# The Neotropical mirid predator *Macrolophus basicornis* uses volatile cues to avoid contacting old, *Trichogramma pretiosum* parasitized eggs of *Tuta absoluta*

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## Abstract

Polyphagous mirid predators are increasingly used in commercial, augmentative biological control. Information about their foraging behaviour is essential, especially if one intends to use several natural enemies to control one or more pests in a crop, to detect if negative intraguild effects occur. We studied a case of intraguild predation (IGP) involving the predator *Macrolophus basicornis* (Stal) (Hemiptera Miridae) of the worldwide invasive South American tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera Gelechiidae) and explored how this predator deals with prey parasitized by *Trichogramma pretiosum* (Riley) (Hymenoptera Trichogrammatidae). Behavioural observations show that *M. basicornis* predators contacted significantly fewer old, parasitized eggs of *T. absoluta* than recently parasitized eggs. Olfactometer tests revealed that predators could smell differences between volatiles of tomato leaves infested with eggs of different qualities to locate suitable prey. They preferred volatiles from leaflets with unparasitized eggs above control leaflets and, moreover, preferred volatiles from leaflets with recently parasitized eggs over volatiles of leaflets with 5-day-old parasitized eggs. When predators and parasitoids are used together to control *T. absoluta*, parasitoids should be introduced one week before predators are released to prevent high levels of IGP.

**Key words:** prey selection, predator repellence by parasitized prey, intraguild predation, competitive exclusion, biological control of invasive pest, South American tomato leaf miner, *Phthorimaea absoluta*.

## Introduction

Biological control, which uses natural enemies to reduce populations of pest organisms, is a powerful nonpolluting method to manage the impacts of (invasive) pests and has been used for centuries but has become particularly popular since the 1880s (DeBach, 1964; van Lenteren, 2005). Recently, a specific guild of natural enemies, polyphagous predatory mirid species, is increasingly used in augmentative, commercial biological control of pests (van Lenteren *et al*., 2021; Urbaneja *et al*., 2022). Although they are applied to large areas (Ferracini *et al*., 2019) and are successfully controlling globally occurring devastating pests such as whiteflies -including *Bemisia* and *Trialeurodes* species (Yao *et al*., 2022)- and lepidopterans - including *Tuta* and *Spodoptera* species (Pineda *et al*., 2020)-, surprisingly little is known about their prey searching behaviour. According to Wheeler (2001), mirids seem to search unsystematically and discover prey by touching it with their antennae and/or tip of the rostrum. Although we know that mirid predators do make use of herbivore-induced plant volatiles in prey finding (Molla, 2013; Lins *et al*., 2014; Silva *et al*., 2018), studies on foraging decisions of hemipteran predators revealing when and how the predator decides to feed or not have rarely been done (Ruberson and Kring, 1991; Wheeler, 2001; Marzieh, 2021).

Understanding the foraging behaviour of mirid predators is of general importance for the design of predator release programs and of particular significance when different species of natural enemies are introduced into the same crop because negative intraguild effects may arise. Intraguild predation (IGP) is a phenomenon in which competing species prey on each other as well as on shared prey (Polis and Myers, 1989), which can influence the success of biological control. An example is the combined use of the mirid predator *Nesidiocoris tenuis* (Reuter) (Hemiptera Miridae) and the egg parasitoid *Trichogramma achaeae* Nagaraja et Nagarkatti (Hymenoptera Trichogrammatidae) to control *Tuta absoluta* (Meyrick) (Lepidoptera Gelechiidae) [= *Phthorimaea absoluta* Meyrick (Chang and Metz, 2021)] in tomato crops in Spain (Cabello *et al*., 2015). Similarly, the use of the Neotropic mirid *Macrolophus basicornis* (Stal) (Hemiptera Miridae) together with the parasitoid *Trichogramma pretiosum* (Riley) (Hymenoptera Trichogrammatidae) is considered for control of the same pest in Brazil (Bueno *et al*., 2023a). These types of interactions are mostly asymmetric and favourable for the predator, particularly for polyphagous predators that consume large amounts of parasitized prey by intraguild predation. However, parasitized prey may become unsuitable for consumption, and in that case, the predator is negatively affected through a process called competitive exclusion (Tilman, 1982).
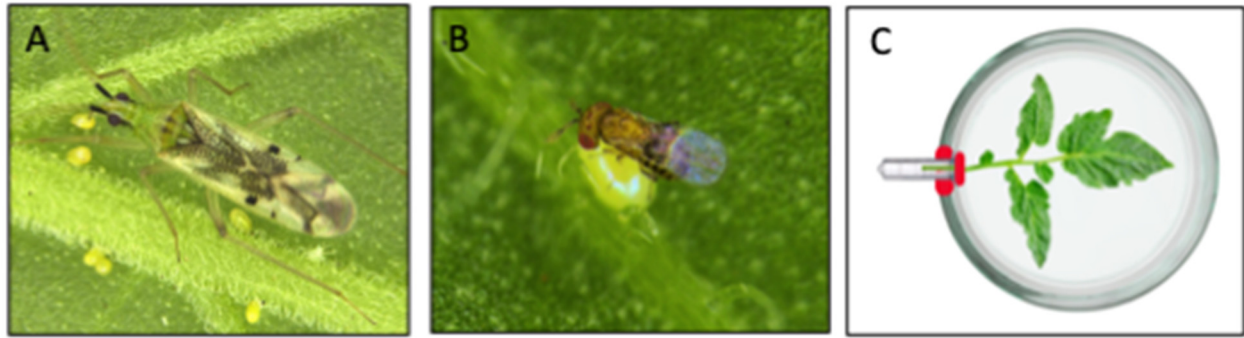
**Figure 1.** Model study organisms used: **A**) mirid predator *M. basicornis* sucking on a *T. absoluta* egg, **B**) female *T. pretiosum* parasitizing a *T. absoluta* egg and **C**) tomato plant leaflet with *T. absoluta* eggs as used in the experiments.

