

The mirid predator *Macrolophus basicornis* smells and avoids eggs of *Tuta absoluta* parasitized by *Trichogramma pretiosum*

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Abstract

Biological control, which uses natural enemies to reduce pest populations, is a non-polluting powerful method to manage impacts of (invasive) pests. Currently, 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 for control of one or more pests in a crop, to detect if negative intraguild effects occur. We studied a case of intraguild predation (IGP) involving a predator, *Macrolophus basicornis*, of the worldwide invasive South American tomato leaf miner *Tuta absoluta*, and explored how this predator deals with prey parasitized by *Trichogramma pretiosum*. Behavioural observations show that *M. basicornis* predators contacted significantly fewer old, parasitized eggs of *T. absoluta* than recently parasitized ones. Olfactometer tests revealed that predators could smell differences between volatiles of tomato leaves infested with eggs of different quality 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 days before predators to prevent high levels of IGP.

Introduction

Sustainable production of food free of negative effects on the environment and without adverse consequences for climate change is high on the agenda of many countries and international organizations¹. One of the approaches to obtain this goal is to replace synthetic chemical pesticides by other, far less or non-polluting pest management methods². Biological control, which uses natural enemies to reduce populations of pest organisms, is a non-polluting powerful method to manage impacts of (invasive) pests and has been used for centuries, but became particularly popular since the 1880s^{3,4}. Recently, a specific guild of natural enemies, polyphagous predatory mirid species, is increasingly used in augmentative, commercial biological control of pests^{5,6}. Though they are applied on large areas⁷ and are successfully controlling globally occurring devastating pests such as whiteflies (including *Bemisia* and *Trialeurodes* species⁸) and lepidopterans (including *Tuta* and *Spodoptera* species⁹), surprisingly little is known about their prey searching behaviour. According to Wheeler¹⁰, 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 finding¹¹⁻¹³, studies on foraging decisions of hemipteran predators revealing when and how the predator decides to feed or not, have rarely been done^{10, 14-15}.

Understanding the foraging behaviour of mirid predators is of general importance for the design of predator release programmes 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 a shared prey¹⁶, 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 and Nagarkatti (Hymenoptera: Trichogrammatidae) to control *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomato crops in Spain¹⁷. Similarly, the use of the Neotropic mirid *Macrolophus basicornis* (Stål) (Hemiptera: Miridae) together with the parasitoid *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) is considered for control of the same pest in Brazil. 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 exclusion¹⁸.

Mirid predators like *N. tenuis* and *M. pygmaeus* 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^{17,19}. When we know how mirids find and evaluate parasitized prey, we might be able to fine tune natural enemy release programmes to prevent or reduce 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* direct observation and by olfactometer experiments (Fig. 1). The main aim of this study was to answer the question whether competitive exclusion by the parasitoid takes place through 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, (2) or that the predator perceives the condition of prey eggs by olfactory cues and avoids to approach them.

Results

Behavioural observation experiments

With the behavioural observations (see Fig. 2 for experimental set-up) we tested the hypothesis if the predator rejects old parasitized *T. absoluta* eggs only after having contacted the prey with its rostrum. The observations revealed several surprising results. First of all, the total number of contacts was significantly lower for old parasitized eggs than for unparasitized eggs during the 2h observation period (GLM unpar vs 1-day par: $z = 9.63$, $P < 0.001$; unpar vs 5-days par: $z = 8.23$, $P < 0.001$; unpar vs 9-days par: $z = 10.85$, $P < 0.001$) (Fig. 3a, Table S1). Thus, it seems that old parasitized eggs are often already rejected before they are contacted. Secondly, the mean number of contacts with unparasitized eggs was significantly different across the three choice combinations (GLM, $\chi^2 = 40.62$, $df = 2$, $P < 0.001$). Similarly, the mean number of contacts with parasitized eggs was also significantly different ($\chi^2 = 81.19$, $df = 2$, $P < 0.001$) as the predators displayed higher numbers of contacts with 1-day old parasitized eggs, intermediate numbers with 5-days old and lower numbers of contacts with 9-days old parasitized eggs (Table S1). However, the average number of contacts before egg consumption was not significantly different in any of the within-choice combinations (unpar vs 1-day par: $z = 1.43$, $P = 0.153$; unpar vs 5-days

