



Feeding preference and intraguild interactions between the parasitoid *Trichogramma achaeae* and the predator *Macrolophus pygmaeus*, two biological agents of *Tuta absoluta*

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Abstract

BACKGROUND: *Tuta absoluta* is an exotic species and a major pest of tomato crops in Europe. *Macrolophus pygmaeus* and *Trichogramma achaeae* are two biocontrol agents widely used in integrated pest management programs of the South American tomato pinworm *Tuta absoluta*. In this study, we evaluated under laboratory conditions the (i) voracity of *M. pygmaeus* females fed on single diets of *Tuta absoluta* eggs parasitized or unparasitized by *Trichogramma achaeae*, (ii) voracity and feeding preference of *M. pygmaeus* females provided with mixed diets of *Tuta absoluta* eggs unparasitized and parasitized by *Trichogramma achaeae* and (iii) effect of competitive and intraguild interactions between *M. pygmaeus* and *Trichogramma achaeae* on the number of *Tuta absoluta* eggs consumed and/or parasitized. Lastly, we assessed under field conditions the effect of interspecific and intraspecific interactions between natural enemies on the number of *Tuta absoluta* eggs consumed and/or parasitized.

RESULTS: *Macrolophus pygmaeus* consumed more unparasitized than parasitized eggs of *Tuta absoluta*. Under mixed diet regimes, Manly indices revealed a feeding preference for unparasitized eggs, and a decrease in the total number of eggs consumed, as the proportion of available parasitized eggs increased, whereas the unparasitized eggs were consumed in direct proportion to their availability. Conspecific interactions between *M. pygmaeus*, in contrast to *Trichogramma achaeae*, revealed the possible occurrence of intraspecific competition. For intraguild heterospecific interactions, the number of *Tuta absoluta* eggs consumed by *M. pygmaeus* and parasitized by *Trichogramma achaeae* was lower than that predicted for additive and non-interactive scenarios. Under field conditions, a significant difference between the conspecific treatment and heterospecific treatments revealed a slightly higher success rate in controlling *Tuta absoluta* when both *M. pygmaeus* and *Trichogramma achaeae* were used simultaneously.

CONCLUSION: *Macrolophus pygmaeus* prefers unparasitized eggs of *Tuta absoluta* but inflicts intraguild predation on *Trichogramma achaeae*. In conspecific experiments, mutual interference between *M. pygmaeus* predators intensifies as the number of individuals increases, but for *Trichogramma achaeae*, it occurs in an unpredictable manner. Adding *Trichogramma achaeae* could significantly increase the level of control of *Tuta absoluta* compared to what could be achieved when only *M. pygmaeus* is present in glasshouse tomatoes.

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Keywords: augmentative biological control; South American tomato pinworm; predatory mirid bug; egg parasitoid; feeding preference; intraguild interactions

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1 INTRODUCTION

Biological control using native natural enemies has proven to be an environmentally sound, sustainable alternative in a wide range of cropping systems,¹ given that exotic natural enemies may pose important concerns for local biodiversity.² An optimal augmentative strategy should consider the simultaneous use of more than one natural enemy. For many agroecosystems, there is growing evidence that more diverse guilds enhance biological control efficacy.^{3,4} The few empirical studies reveal variable results, however, as intraguild predation and/or behavioral interference may disrupt pest control. These varying outcomes are due to idiosyncrasies that demand case-by-case consideration, once it may be dependent on the agroecosystems, landscape complexity, predators' identity and species abundance and the mechanisms underlying their functioning.^{5–10} Interactions between multiple biocontrol agents can be (i) antagonistic, (ii) additive/non-interactive, or (iii) synergistic. Additive/non-interactive interactions result in a summation effect, while an antagonistic interaction results in a decreased overall effect of biocontrol agents, that is less than additive effect. Synergetic effect occurs when the combined effect is greater than the expected additive effect. Despite the possibility that any of these three general scenarios may occur between biocontrol agents in integrated pest management programs, extrapolative experiments are rarely conducted to determine which of these various scenarios may apply.

When confronted with two alternative preys, biocontrol agents often display a feeding preference for one of them and that response is strongly affected by the relative abundance of the two preys.^{11–13} When tested with different prey proportions, a predator can display four types of response: (i) a constant preference for one prey species, (ii) no preference, wherein the ratio of consumed prey is equal to the ratio of prey individuals in the environment (i.e., null switching),¹⁴ (iii) a switching behavior, wherein the predator eats disproportionately more of the more abundant prey¹¹ and (iv) an anti-switching behavior, wherein the predator eats disproportionately more of the less abundant prey.¹⁴ However, testing for such responses is rarely carried out before initiating a program of inoculative or augmentative biological control.

The South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a major pest of tomatoes, causing considerable fruit quality reduction¹⁵ and worldwide economic losses to growers.¹⁶ This species, native to South America, has already invaded more than 100 countries.¹⁷ In the Portuguese mainland, this invasive pest was reported for the first time in 2009 in glasshouse tomato crops.^{18–20} *Tuta absoluta* was accidentally introduced in the Azores archipelago (Portugal); it was first reported in São Miguel Island, during the 2009–2010 growing season²¹ and it has spread to Terceira, Faial and Pico Islands²² and Graciosa Island (António O. Soares, personal observations).