Mirid predators such as *N. tenuis* and *Macrolophus pygmaeus* (Rambur) (Hemiptera Miridae) are known to consume recently parasitized prey eggs as well as unparasitized eggs, but hardly feed on parasitized prey eggs containing prepupal or pupal stages of the parasitoid (Chailleux *et al*., 2013; Cabello *et al*., 2015). When we know how mirids find and evaluate parasitized prey, we might be able to fine-tune natural enemy release programs to prevent or reduce the consumption of parasitized prey eggs.

Hence, we studied the prey selection of *M. basicornis* when exposed to eggs of *T. absoluta* that are unparasitized or parasitized by the egg parasitoid *T. pretiosum* through direct observation and by olfactometer experiments (figure 1). The main aim of this study was to answer the question of whether competitive exclusion by the parasitoid takes place by influencing the mirid's foraging behaviour when encountering parasitized eggs. We hypothesize that (1) either *M. basicornis* rejects old, parasitized prey after having contacted them with their rostrum, or (2) the predator perceives the condition of these eggs by olfactory cues and avoids approaching them.

**Materials and methods**

Plants and insects

Tomato plants *Solanum lycopersicum* cv. Santa Clara L. (Solanaceae) were reared in pots and used in pest insect rearing after they reached a height of 30 cm. Adults of the pest insect *T. absoluta* were collected from tomato plants in Sao Paulo State, Brazil, and maintained in mesh cages (90 × 70 × 70 cm) with tomato plants in the laboratory according to Pratissoli and Parra (2001). New tomato plants were regularly placed into cages to maintain a stock rearing of *T. absoluta* at 25 ± 2 °C, RH 70 ± 10% and 12 hours photophase. Newly emerged adults from this rearing were collected and allowed to lay eggs for use in experiments.

The predator *M. basicornis* was collected on tobacco *Nicotiana tabacum* (L.) (Solanaceae) near Lavras, Minas Gerais, Brazil and reared as previously described (Bueno *et al*., 2013; Bueno *et al*., 2018) using tobacco plants as oviposition substrate and with UV-irradiated *Ephestia kuehniella* Zeller (Lepidoptera Pyralidae) eggs as prey in

climate rooms at 25 ± 2 °C, 70 ± 10% RH and a photoperiod 14:10 (L:D). Female *M. basicornis* predators of up to seven days old were used in the experiments and had been starved for 24 hours but had access to water. The parasitoid *T. pretiosum* was obtained from Koppert Biological Systems Brazil and then reared on UV-irradiated eggs of *E. kuehniella* in climate rooms at 25 ± 1 °C, 70 ± 5% RH and a photoperiod of 16:8 (L:D) (Parra, 1997). Adult parasitoids used in the experiments were less than 24 hours old. All plant and insect rearing, as well as the behavioural experiments were performed in the Laboratory of Biology of Insects, while the olfactometer experiments were performed at the Laboratory of Chemical Ecology and Insect Behavior. Both laboratories are part of the Department of Entomology and Acarology at the College of Agriculture "Luis de Queiroz" (ESALQ), University of São Paulo (USP), Piracicaba, Brazil.

Behavioural observation of prey searching and predation

To test the first hypothesis - the predator rejects old, parasitized prey after having contacted them with their rostrum - the behaviour of *M. basicornis* towards five unparasitized versus five parasitized eggs of *T. absoluta* was observed and video recorded for 2 hours with the aid of a DinoCapture 2.0 microscope (magnification 7×) connected to a laptop computer. *T. absoluta* eggs were placed with a fine paint brush on a tomato leaflet, and their position and condition (parasitized or not) were noted (figure 2). The interval between introduction of the predator into the Petri dish and the start of feeding, the number of contacts and the type of contact with the prey (encounter, feeding) were recorded. In this choice experiment, 1-day unparasitized eggs were tested against eggs at 1-, 5- and 9-days after parasitism by *T. pretiosum*. The number of replicates varied between 28 and 36 (supplemental material table S1).

