have both been demonstrated to be efficient natural enemies of *B. tabaci* and can be released simultaneously for control of this pest.^{45,46} However, it is known that *M. pygmaeus* can engage in IGP on immatures of this wasp (Desneux N, unpublished data) and those of *E. mundus* in the laboratory.³⁷ Therefore, it is possible that IGP interactions could take place between these natural enemies in the field. In addition, understanding the nature of this interaction is crucial for both pest biological control and behavioural ecology studies. The aim of the present study was to determine whether and how *E. eremicus* females are capable of modifying their foraging behaviour in order to avoid the risk of falling victim to IGP.

2 MATERIALS AND METHODS

2.1 Biological material

2.1.1 Tomato plants

Commercial tomato seeds (*Solanum lycopersicum*) var. Marmande were used. These were planted in plastic pots (7 cm × 7 cm × 10 cm) containing 50% organic soil (TONUSOL®; Agriver, Saint Papoul, France) and 50% perlite (Perlite Italiana®; AGRILIT, Corsico, Italy) and fertilised with a nutrient solution composed of mineral salts, trace elements and macro- and micronutrients. Pesticide applications were strictly avoided. The plants were used when they reached the 5–7-leaf developmental stage. The plants were maintained free of herbivores until their use in the experiments, under the following controlled conditions: temperature 24 ± 2 °C, relative humidity 70 ± 5% and a 16:8 h light:dark photoperiod.

2.1.2 *Bemisia tabaci*

The whiteflies used were from the colony maintained at the facilities of the Theoretical Ecology and its Applications to Protect Environment Agrosystems research unit of the National Institute of Agronomic Research (INRA, Sophia Antipolis, France). This colony was established in 2010, starting from individuals provided by Dr Olivier Bonato of the CBGP (Centre de Biologie pour la Gestion des Populations) of Montpellier, France. The virus-free whiteflies were

maintained on tobacco plants (*Nicotiana tabacum* L.) under the following controlled conditions: temperature 24 ± 2 °C, relative humidity 70 ± 5% and a photoperiod of 16:8 h (light:dark).

2.1.3 *Eretmocerus eremicus*

The parasitoids used in these experiments were provided by Koppert B.V., the Netherlands (ERCAL®; cardboard strips with parasitised whitefly pupae). Upon their arrival in the laboratory, they were maintained in transparent plastic cylinders (20 cm in diameter × 40 cm in length), and the emerged adults were fed with pure honey droplets (Le Rucher aux Marmottes, Antibes, France) placed on a petri dish lid (4 cm in diameter), and were provided with tap water through a soaked paper napkin (1 cm²; Sopalin, Groupe Délipapier-Sofidel, France) in the base of the petri dish. The parasitoids were kept in the laboratory under the previously described environmental conditions. To increase the probability of mating, groups of 30 parasitoids, 15 females and 15 males, were placed together in petri dishes (9.5 cm in diameter) on the first day after emergence. In these petri dishes, the parasitoids were provided with honey diluted with water, in a 7:3 ratio (honey:water), with water via a soaked piece of napkin (~1 cm²) and with a tomato leaf infested with whitefly nymphs, with the objective of the wasps having access to hosts and avoiding reabsorption of their eggs.⁴⁷ Individual *E. eremicus* parasitoids were used in the experiments when they were 2–5 days old, as it is known that they are able to oviposit when they are 1 day old.⁴⁷

2.1.4 *Macrolophus pygmaeus*

The individual predators (one-week-old adults) were provided by BIOTOP (Valbonne, France). Upon their arrival, they were maintained in acrylic cages (60 cm × 30 cm × 30 cm) containing two tomato plants. The cages were kept in a climate-controlled room under the following conditions: temperature 25 ± 2 °C; relative humidity 70 ± 5% and a 16:8 h light:dark photoperiod. Individual *M. pygmaeus* were fed eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) provided by BIOTOP, France. The *E. kuehniella* eggs were provided to the predator inside the cage on the leaves of the tomato plants, and in the base of two petri dishes (9.5 cm in diameter) as well. Every third day, approximately 3 g of prey eggs was placed in the cage. The predators were used when they were 7–17 days old, as it is known that they require a preoviposition period of 5 days.³⁶