par: $z = 0.52$, $P = 0.600$; unpar vs 9-days par: $z = 1.17$, $P = 0.243$). The numbers of contacts with unparasitized eggs across the three choice combinations was not significantly different ($\chi^2 = 3.17$, $df = 2$, $P = 0.283$). Similarly, also the number of contacts with parasitized eggs was not different among 1,5 and 9-day old parasitized eggs ($\chi^2 = 1.31$, $df = 2$, $P = 0.329$) (Fig. 3, Table S1). Thirdly, in those cases where an old, parasitized egg has not been rejected before it was contacted, the percentage of eggs rejected for feeding is not higher for parasitized than for unparasitized eggs. This indicates that the old parasitized can still be penetrated by the predator. The percentage acceptance for feeding after encountering a prey varied between 29 and 54 percent for unparasitized egg, while it was 45, 50 and 63 percent for 1-, 5- and 9-day old parasitized egg, respectively (Fig. 3c, Table S1), which suggests 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 (Fig. 3d, Table S1). In fact, in each within-choice combination between unparasitized and parasitized eggs, no significant differences were found (unpar vs 1-day par: $F = 1.58$, $df = 1.70$ $P = 0.131$; unpar vs 5-days par: $F = 0.853$, $df = 1.44$ $P = 0.123$; unpar vs 9-days par: $F = 0.33$, $df = 1.37$ $P = 0.205$). The average time intervals between introduction of the predator and first time feeding was also not different when comparing unparasitized eggs across the choice combinations ($F = 1.29$, $df = 2.72$ $P = 0.280$). On the contrary, a significant effect was found when comparing 1, 5 and 9-day old parasitized eggs across the three choice combinations ($F = 6.04$, $df = 2.41$ $P = 0.02$).

Olfactometer experiments

The olfactometer experiments were done to test the hypothesis that the predator perceives the condition of prey eggs by olfactory cues. *Macrolophus basicornis* preferred volatiles from tomato leaflets laden 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 they 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, the *M. basicornis* preferred volatiles of leaflets with 1-day old parasitized eggs over leaflets with 5-day old parasitized eggs ($\chi^2 = 2.13$; $df = 1$; $P < 0.05$) (Fig. 4).

Discussion And Conclusions

The results from our behavioural observation experiments clearly indicate that *M. basicornis* consumes parasitized prey eggs equally often as unparasitized prey eggs when the developing parasitoid in the prey egg is still young. However, the predator shows strongly reduced predation rates when parasitized *T. absoluta* eggs are older and the *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.²⁰ show 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, this study did not reveal if 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 randomly¹⁰. In this view, old parasitized eggs are rejected after contact because they can no longer be penetrated by the rostrum 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 eggs by *M. basicornis* appears to differ from that of the mirid species referred to in Wheeler¹⁰. 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 development²¹. *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) 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 experiments¹⁹. Also, in laboratory experiments with the mirid *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) and the egg parasitoid *T. achaeae*, *N. tenuis* consumed significantly more unparasitized eggs than parasitized eggs, and significantly more parasitized eggs younger than 4-day old than eggs parasitized more than 4-days ago¹⁷. Eggs of many Lepidoptera parasitized by *Trichogramma* spp. become dark due to the deposition of melanin to the inner surface of the host egg chorion at the end of the larval stages and the start of prepupa formation of the parasitoid²²⁻²⁶. In general, melanin protects the insect egg against desiccation, UV light and natural enemies^{25,27}. 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 about 6.1 days long²⁸. No detailed data are available for development of *T. pretiosum* in *T. absoluta*, but the total immature development time at 25°C of 10.3 days in *T. absoluta*²⁹ is very similar to that of 10.4 days in *E. kuehniella*. So, in our experiments, *M. basicornis* was exposed to *T. absoluta* eggs with the parasitoid in the egg-larval stage (1- and 2-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).