Although *T. absoluta* egg development takes up to 4 days (Silva *et al*., 2015), only unparasitized eggs of maximally 24 hours old were used in all tests. To be sure those older, unparasitized eggs are not of inferior quality, we performed a choice test offering young and old unparasitized prey eggs. *T. absoluta* eggs of 3-4 days old were consumed as frequently as eggs of maximally 1-day-old (for details, see Bueno *et al*., 2023b).
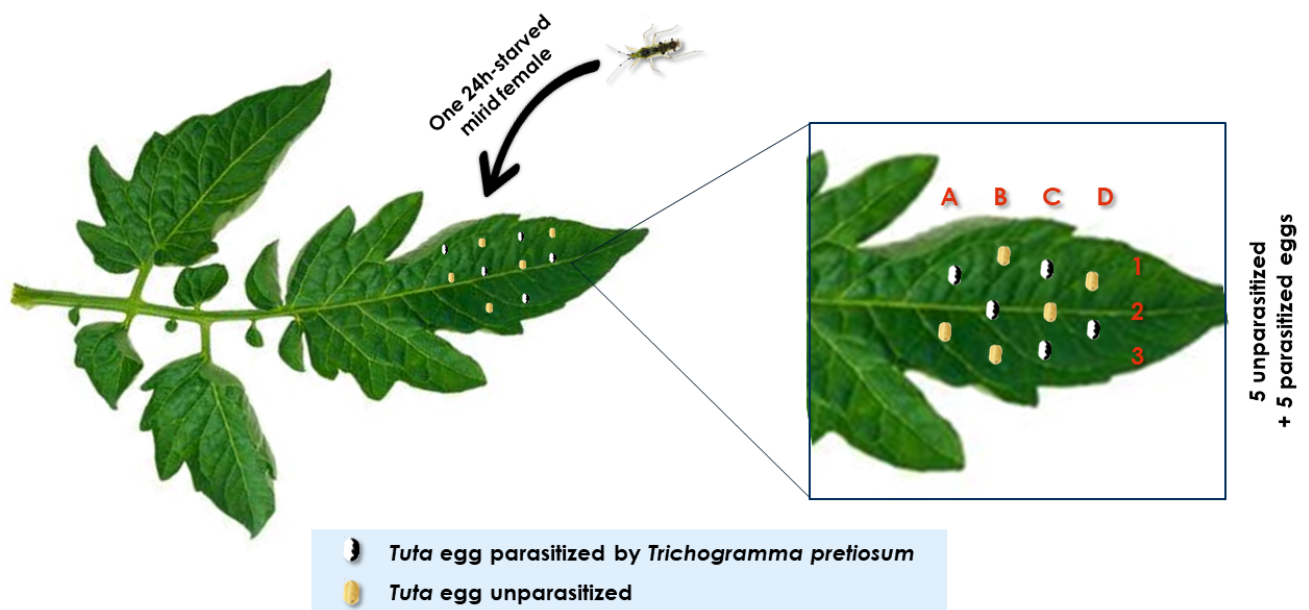
**Figure 2.** Experimental setup of the behavioural observation experiment. Tomato leaflet with five unparasitized and five parasitized eggs of *T. absoluta.* Letters and numbers are used to follow the position of the predator *M. basicornis* during the 2 hours observation period.

To obtain parasitized eggs, *T. absoluta* eggs laid during the previous 24 hours were exposed to *T. pretiosum* parasitoids for 24 hours. After 24 hours, the parasitoids were removed, and the 24-48 h-old *T. absoluta* eggs, which thus contain parasitoid eggs of 0-24 hours, were offered to the predator *M. basicornis*. In the test with 5-day-old parasitized eggs, the parasitoids were 96-120 hours old, and in the test at 9-day-old parasitized eggs, the parasitoids were 192-216 hours old. Egg-adult development of *T. pretiosum* takes on average 10 days at 25 °C (Consoli *et al*., 1999). The immature parasitoid is in the egg-larval stage in 1-day-old parasitized prey eggs, in the early pupal stage in 5-day-old parasitized eggs, and in the late pupal stage in 9-day-old parasitized eggs when reared at 25 ± 1 °C, 70 ± 5% RH and a photoperiod of 16:8 (L:D). In 1-day-old parasitized eggs, it is not possible to see whether the *T. absoluta* eggs have all been parasitized. To determine the average percentage parasitism of these eggs, 18 batches of 100 eggs were exposed to *T. pretiosum* for 24 hours, and the parasitism results were determined after 5 days when the parasitized eggs had turned black. The average percentage parasitism of the 1800 eggs in the 18 replicates was 91.00% (SE ± 1.43). Thus, in the experiments with 1-day-old parasitized prey eggs, on average less than 10% of the eggs were unparasitized. In the tests with 5- and 9-day-old parasitized eggs, the parasitism percentage was always 100%, as only dark-coloured parasitized eggs were transferred to a tomato leaflet for exposure to the predator.

## Olfactometer experiments

To test the second hypothesis - predators perceive the parasitism condition of prey eggs by olfactory cues - we assessed the responses of female *M. basicornis* to volatiles produced by the host plant and by unparasitized or parasitized eggs on the host plant.