2.2 Foraging behaviour of *E. eremicus*, no-choice bioassay

Each day, between 8:30 a.m. and 12:30 p.m., a female parasitoid was introduced into a petri dish (containing an 8.5 cm diameter moist napkin on the bottom). This petri dish will henceforth be referred to as the 'no-choice arena'. Each no-choice arena contained one of the following three treatments: (1) the control (C); (2) previous exposure to the predator (PEP); and (3) the presence of the predator (PP). Treatment C consisted of a tomato leaf with 60–80 second–third stage whitefly nymphs. Before their use in the no-choice arena, the tomato leaves with whitefly nymphs were kept inside plastic jars (2 cm in diameter × 10 cm in height) containing wet floral foam at their bases (Oasis®; Smithers-Oasis, Kent, OH), in which the petiole of the leaf was inserted in order to maintain hydration.⁴⁸ The lid of the jar had a hole (1.5 cm in diameter) covered with organdy in order to allow ventilation. This plastic jar with the tomato leaves and whitefly nymphs, prepared 24 h before the observation, will henceforth be referred to as the

'preparative jar'. Before the initiation of the observation, the leaf with whitefly nymphs was removed from the preparative jar and placed in the no-choice arena.

For the PEP treatment, the preparative jars were prepared as for the C treatment, but at the same time, a female predator was added and allowed to prey upon the nymphs for 24 h. During this period of time, predators only marginally preyed upon nymphs; therefore, it was assumed that the density of prey was similar in all treatments. The female predator was removed from the preparative jar 10 min before the initiation of observation, and the leaf with the whitefly nymphs and traces of the predator (e.g. faeces and other traces than can serve as cues for the parasitoid) was placed in the no-choice arena.

For the PP treatment, the preparative jar was prepared as for the C treatment, but 10 min before initiation of observation, the leaf with the nymphs was transferred to the no-choice arena, and at the same time a female predator was introduced into the arena and allowed to forage concomitantly with the wasp. In all of the treatment conditions, observation was initiated when the female parasitoid was introduced into the arena. We followed a randomised block design in which time was considered to be the blocking factor. Each treatment was replicated 20 times. For each replication, we used new insects and new leaves in order to avoid pseudoreplication.

For each parasitoid female, the number of attacks and ovipositions on whitefly nymphs was recorded, along with the residence time (i.e. the time the parasitoid spent foraging on the leaf in each of the individual treatments) and the behavioural sequence of foraging on the nymphs. To discriminate between an attack and an oviposition, we used information reported by Ardeh *et al.*¹⁰ Based on this information, an attack occurred when the wasp ovipositor was inserted into a whitefly nymph for 50 s or less. Meanwhile, oviposition occurred when the insertion of the ovipositor was greater than 50 s. These response variables were recorded for each female parasitoid for 1 h of continuous observation. Wasp behaviour was observed through a digital CMOS camera integrated into a stereomicroscope (Leica EZ4 HD) and connected to a digital monitor. Based on preliminary observations, Table 1 was constructed and shows the observed foraging behaviour of *E. eremicus* on tomato leaves infested with whitefly nymphs. The occurrence, frequency and duration of all of the observed behaviours were recorded using the software Etholog v. 2.2.⁴⁹

Datasets were first tested for normality and homogeneity of variance using the Kolmogorov–Smirnov *D*-test and Cochran's test respectively, and transformed (using $\sqrt{x} + 0.5$) when needed. Then, data were analysed using linear mixed models with restricted maximum likelihood estimation.⁵⁰ The dependent variables were: (1) the number of attacks; (2) the number of ovipositions; (3) the residence time; (4) the duration of attacks; (5) the duration of oviposition. The fixed factor in all five cases was the treatment conditions with respect to presence, absence or signs of the IG predator (C: control; PEP: previous exposure to predator; PP: predator presence). Blocks were considered to be random effects, except in the model used for studying residence time, in which the number of ovipositions was considered to be a random effect. When the effect of the fixed factor was significant, pairwise comparisons performed to study different treatments for a given dependent variable were carried out using the least significant difference (LSD) adjustment method for multiple comparisons.