In order to be able 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 takes 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 to penetrate 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 to contact old parasitized eggs. However, in the few cases that they do contact an old parasitized egg, they will penetrate it with the same probability as unparasitized eggs. Apparently, melanin deposition and sclerotization by *Trichogramma*²⁵ does 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 a synomone - 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 herbivore insect oviposition induces plant volatiles (OIPVs) attract egg and larval parasitoids and repel ovipositing herbivores (reviewed by ^{27,30,31,32}) 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 the plant volatile blends³³. 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. If the volatiles resulting in repellence of the 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 indicates that OIPV blends change when eggs are parasitized: volatiles of rice plants infested with eggs of the brown plant hopper (*Nilaparvata lugens*) and parasitized by *Anagrus nilaparvatae* Pang and Wang (Hymenoptera: Mymaridae) were less attractive to the conspecific parasitoids when compared to volatiles from plants with unparasitized eggs³⁴. 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 show that predators they use of herbivore-induced plant volatiles (HIPVs) for prey location^{35,36}. For two European and three Neotropical mirid predators, including *M. basicornis*, we previously shown that they use volatile cues in their prey finding process^{12,13}. Yet, the Neotropical mirid predators did not discriminate between volatiles of tomato plants infested with eggs of *T. absoluta* and volatiles of clean tomato plants¹³. Nevertheless, oviposition on tomato plants by *T. absoluta* triggered

emission of OIPVs attracting *Trichogramma* wasps³⁷. 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 genotypic differences in volatile emission between tomato plant cultivars. 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 the rare occasion when such eggs are encountered, and they 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. In order to strongly reduce IGP, predators should be released a week after introduction of the 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 old parasitized eggs before contacting them

Material 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 at the campus of the Federal University of Lavras, Minas Gerais, Brazil, and maintained in mesh cages (90 x 70 x 70 cm) with tomato plants in the laboratory. New tomato plants were regularly placed into the cages to keep a stock rearing of *T. absoluta* at $25 \pm 2^\circ\text{C}$, RH $70 \pm 10\%$ and 12h 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^{38,39} using tobacco plants as oviposition substrate and with UV-irradiated *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs as prey in climate rooms at $25 \pm 2^\circ\text{C}$, $70 \pm 10\%$ RH and a photoperiod 14: 10 (L:D). Adult female *M. basicornis* predators of up to seven days old were used in the experiments and had been starved for 24 h, 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 $25 \pm 1^\circ\text{C}$, $70 \pm 5\%$ RH and a photoperiod of 16:8 (L:D)⁴⁰. Adult parasitoids used in the experiments were less than 24 h old. All

rearing and behavioural experiments were done in the Laboratory of Biology of Insects, while the olfactometer experiments were done 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.

Research involving plants. The plants used in the experiments were commercially available cultivars and did not involve plant species at risk of extinction or species of the wild flora. Our research complied with local and national regulations—Formal ethical approval was not required.

Behavioural observation of prey searching and predation

To test the first hypothesis – predators cannot penetrate the older parasitized eggs - the behaviour of the predator *M. basicornis* towards five unparasitized versus five parasitized eggs of *T. absoluta* was observed and video recorded during 2h. with the aid of a DinoCapture 2.0 microscope (magnification 7x) connected to a laptop computer. *Tuta absoluta* eggs were placed with a fine paint brush on a tomato leaflet, and their position and condition (parasitized or not) was noted (Fig. 2). The interval between introduction of the predator into the Petri dish and the start of feeding, the number of contacts as well as the type of contact with the prey (encounter, feeding) was recorded. In this choice experiment, 1-day unparasitized eggs were tested against eggs of 1-, 5- and 9 dpp by *T. pretiosum*. The number of replicates varied between 28 and 36 (see Table S1, Supplementary information).