Y-tube olfactometer setup

We used a dynamic airflow Y-tube olfactometer set-up made from glass as described in Silva *et al*. (2018). The olfactometer device was vertically positioned, and tests with mirids (see treatments below) were conducted following the methodology previously described (Moayeri *et al*., 2006; Ingegno *et al*., 2011; Lins *et al*., 2014; Silva *et al*., 2018). Naïve 1- to 7-day-old female *M. basicornis* predators starved for 24 hours but with access to water were used in the assays, i.e., individuals that had neither been exposed to tomato volatiles nor had preyed on *T. absoluta* eggs before the tests, because they were reared on tobacco plants with *E. kuehniealla* as prey. A single female was introduced in the main arm of the olfactometer and observed maximally for 10 minutes. A choice was considered to be made when a female crossed a line drawn 13 cm from the branching point of the Y-tube. Females not choosing a side arm within 10 minutes were considered nonresponding and were excluded from the data analysis. Each female was tested only once, and after every replicate, the olfactometer side arms were switched to minimize positional bias. After testing ten females, the Y-tube and glass vessels were washed with neutral soap (Extran®) and ethanol (70%) and dried. Bioassays took place in a climatized room at 25 ± 1 °C and 70 ± 10% RH between 10-12 AM and 2-4 PM.

Treatments

Twenty pairs of 1- to 3-day-old *T. absoluta* adults were introduced for 24 hours into an acrylic cage (60 × 30 × 30 cm) with a tomato leaflet (2 cm long). After removal of the *T. absoluta* adults, the leaflets with circa 200 eggs were either used in the olfactometer, or the leaflets were exposed for 24 hours to a few hundred adult *T. pretiosum* parasitoids to obtain high rates of parasitism. The leaflets with parasitized *T. absoluta* eggs were after removal of

the adult parasitoids used immediately for the test with 1-day-old parasitized eggs or later for the test with 5-day-old parasitized eggs. The petioles of the leaflets were kept in a 2 ml Eppendorf tube with water to maintain the leaf in good condition. On leaflets to be used in tests with 5-day-old parasitized eggs, the few unparasitized eggs were removed after three days to prevent larval emergence and damage due to feeding on the leaflet by *T. absoluta* larvae. Tomato leaflets were replaced with fresh leaflets after testing 10 predators.

We tested the following combinations of treatments with female *M. basicornis* predators:
- Uninfested leaflet vs leaflet infested with unparasitized eggs,
- Leaflet with unparasitized eggs vs leaflet with 1-day-old parasitized eggs,
- Leaflet with unparasitized eggs vs leaflet with 5-day-old parasitized eggs,
- Leaflet with 1-day-old parasitized eggs vs leaflet with 5-day-old parasitized eggs.

For each treatment, tests were continued until 30 female predators had responded. The total number of replicates, i.e. the total number of predators tested, varied between 33 and 46.

## Statistics

### Behavioural observation experiments

General linear models (GLMs) with Poisson error distribution and log link function were used to analyse count response variables (i.e., numbers of eggs consumed, numbers of contacts with eggs) fitting the treatments (unparasitized eggs, eggs parasitized at different time points) as categorical fixed factors. When overdispersion was detected, we corrected this by fitting quasi-Poisson GLMs. The significance of the explanatory variables was tested with likelihood-ratio tests (LRTs) (Crawley, 2012). GLMs with gamma error distribution and reciprocal link function were used to analyse time-to-event data (i.e., time before the first feeding occurs), fitting the treatment (unparasitized eggs, eggs parasitized at different time points) as a fixed factor. The significance of the explanatory variables was obtained with *F*-tests (Crawley, 2012).

If the GLMs detected significant differences among factor levels, we proceeded to pairwise comparisons to determine which ones differed from each other using the *glht* function in the *multcomp* package of R software (Bretz *et al*., 2010). Model fit was assessed with residual plots. All statistical analyses were performed using R statistical software version 3.6.2 (R-Core-Team, 2018).

### Olfactometer tests

The response variable used in the test was the proportion of insects responding to one of the volatile sources. For all experiments, we tested whether the predator's choice was significantly different from a 50% distribution. Separate analyses for each pair of choices were carried out. The significance of the response was tested using a $\chi^2$ test. Statistical analyses were performed using R statistical software version 3.6.2. (R-Core-Team, 2018).

All raw data of the experiments are provided in the supplemental material.

## Results

### Behavioural observation experiments

With the behavioural observations we tested the hypothesis that the predator rejects old, parasitized *T. absoluta* eggs only after having contacted the prey with its rostrum. The observations revealed several surprising results.

First, the average of the numbers of contacts was significantly lower for parasitized eggs than for unparasitized eggs during the 2 hours observation period (GLM, unpar vs 1-day par: z = 9.63, *P* < 0.001; unpar vs 5-day par: z = 8.23, *P* < 0.001; unpar vs 9-day par: z = 10.85, *P* < 0.001) (figure 3A, supplemental material table S1). Thus, it seems that parasitized eggs are in a number of cases already rejected before they are contacted.