The behavioural sequences were analysed following the procedure described in detail by Ramirez-Romero *et al.*⁵¹ This method

Table 1. Foraging behaviours of the parasitoid *Eretmocerus eremicus* on a tomato leaf harbouring *Bemisia tabaci* nymphs, as analysed in this study

Behaviour	Symbol	Description
Antennation	A	The parasitoid touches the whitefly nymphs with its antennae
Attack	K	The parasitoid attacks the whitefly nymphs with its ovipositor for both probing or oviposition
Walking	W	The parasitoid walks on the leaf
Cleaning	C	The parasitoid cleans its antennae, legs, ovipositor or wings
Resting	R	The parasitoid pauses and stops activity
Ovipositor dragging	O	The parasitoid withdraws its ovipositor and drags it on the leaf
Legs	L	The parasitoid touches the nymphs with its legs
Suction	S	The parasitoid inserts its mouthparts into the nymph and sucks its hemolymph

consists of analysing the behavioural transitions and grouping them into a global matrix, which is then compared with an expected matrix using a G-test. The results of this analysis were then represented graphically through ethograms.^{44,51}

2.3 Foraging behaviour of *E. eremicus*, choice bioassay

In this bioassay, one parasitoid female was introduced into a plastic petri dish (9.5 cm in diameter), similar to that described in detail by Velasco-Hernández *et al.*⁴⁴, which will henceforth be referred to as the 'choice arena'. Briefly, this arena simultaneously contained all three treatments described for the no-choice bioassay (C, PEP and PP), prepared with the same methodology and materials. The treatments were physically separated with plastic dividers. Each division contained 56 holes, ~2 mm in diameter each, which allowed the passage of the wasp, but not the predator, between treatments. Each parasitoid female was released in the centre of the arena.

Each day, between 8:30 a.m. and 12:30 p.m., a female parasitoid was introduced into the choice arena and observed over 8 h as described below. Each female was observed for the first 8 min of each observation hour, according to a modified variation of the instantaneous sampling method.⁵² This procedure was adopted on the basis of pilot observations and other published works on behaviour (e.g. Velasco-Hernández *et al.*⁴⁴ and Batchelor *et al.*⁵³). Each female was then observed for a total of 64 min, and this constituted one replication. This bioassay was replicated 30 times (about 25 h of observation). In order to avoid pseudoreplication, for each new replication, new insects and new leaves were used. Choice bioassays were conducted under the same environmental conditions and with the same tools as those previously described for the no-choice experiment.

For each female parasitoid, the following parameters were recorded: the residence time (i.e. the time spent by the parasitoid foraging on the leaf in the distinct treatments), the number of attacks on the whitefly nymphs and the frequency of choice of each treatment by the wasp. The number of attacks and the residence time were analysed as described for the no-choice bioassay, with the exception that, for residence time, we considered the number of attacks to be a random factor. Proportions of selection

were compared using the Marascuilo procedure.⁵⁴ All statistical analyses were performed using SPSS® software v.20.0.0 (IBM SPSS Statistics, Armonk, NY).

3 RESULTS

3.1 No-choice bioassay

The number of wasp attacks differed significantly according to treatment ($F_{2,38} = 8.591$; $P = 0.001$). In particular, the number of parasitoid attacks was significantly higher when the patch was previously exposed to the predator (Fig. 1a). Significant differences among treatments were also found for the time spent by the parasitoids attacking, the longest time being in treatment C ($F_{2,503} = 3.186$; $P = 0.042$) (Fig. 1b). In contrast, no significant differences among treatments were found for the number of ovipositions ($F_{2,38} = 0.782$; $P = 0.465$) (Fig. 1c) or oviposition duration ($F_{2,411} = 0.770$; $P = 0.464$) (Fig. 1d). Similarly, no significant differences among treatments were recorded for the time of residence ($F_{2,38} = 0.940$; $P = 0.347$) (Fig. 1e).

Plotting of the foraging behaviour of *E. eremicus* revealed a relatively simple, stereotyped pattern of behaviour by the parasitoids (Fig. 2). Under the three treatment conditions, the female parasitoids showed four principal behaviours: walking, antennation, cleaning and attacking. The remainder of the behaviours (see Table 1) are not presented graphically because they occurred at relatively low frequency (<0.03). The behavioural sequence of the wasps in treatment C (Fig. 2a) was as follows: the wasp began by walking, then proceeded either to clean (39.1%) or to antennate (60.9%). If the wasp cleaned itself, afterwards it antennated (19.6%) or returned to walking (80.4%). If the wasp performed antennation after the initial walking behaviour, it then either returned to walking (26.2%) or performed an attack (73.8%). After finishing an attack, the wasp generally returned to cleaning (63.1%) or, less frequently (36.9%), to walking. The female parasitoids in the PEP treatment exhibited a sequence similar to the control group, with one difference: when finishing an attack, the wasp performed walking or cleaning with equal frequency (39.1%), or, less frequently (21.8%), antennation; this last sequence (attacking–antennation) is a random sequence (Fig. 2b). In the PP treatment, the behavioural sequences were also similar to those seen in the control treatment, with the sole difference also taking place after the attack behaviour. After finishing an attack, the wasp frequently tended to clean (46.2%), and less frequently, walked (32.6%) or performed an antennation (21.2%), resulting in these two final, random sequences (Fig. 2c).