To obtain 1-day old parasitized eggs, *T. absoluta* eggs laid during the previous 24 h were exposed to *T. pretiosum* parasitoids during 24 h. After 24 h the parasitoids were removed and the 24–48 h old *T. absoluta* eggs, which thus contain parasitoid eggs of 0–24 h, were offered to the predator *M. basicornis*. Reasoning in the same way, in the 5-day test, the parasitoids were 96–120 h old and in the 9-day test, the parasitoids were 192–216 h old. Egg-adult development of *T. pretiosum* takes on average 10 days^{28, F.C. Montes personal communication}. The immature parasitoid is in the egg-larval stage 1–2 dpp of *T. absoluta* prey eggs, in the early pupal stage in 5 dpp, and in the late pupal stage in the 9 dpp when reared at 25 ± 1 °C, 70 ± 5 % RH and a photoperiod of 16:8 (L:D). In 1- and 2- day old parasitized eggs, it is not possible to see whether the *T. absoluta* eggs have all been parasitized. To determine what the average percentage parasitism of these eggs is, 18 batches of 100 eggs were exposed to *T. pretiosum* for 24 h and the result of parasitism was 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% (S.E. \pm 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 percentage parasitism 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 set-up. We used a dynamic airflow Y-tube olfactometer set-up made from glass as described in Silva et al.¹³. The olfactometer device was vertically positioned, and tests with mirids (see treatments below) were conducted following the methodology previously described^{12,13,41,42}. Naïve 1–7 day old female *M. basicornis* predators were used in the assays, i.e. individuals that had neither been exposed to tomato volatiles, nor had preyed on *T. absoluta* eggs before tests. A single female was introduced in the main arm of the olfactometer and observed maximally 10 min. 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 min were considered as non-responding 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 $25 \pm 1^\circ\text{C}$ and $70 \pm 10\%$ RH between 10–12 AM and 2–4 PM.

Treatments. Twenty pairs of 1–3 day old *T. absoluta* adults were introduced for 24 h into an acrylic cage (60x30x30 cm) with a tomato leaflet (2cm long). After removal of the *T. absoluta* adults, the leaflets with eggs were either used in the olfactometer, or the leaflets were exposed for 24 h to adult *T. pretiosum* parasitoids to obtain high rates of parasitism. The leaflets with parasitized *T. absoluta* eggs were, after removal of the adult parasitoids, then used immediately for the test with 1-day old parasitized eggs or later as 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 ones after testing ten predators.

We tested the following combinations of treatments with female *M. basicornis* predators:

- Uninfested leaflet vs. leaflet infested with unparasitized *T. absoluta* eggs
- Leaflet with unparasitized eggs vs. leaflet with 1-day old parasitized *T. absoluta* eggs
- Leaflet with unparasitized eggs vs. leaflet with 5-day old parasitized *T. absoluta* eggs
- Leaflet with 1-day old parasitized *T. absoluta* eggs vs. leaflet with 5-day old parasitized *T. absoluta* eggs

For each treatment, tests were continued till 30 predators had responded. Each predator was only used once in a test. The total number of replicates, i.e. the total number of predators tested, varied between 33 and 48 (see Fig. 2).

Statistics

Behavioural observation experiments. General linear models (GLMs) with Poisson error distribution and log link function were used to analyze count response variables (i.e., numbers of egg consumed, numbers of contacts with eggs) fitting the treatments (unparasitized eggs, eggs parasitized at different time points) as categorical fixed factor. When over dispersion was detected, we corrected this by fitting quasi-Poisson GLMs. Significance of the explanatory variables were tested with likelihood-ratio tests (LRTs)⁴³. GLMs with gamma error distribution and reciprocal link function were used to analyze time-to-event data (i.e., time before the first feeding occurs), fitting the treatment (unparasitized eggs, eggs parasitized at different time points) as fixed factor. Significance of the explanatory variables was obtained with *F*-tests⁴³.

If the GLMs detected significant differences amongst factor levels, we proceeded to pairwise comparisons to determine which differed using the *glht* function found in the *multcomp* package of the R software⁴⁴. Model fit was assessed with residual plots. All statistical analyses were performed using R statistical software version 3.6.2⁴⁵.

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 χ^2 test. Statistical analyses were performed using R statistical software⁴⁵.

All raw data of the experiments are provided Table S2 in the Supplementary information.