Second, the mean number of contacts with unparasitized eggs was significantly different across the three choice combinations (GLM, $\chi^2$ = 40.62, df = 2, *P* < 0.001) and decreased when offered together with increasingly older parasitized eggs. The mean number of contacts with parasitized eggs was also significantly different (GLM, $\chi^2$ = 81.19, df = 2, *P* < 0.001): the predators displayed higher numbers of contacts with 1-day-old parasitized eggs, intermediate numbers with 5-day-old parasitized eggs and lower numbers with 9-day-old parasitized eggs (supplemental material table S1). However, the average number of contacts before egg consumption was not significantly different in any of the within-choice combinations (GLM, unpar vs 1-day par: z = 1.43, *P* = 0.153; unpar vs 5-day par: z = 0.52, *P* = 0.600; unpar vs 9-day par: z = 1.17, *P* = 0.243, figure 3B). The number of contacts with unparasitized eggs across the three choice combinations were not significantly different (GLM, $\chi^2$ = 3.17, df = 2, *P* = 0.283). Similarly, the number of contacts with parasitized eggs was not different among 1-, 5- and 9-day-old parasitized eggs (GLM, $\chi^2$ = 1.31, df = 2, *P* = 0.329) (figure 3B, supplemental material table S1).

Third, in those cases where an old parasitized egg was not rejected before it was contacted, the percentage of eggs rejected for feeding was not higher for parasitized than for unparasitized eggs. This indicates that the old, parasitized prey eggs can still be penetrated by the predator. The acceptance for feeding after encountering prey varied between 29 and 54% for unparasitized eggs, while it was 45, 50 and 63% for 1-, 5- and 9-day-old parasitized eggs, respectively (figure 3C, supplemental material table S1), which shows that old parasitized eggs are still accepted as prey.

The average time interval between introduction of the predator into the Petri dish and the first time it was feeding on the different categories of eggs varied widely (29-78 minutes) and did not show a relationship with the type of egg on which the predator was feeding (figure 3D, supplemental material table S1). In fact, in each within-choice combination between unparasitized and parasitized eggs, no significant differences were found (GLM, unpar vs 1-day par: *F* = 1.58, df = 1.70 *P* = 0.131; unpar vs 5-day par: *F* = 0.853, df = 1.44 *P* = 0.123; unpar vs 9-day par: *F* = 0.33, df = 1.37 *P* = 0.205). The average time intervals between introduction of the predator and first feeding were also not different when comparing
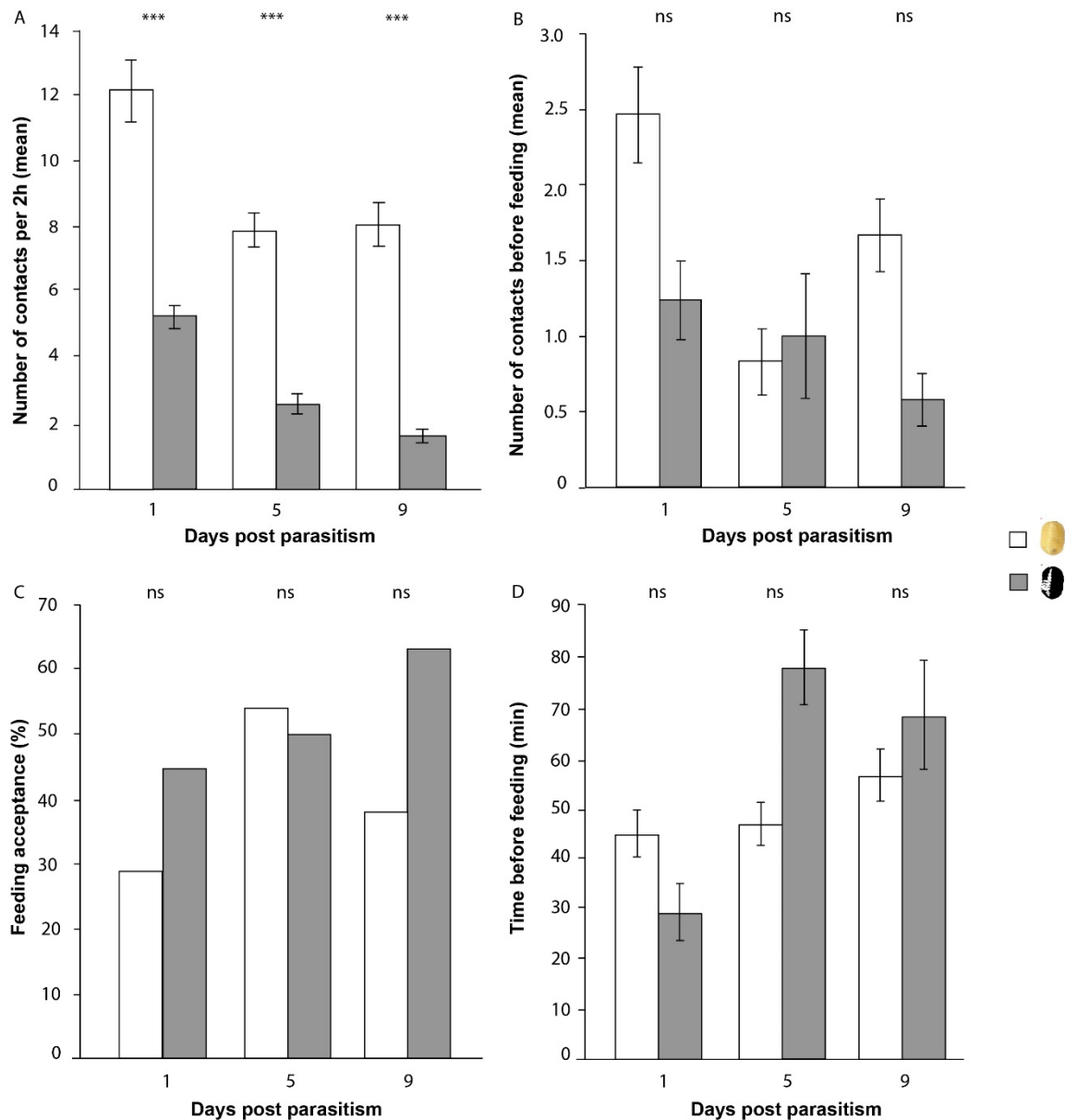
**Figure 3.** Behavioural observations of *M. basicornis* exposed to unparasitized and *T. pretiosum* parasitized *T. absoluta* eggs. Unparasitized eggs were always tested 1-day old (white columns), while parasitized eggs were tested either 1-, 5- or 9-days after parasitism (grey columns). **A**) Average of numbers of contacts with eggs per replicate (± SE) during 2 hours of exposure. **B**) Average of number of contacts before feeding per replicate (± SE). **C**) Percentage feeding acceptance after encountering unparasitized or parasitized prey. **D**) Mean time interval (± SE) between the introduction and first consumption of unparasitized or parasitized prey. *** = p < 0.001, ns = not significant (GLM). Number of replicates: unparasitized vs 1-day-old parasitized = 36, unparasitized vs 5-day-old parasitized = 28, unparasitized vs 9-day-old parasitized = 33.