During our observations, we found that, in the PP treatment, only two adult wasps out of 20 (10%) were directly attacked by the predator.

3.2 Choice bioassay

No significant differences were recorded between treatments in the number of parasitoid attacks ($F_{2,87} = 0.051$; $P = 0.950$) (Fig. 3a), time spent by the parasitoid foraging on the patch ($F_{2,87} = 1.278$; $P = 0.284$) (Fig. 3b) or proportion of selections (Fig. 3b) when C was compared with PEP ($\chi^2_1 = 0.263$; $P = 0.608$) or PP ($\chi^2_1 = 0.000$; $P = 0.999$). A similar result was obtained when comparing the proportion of selection between PEP and PP treatments ($\chi^2_1 = 0.263$; $P = 0.608$) (Fig. 3c). During our observations, in the PP treatment, three adult wasps out of 30 (10%) were directly attacked by the predator.

4 DISCUSSION

M. pygmaeus and *E. eremicus*, both natural enemies of whiteflies, are often used together for the control of *B. tabaci*.^{45,46} However, it is known that *M. pygmaeus* can engage in IGP on immatures of this wasp (Desneux N, unpublished data) and those of *E. mundus* in the laboratory.³⁷ Therefore, understanding the nature of this interaction is crucial for both pest biological control and behavioural ecology studies. In the present study, we analysed, in the laboratory, the behavioural response of the parasitoid to conditions characterised by the risk of IGP. Overall, our results show that *E. eremicus* does not modify its foraging behaviour in situations in which the risk of IGP exists. Parameters such as the number and duration of ovipositions, the residence time and the behavioural sequence were similar in all of the treatments. The only parameters that were modified were the number and duration of attacks. We observed that the predator did exert IGP on *E. eremicus* adults, even when whitefly prey were available, but that the frequency of attacks was relatively low (only 10% of the adult wasps were attacked in both bioassays).

4.1 No-choice bioassay

Various ecological factors can modulate IGP; for example, the availability of the primary prey, the host plant, the presence of competitors and, notably, the behaviour of the IG prey.^{26,55–58} The behaviour of an IG prey, when faced with the risk of IGP, can be modified or not. Previous studies have demonstrated that some IG prey under risk of IGP modify their behaviour in order to avoid predation.^{20,59} Such behavioural modifications can include a decrease in certain parameters (e.g. foraging time and residence time) or an increase in others (e.g. the rate of parasitism and oviposition).^{26,27,44} It has been postulated that these behavioural modifications are related to the presence of chemical cues left by the IG predator and their detection by the IG prey.^{21–26} In these cases, the results are intuitive, as behavioural modifications leading to avoidance of IGP (e.g. reducing residence time and foraging time in threatening situations) should be adaptive for the IG prey.^{21,23} However, evasion of the IG predator is not the sole behavioural response of the IG prey; for example, some IG prey remain in the high-risk zone and increase certain behaviours, such as rates of attack and oviposition (e.g. Taylor *et al.*,²² Martinou *et al.*,²⁷ Velasco-Hernández *et al.*⁴⁴ and the present results). Although this could seem counterintuitive, the fact that the IG prey does not avoid the IG predator is not necessarily counteradaptive. Indeed, if instead of avoidance, the IG prey increases its rate of parasitism under IGP risk, and thereby the number of surviving descendents, its fitness will be increased overall. In light of this, it would be interesting empirically to analyse both behavioural possibilities (i.e. avoidance versus confrontation) in terms of their impact on fitness or parasitoid demography, which to our knowledge has not been done.