Declarations

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Author Contributions

J.C.v.L., V.H.P.B. and N.E.F. wrote the original draft of the manuscript. All authors reviewed and edited the manuscript. J.C.v.L., M.S.S., F.C.M. and N.E.F. prepared the figures. J.C.v.L. and V.H.P.B. developed the idea and methodology for the manuscript. J.C.v.L., V.H.P.B., M.S.S. and F.C.M. were involved in execution of the experiments and data preparation. A.C. and J.C.v.L. analysed the data and performed the statistical analyses. J.C.v.L. and V.H.P.B supervised all phases of this project. All authors have read and agreed to this version of the manuscript, including the order of the authors.

Data availability

The online version contains supplementary material available at [https:// doi. org/****](https://doi.org/****)

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Competing interests

The authors declare no competing interests

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Figures



Figure 1

Model study organisms used: a) mirid predator *Macrolophus basicornis* sucking on an *T. absoluta* egg, b) female *Trichogramma pretiosum* parasitizing a *T. absoluta* egg and c) tomato plant leaflet with *T. absoluta* eggs as used in the experiments.

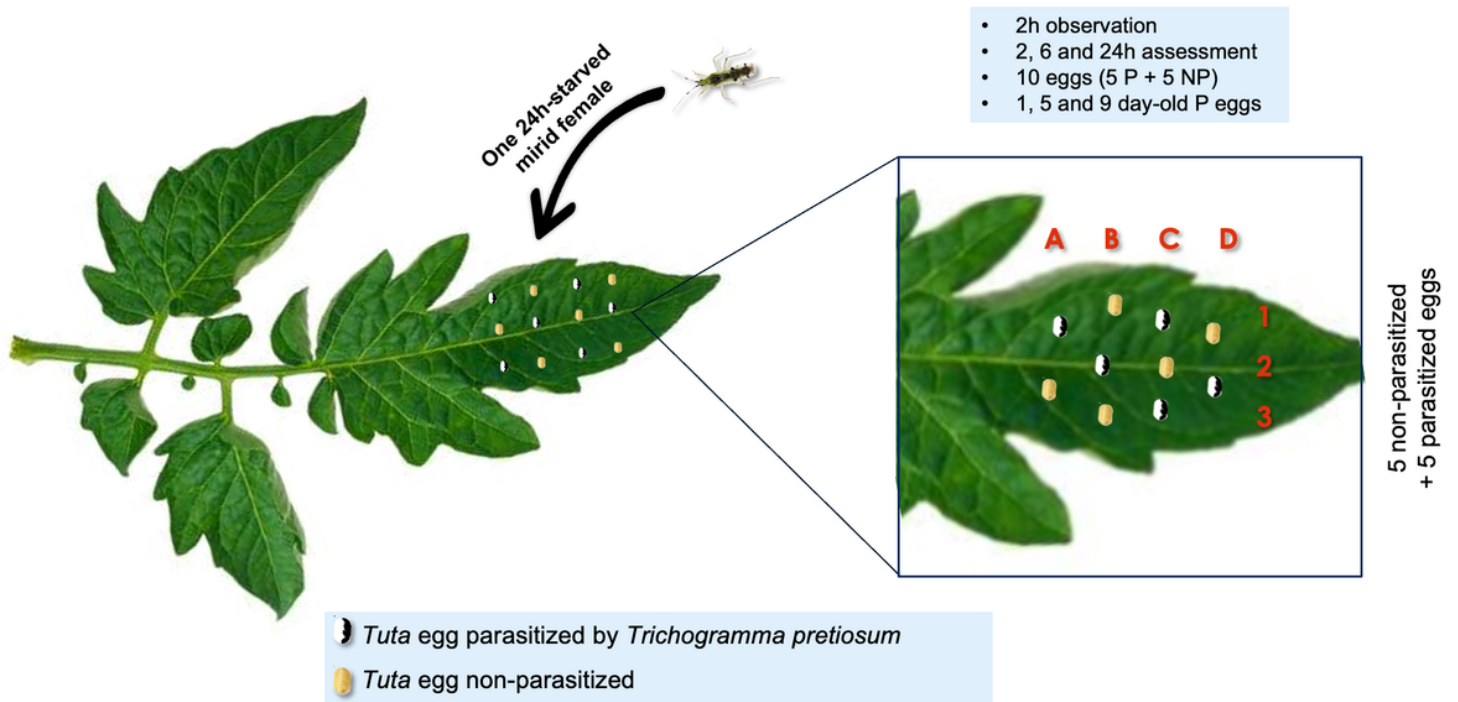


Figure 2

Experimental set-up of behavioural observation experiment. Tomato leaflet with five unparasitized and five parasitized eggs of *Tuta absoluta*. Letters and numbers are used to follow the position of the predator *Macrolophus basicornis* during the 2h observation period.