unparasitized eggs across the choice combinations (GLM, $F = 1.29$, df = 2.72 $P = 0.280$). In contrast, a significant effect was found when comparing 1-, 5- and 9-day-old parasitized eggs across the three choice combinations (GLM, $F = 6.04$, df = 2.41 $P = 0.02$).

### Olfactometer experiments

Olfactometer experiments were performed to test the hypothesis that the predator perceives the condition of prey eggs by olfactory cues. *M. basicornis* preferred volatiles from tomato leaflets with unparasitized *T. absoluta*

eggs above uninfested leaflets ($\chi^2 = 6.53$; df = 1; $P < 0.05$). The predator did not discriminate between volatiles of leaflets with unparasitized eggs and volatiles of leaflets with 1-day-old parasitized eggs ($\chi^2 = 0.53$; df = 1; $P > 0.05$), while it did prefer volatiles of leaflets with unparasitized eggs over volatiles of leaflets with 5-day-old parasitized eggs ($\chi^2 = 4.80$; df = 1; $P < 0.05$). Similarly, *M. basicornis* preferred volatiles of leaflets with 1-day-old parasitized eggs over leaflets with 5-day-old parasitized eggs ($\chi^2 = 2.13$; df = 1; $P < 0.05$) (figure 4).
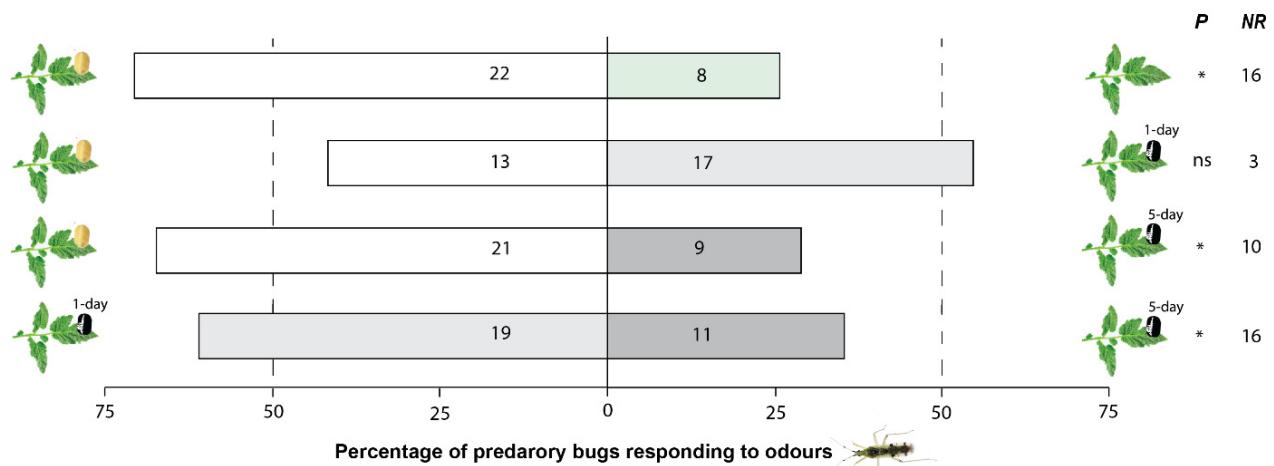
**Figure 4.** Percentage of *M. basicornis* females responding to odours of *T. absoluta* eggs deposited on leaflets in a Y-tube olfactometer. Leaflets with either no eggs (clean), unparasitized eggs (always 1-day-old) or 1- or 5-day-old parasitized by *T. pretiosum* were offered to the predators. The horizontal axis represents the percentage of *M. basicornis* that moved toward the volatile sources in the corresponding choice trials. N = 30 responding *M. basicornis* females per test. NR = number of nonresponding individuals. Numbers in columns = number of responding predators. Light green bar = leaflet with no eggs; white bar = leaflets with unparasitized eggs; light grey bar = leaflets with 1-day parasitized eggs; medium grey bar = leaflets with 5-day parasitized eggs; * $P < 0.05$, ns = not significant ($\chi^2$ test).