In our study, we observed that the predator *M. pygmaeus* left certain traces that might allow detection, such as excrement and eggs (PEP treatment), which could represent chemical footprints.^{21–23} Additionally, these traces could also be present together with the IG predator itself (PP treatment). Our results, however, showed that *E. eremicus* did not change its behaviour significantly in the treatments with risk of IGP (i.e. PEP and PP) compared with the control. The only behavioural change that was detected was that in the PEP treatment the wasp increased the number of attacks on the whitefly nymphs (Fig. 1a) and reduced the duration of these attacks (Fig. 1b). However, we do not believe that these behavioural

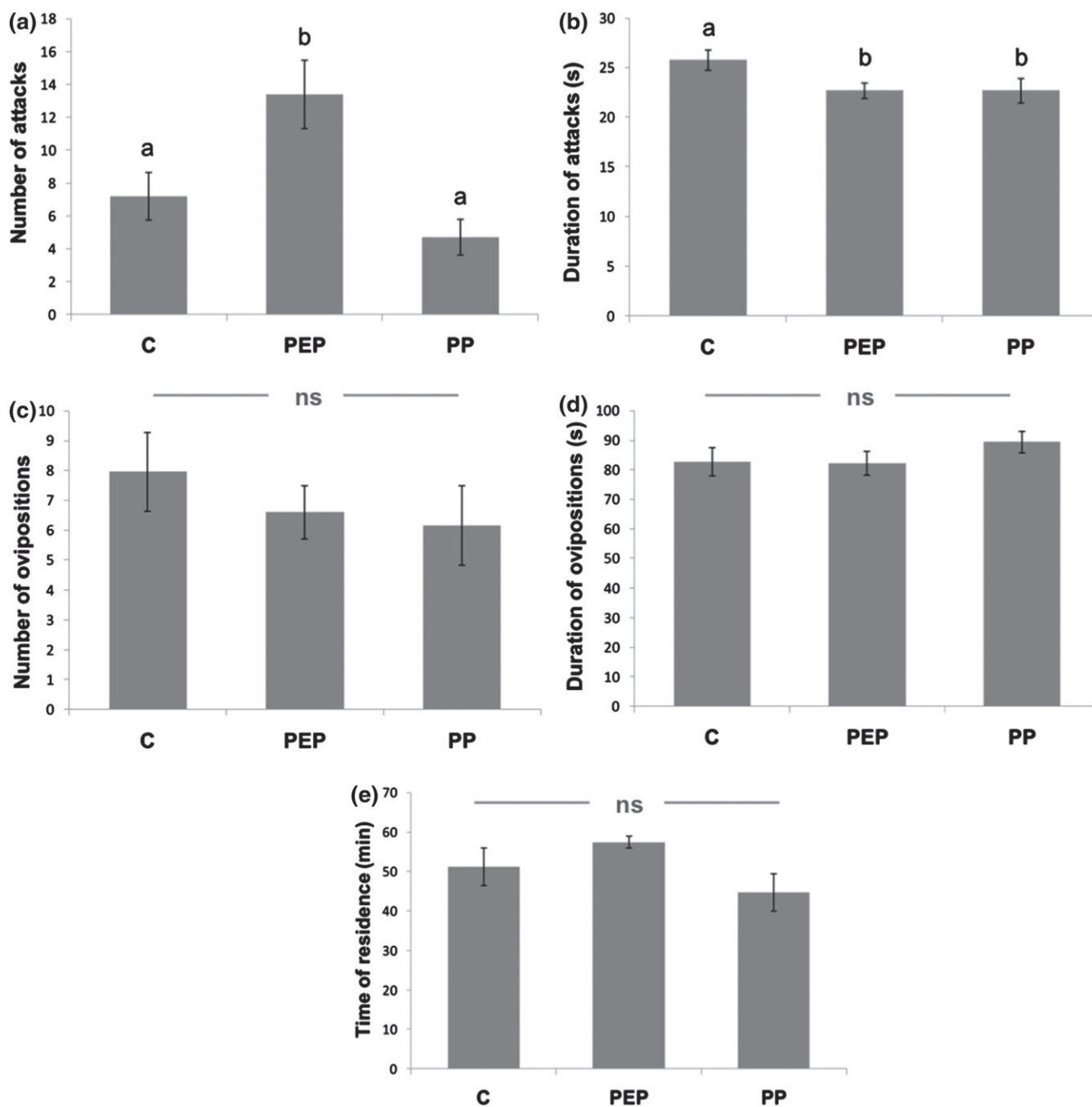


Figure 1. Behavioural parameters of *E. eremicus*, measured in the presence of *B. tabaci* nymphs in a no-choice bioassay. The wasps were subjected to three treatments: C = control; PEP = previous exposure to the predator; PP = predator presence. *M. pygmaeus* was used as the predator. (a) Number of attacks; (b) duration of attacks; (c) number of ovipositions; (d) duration of oviposition; (e) residence time. Columns bearing different letters represent significant differences (at $P < 0.05$) among treatments. ns = non-significant differences (at $P < 0.05$).