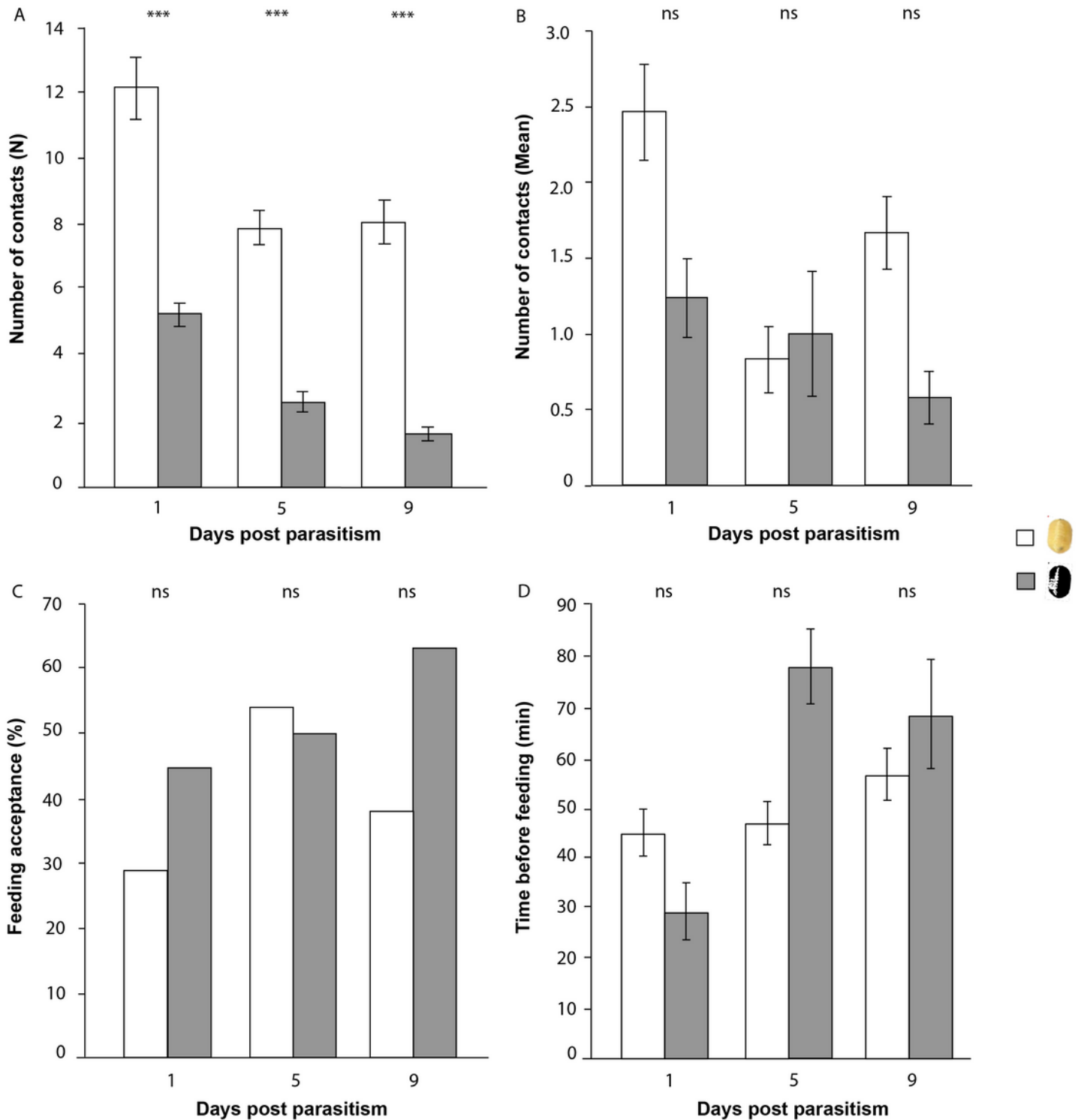


Figure 3

Two-choice contact bioassays with *Macrolephus basicornis* choosing *Tuta absoluta* eggs, either unparasitized or *Trichogramma pretiosum*-parasitized. Unparasitized eggs were always tested 1-day old (white columns), while parasitized eggs were tested either 1, 5 or 9-days after parasitization (grey columns). a) Mean number of contacts with eggs per replicate (\pm S.E) during 2h exposure, b) Mean number of contacts before feeding per replicate (\pm S.E), c) Percentage feeding acceptance after

encountering an unparasitized or parasitized prey, d) Mean time interval (\pm S.E) between introduction and first consumption