## Discussion and conclusions

The results from our behavioural observation experiments clearly indicate that *M. basicornis* accepts 1-day-old parasitized eggs equally well as unparasitized eggs. However, the predator shows strongly reduced predation rates when parasitized *T. absoluta* eggs are older and *T. pretiosum* larvae start to pupate inside the host egg. Apparently, rejection of older parasitized prey eggs takes place before contact and not because they could not be penetrated by the predator's stylets or were judged to be no longer suitable for consumption. Our results of the Y-tube olfactometer tests indicate a role of volatile cues specifically emitted by leaflets infested with older parasitized eggs that could repel the predators to avoid contacting prey eggs with pupae of the parasitoid.

Bueno *et al.* (2023b) also provide extensive data that *M. basicornis* consumes *T. absoluta* eggs recently parasitized by *T. pretiosum* in equal numbers as unparasitized eggs but hardly attacks eggs that contain pupal stages of the parasitoid. However, the Bueno *et al.* (2023b) study did not reveal whether the predator rejected these old, parasitized eggs or what prevented them from eating these eggs. The currently held opinion about prey searching and evaluation behaviour by mirid predators, though backed by very limited evidence, is that they do not search by vision or smell but encounter prey randomly (Wheeler, 2001). In this view, old, parasitized eggs are rejected after contact because they can no longer be penetrated by the stylets of the predators. Our observations of the behaviour of *M. basicornis* revealed a much lower number of contacts with old parasitized eggs than expected from random search behaviour. Apparently, rejection of these old, parasitized eggs takes place before they are encountered. However, if encountered, they were as easily penetrated as unparasitized eggs. Thus, prey

searching and penetration of old parasitized eggs by *M. basicornis* appears to differ from that of the mirid species referred to in Wheeler (2001). The olfactometer tests show that volatile cues specifically emitted by tomato leaflets infested with old, parasitized eggs may repel the predators to avoid contacting less suitable prey.

Other Heteroptera, including several mirid species, also reject older parasitized eggs but generally do not distinguish between unparasitized eggs and eggs containing parasitoids early in their development (Rosenheim and Harmon, 2006). *M. pygmaeus* preferentially preys on unparasitized or recently (< 4 days exposed to parasitoids) *T. absoluta* eggs parasitized by *T. achaeae* when the eggs are still yellow, but hardly preys on old, black parasitized eggs in laboratory experiments (Chailleux *et al.*, 2013). Additionally, in laboratory experiments with the mirid *N. tenuis* and the egg parasitoid *T. achaeae*, *N. tenuis* consumed significantly more unparasitized eggs than parasitized eggs and significantly more parasitized eggs younger than 4 days old than eggs parasitized more than 4 days ago (Cabello *et al.*, 2015). Eggs of many Lepidoptera parasitized by *Trichogramma* spp. become dark due to the deposition of melanin on the inner surface of the host egg chorion at the end of the larval stages and the start of prepupa formation of the parasitoid (Clausen, 1940; Metcalfe and Brenière, 1969; Alrouechdi and Voegelé, 1981; Pintureau *et al.*, 1999; Knutson, 2005). In general, melanin protects the insect egg against desiccation, UV light and natural enemies (Pintureau *et al.*, 1999; Hilker *et al.*, 2023). The detailed description of the development of *T. pretiosum* in host eggs of *E. kuehniella* at 25 °C - the same temperature we used - shows that the egg-larval stage of the parasitoid takes on average 2.9 days, the prepupal stage lasts 1.4 days and the pupal stage is approximately 6.1 days long (Consoli *et al.*, 1999). No detailed data are available for the development of

*T. pretiosum* in *T. absoluta*, but the total immature development time at 25 °C of 10.3 days in *T. absoluta* (Pratissoli and Parra, 2001) is very similar to that of 10.4 days in *E. kuehniella*. Therefore, in our experiments, *M. basicornis* can be expected to have been exposed to *T. absoluta* eggs with the parasitoid in the egg-larval stage (1-day-old parasitized prey eggs) and to the early and late pupal stages of the parasitoid in the prey egg (5- and 9-day-old parasitized eggs).