modifications necessarily confer an advantage for the wasp in the presence of IGP risk. In terms of evolutionary fitness, these behavioural modifications do not confer a clear benefit, as an attack does not necessarily represent the depositing of an egg.^{43,60} Nevertheless, to support or refute this hypothesis, it will be necessary to determine whether there are a number of eggs laid during the attacks and the consequent emergence rate. On the one hand, if this behavioural modification is not adaptive, it is possible that it could just be a behavioural reaction to the stimulus of proximity of the IG predator. Roitberg *et al.*⁶¹ mention that the

decision to attack will depend on the degree of danger for the parasitoid, because if the parasitoid detects a high degree of danger, it can decide to increase its rate of attacks (Fig. 1a). In addition, reduction in attack time in the PEP and PP treatments could be related to the aforementioned stimulus, because in the control treatment (C) the wasp took more time for its attacks (Fig. 1b). The same could hold true for the behavioural sequences (Fig. 2), as the parasitoids presented stereotypical behavioural sequences in the control treatment, but in the PEP and PP treatments they showed random sequences. This could indicate that the presence of the

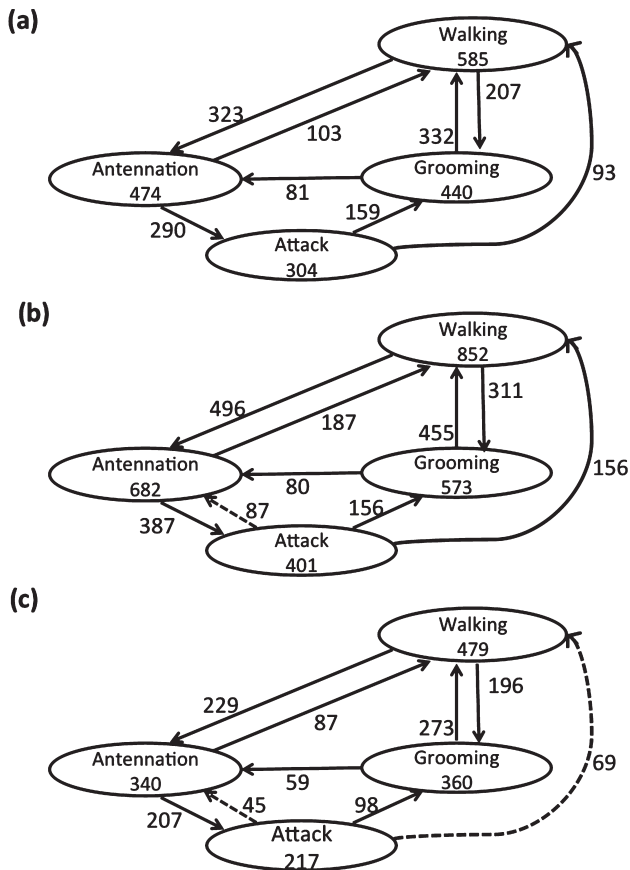


Figure 2. Ethograms of the no-choice bioassay, showing the foraging behavioural sequence of the parasitoid *E. eremicus* in each of the treatments: (a) control (C); (b) previous exposure to the predator (PEP); (c) presence of the predator (PP). The numbers in circles represent behaviour repetitions, and the numbers beside the arrows represent the number of transitions. Solid arrows represent those transitions that were significantly different from the expected (not random), and dotted lines represent transitions that were not significantly different from the expected (random). Transitions with a relative frequency of 3% or less are not shown.