of an unparasitized or parasitized prey. *** = $p < 0.001$, ns = not significant, (GLM). Number of replicates: unparasitized vs. 1 dpp = 36, unparasitized vs. 5 dpp = 28, unparasitized vs. 9 dpp = 33.

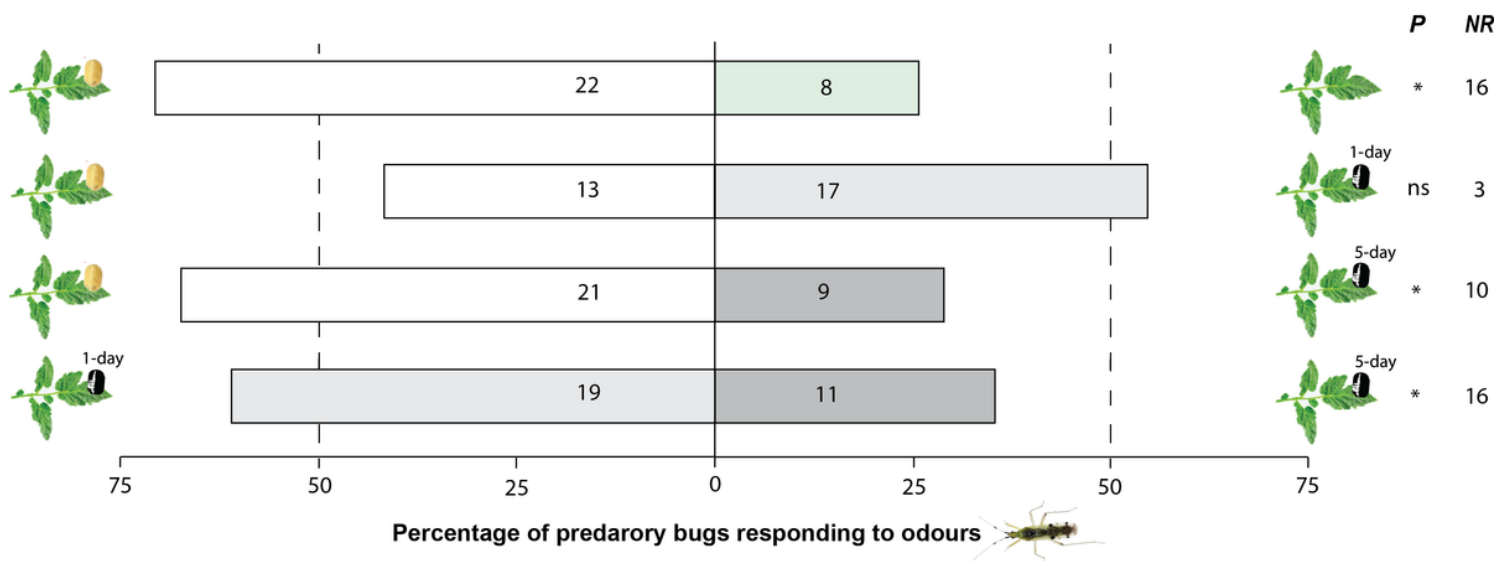


Figure 4

Percentage of *Macrolophus basicornis* females responding to odours of *Tuta 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 *Trichogramma 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 non-responding individuals. Numbers in columns = number of responding wasps. 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-square test).

Supplementary Files

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