To draw conclusions about preference for one category of prey over another, prey searching and selection behaviour should be known, e.g., does prey selection take place before arrival on a host plant, after landing on the plant or only after contact with the prey? Based on the present opinion that mirids search unsystematically, we initially supposed that the lower consumption rates of 5- and 9-day-old parasitized eggs were the result of difficulties in penetrating the melanized chorion of the prey egg and/or the consequence of rejection of these eggs for consumption. The results of the behavioural and olfactometer tests show that *M. basicornis* avoids contact with old, parasitized eggs. However, in the few cases in which the predator does contact an old, parasitized egg, it will penetrate it with the same probability as unparasitized eggs. Apparently, melanin deposition and sclerotization by *Trichogramma* spp. (Pintureau *et al*., 1999) do not prevent *M. basicornis* from penetrating these eggs. Thus, *M. basicornis* does not search unsystematically and does not decide to reject a certain type of egg only after having made physical contact. The results of olfactometer experiments show that volatiles - in this case synomones - play a role in prey selection because the predators prefer tomato leaflets with unparasitized eggs over leaflets with 5-day-old parasitized eggs.

Numerous studies have shown that phytophagous insect oviposition induces plant volatiles (OIPVs) to attract egg and larval parasitoids and repel ovipositing phytophages (reviewed by Hilker and Fatouros, 2015; Fatouros *et al*., 2016; Greenberg *et al*., 2022; Hilker *et al*., 2023). Lepidopteran oviposition, including that by *T. absoluta*, does not cause obvious damage to plants. Nevertheless, egg deposition of several lepidopteran species induces quantitative changes in plant volatile blends (Bertea *et al*., 2020). The finding that *M. basicornis* prefers volatiles of leaflets with unparasitized or 1-day-old parasitized eggs over volatiles of 5-day-old parasitized eggs indicates that the predator uses volatile information produced by the combination 'old, parasitized eggs-tomato leaflet'. Whether the information derives from volatiles emitted by the plant and/or volatiles from the *T. absoluta* eggs needs further investigation. Whether the volatiles resulting in the repellence of predators are derived purely from the developing parasitoid inside the egg is another intriguing question.

As far as we know, it was hitherto unknown that eggs of *T. absoluta* parasitized by *T. pretiosum* are rejected by a mirid predator before having made physical contact. A recent study indicated that OIPV blends change when eggs are parasitized: volatiles of rice plants infested with eggs of the brown plant hopper *Nilaparvata lugens* (Stal) (Hemiptera Delphacidae) and parasitized by *Anagrus nilaparvatae* Pang et Wang (Hymenoptera Mymaridae)

were less attractive to conspecific parasitoids than volatiles from plants with unparasitized eggs (Li *et al*., 2020). Plants infested with parasitized eggs showed increased levels of some volatile compounds, such as linalool or methyl salicylate.

Whether predatory insects can make use of OIPVs for prey location has hardly been shown yet. However, numerous studies have shown that predators use herbivore-induced plant volatiles (HIPVs) for prey location (Dicke and Sabelis, 1988; Kessler and Baldwin, 2001). For two European and three Neotropical mirid predators, including *M. basicornis*, we previously showed that they use volatile cues in their prey finding process (Lins *et al*., 2014; Silva *et al*., 2018). Yet, the mirid species did not discriminate between volatiles of tomato plants infested with eggs of *T. absoluta* and volatiles of clean tomato plants (Silva *et al*., 2018). Nevertheless, oviposition on tomato plants by *T. absoluta* triggered emission of OIPVs attracting *Trichogramma* wasps (Milonas *et al*., 2019). We argue that the lack of attraction of *M. basicornis* and several other mirid predators to *T. absoluta* egg-infested plants may be due to differences in volatile emissions between genetically different tomato plant cultivars, and/or to differences in experimental set up, such as variation in the number of prey eggs per leaf. That a difference in prey infection rate may play a role in attraction or not is illustrated in a study by Mollá (2013), in which the mirid *N. tenuis* preferred volatiles of plants with *T. absoluta* eggs over clean plants, but in his experiments egg numbers per plant were about four times higher than in the experiment by Silva *et al*. (2018). In the olfactometer experiments described in this paper, the tomato leaflets carried about 10-20 times the amount of unparasitized *T. absoluta* eggs than the tomato plants in the experiment by Silva *et al.* (2018). Such a difference in herbivore density has previously been shown to effect HIPVs and attraction of the aphid parasitoid *Diaeretiella rapae* (M'Intosh) (Cascone *et al*., 2019) and could explain the preference of the mirids for leaflets with unparasitized eggs over clean plants in our experiment. A next step would be to analyse the volatiles that are emitted by tomato leaflets infested with unparasitized and parasitized *T. absoluta* eggs.

Concluding remarks:

1- The predator *M. basicornis* can penetrate old, parasitized eggs on rare occasions when such eggs are encountered, and these eggs are accepted for consumption at the same rate as unparasitized eggs.

2- The predator uses volatile information emitted by old, parasitized eggs on tomato leaflets to prevent encounters with old parasitized eggs.

3- Due to IGP, young parasitoid eggs and larvae are killed by the predator when both natural enemies are released at the same time. To strongly reduce IGP, predators should be released a week after the introduction of parasitoids.

4- The current belief that mirids search unsystematically, discover and reject prey only after having physically encountered them has to be modified for *M. basicornis*, as they do not search randomly and reject the majority old parasitized eggs before contacting them.

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