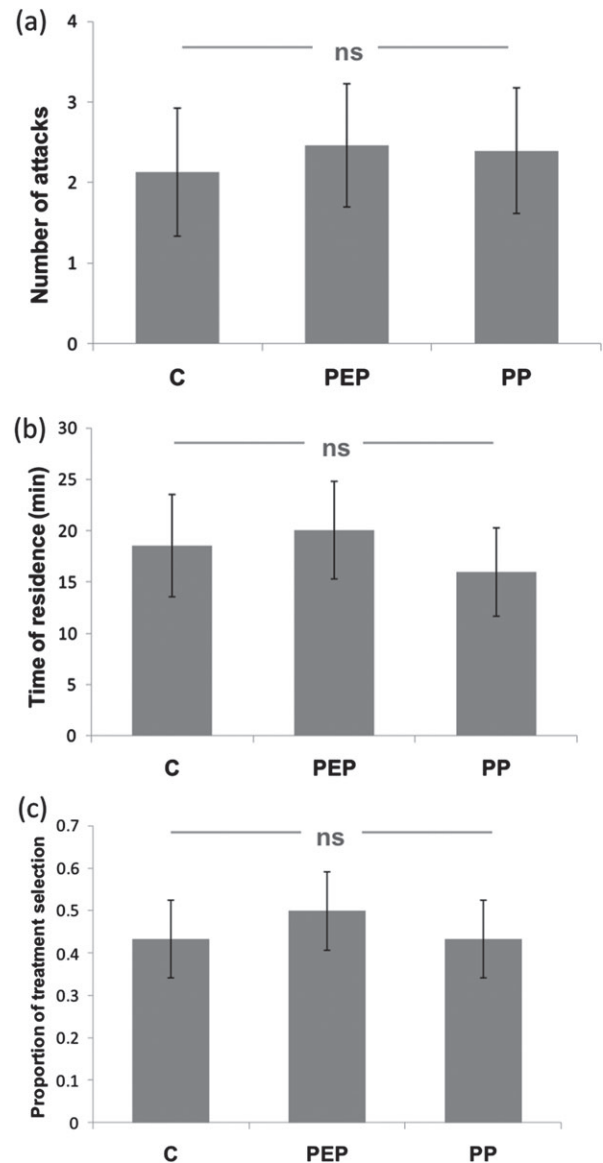


Figure 3. Behavioural parameters of *E. eremicus* in the presence of *B. tabaci* nymphs under a choice bioassay. The wasps were subjected to three treatments: C=control; PEP=previous exposure to the predator; PP=presence of the predator. *M. pygmaeus* was used as the predator. (a) Number of attacks; (b) time of residence; (c) proportion of time spent in the treatment selection. ns = non-significant differences (at $P < 0.05$).

predator or its traces may cause the wasp to switch to a different, haphazard behaviour pattern.

M. pygmaeus is a generalist predator that is used for the biological control of various species of pests.^{17,32} However, it has been reported as an IG predator of various species of parasitoids.^{27,37–39} In our observations we found that, although *M. pygmaeus* attacked *E. eremicus* adults, it only did so in 10% of the exposed individuals. This contrasts with the observation of Velasco-Hernández *et al.*⁴⁴ of a high rate of IGP of *G. punctipes* on *E. eremicus* adults (90%). This indicates that, for *E. eremicus*, the rate of IGP on adult wasps is particularly influenced by the IG predator species with which they are faced.

4.2 Choice bioassay

When the parasitoid was exposed in the choice bioassay, the number of attacks, the residence time and the proportion of time in each treatment choice were similar among the treatments. This result supports the observation in the no-choice bioassay that the parasitoid does not significantly modify its behaviour when the predator or its traces are present. In the choice bioassay we also found that relatively few adult wasps were attacked by the predator (10%), suggesting that the parasitoid and the IG

predator can coexist if released together for biological control. This low rate of attack may also help to explain the lack of any significant behavioural modification by *E. eremicus* in response to *M. pygmaeus*, even in the choice bioassay. In terms of coexistence, if this predator is only a minor threat to the wasp, there may not have been sufficient selection pressure for the development of specific avoidance behaviours. This bodes well for biological control applications, as it suggests that releasing these two natural enemies together would have null or only minimal effects on the foraging behaviour of *E. eremicus*.

5 CONCLUSIONS

Our results show that the parasitoid *E. eremicus* does not significantly modify various parameters of its behaviour when faced with

the risk of IGP by *M. pygmaeus*. However, the number of attacks and their duration were modified when the wasp was confronted with a treatment in which traces left by the predator were present. Finally, we found that *M. pygmaeus* exerted relatively low rates of IGP on *E. eremicus* adults. Although further studies in real field conditions may be needed before drawing final conclusions, taking our results as a whole, we have reasons to believe that the simultaneous release of these two biological control agents can be successfully employed for whitefly biological control programmes.

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