In the Mediterranean area, native parasitoids and predators can play an important role in attacking *Tuta absoluta* eggs.^{23–26} This seems to be the case of *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae)^{27–30} and *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae).^{31–33} Laboratory and field studies using *Trichogramma achaeae* against *Tuta absoluta* have shown promising results in glasshouses tomatoes of the Azores,³³ but an effective control of pest populations was not achieved due to a low rate of parasitism. In this context, it is crucial to explore more effective solutions, by augmentation of one single biocontrol candidate or, alternatively, by multispecific releases.

The general aim of our study was to evaluate the potential of feral populations of *M. pygmaeus* and *Trichogramma achaeae*, collected in the Azores, Portugal, as biocontrol agents against *Tuta absoluta*. For such, we (i) compared the voracity of *M. pygmaeus* fed on single diets of *Tuta absoluta* eggs unparasitized or parasitized by *Trichogramma achaeae*, (ii) compared the total voracity and feeding preference of *M. pygmaeus* when provided with mixed diets of *Tuta absoluta* eggs both unparasitized and parasitized, (iii) assessed, under controlled laboratory conditions, the effect of the density of either *M. pygmaeus* or *Trichogramma achaeae* on the number of *Tuta absoluta* eggs consumed and/or parasitized (conspecific experiments versus heterospecific experiments) and (iv) evaluated, under field conditions, the effect of *M. pygmaeus* and/or *Trichogramma achaeae* on the number of *Tuta absoluta* eggs consumed and/or parasitized (conspecific experiments versus heterospecific experiments).

2 METHODOLOGY

2.1 Biological material

Macrolophus pygmaeus individuals were collected on tomato plants, in São Miguel Island, in five locations (Pico da Pedra: 37° 47' 48.1" N 25° 35' 51.7" W; Lagoa: 37° 45' 4.3" N 25° 34' 27.2" W; Ribeira Grande: 37° 48' 49.59" N 25° 31' 41.10" W; two locations in Arrifes: 37° 45' 1.54" N 25° 41' 28.70" W; 37° 45' 1.54" N 25° 41' 28.70" W). A laboratory stock population was maintained under controlled conditions. The insects were kept in rearing cages (40 cm × 40 cm × 40 cm) covered with mousseline fabric. Eggs of *Ephesia kuehniella* (Zeller) (Lepidoptera: Pyralidae) and a potted tomato plant were provided as food sources. *Ephesia kuehniella* eggs were placed every 2 days onto the tomato plant leaflets. To supplement the diet, droplets of honey diluted in water were placed every week on the tomato plant leaflets.

For all experiments, predatory females were 9–12-day-old to ensure that they were ovipositing. To obtain these females, approximately 20 couples of *M. pygmaeus* adults were transferred from the stock population to a new rearing cage containing a tomato plant leaf inserted in a water pot on which *Ephesia kuehniella* eggs and droplets of honey diluted in water were provided. The new females used in our experiments were obtained from the eggs laid by these couples. After 10 days, the adults were removed to another rearing cage. In the days following removal of adults, predatory nymphs started to hatch, completing development approximately 18 days later. At this time, newly emerged adults were collected every 2 days and transferred to new cages. Females that were 9–12-day-old were then selected to be used in the experiments. This procedure was repeated for as long as necessary to obtain enough predatory females for all the experiments.

Ephesia kuehniella eggs and *Trichogramma achaeae* parasitoids were supplied by the facility of the Biotechnology Center of Azores (University of the Azores, Portugal) and *Tuta absoluta* was collected in a glasshouse in São Miguel Island to establish a stock population in the laboratory, as described in Borges et al.³⁰ All experiments and laboratory populations were maintained at 25 ± 1 °C, 75 ± 5% relative humidity and 16 h:8 h light/dark photoperiod.

2.2 Voracity of *M. pygmaeus* fed on single diets of *Tuta absoluta* eggs unparasitized or parasitized by *Trichogramma achaeae*

To obtain *Tuta absoluta* eggs parasitized by *Trichogramma achaeae*, fresh eggs (< 24 h) were carefully removed from the

tomato plant leaf used for egg collection (see Borges *et al.*³⁰) and spread over individual tomato plant leaflets (500–1000 eggs per leaflet), using a dissection needle under the microstereoscope. The leaflets bearing the eggs were then put in plastic boxes (10 cm diameter × 2 cm height) along with *Trichogramma achaeae* individuals that had emerged that day (approximately 400 individuals per leaflet). After 6–7 days, parasitized eggs turned black and thus were ready for use in the experiments. During the 24 h before experiments began, *M. pygmaeus* females were only fed on tomato and honey diluted in water. The next day, at the start of the experiment, these 9–12-day-old predatory females were offered 100 parasitized (treatment 1) or unparasitized (treatment 2) eggs of *Tuta absoluta* eggs ($N = 15$ per treatment) evenly distributed over a tomato plant leaflet, for 24 h. The number of parasitized and unparasitized *Tuta absoluta* eggs consumed was recorded.

2.3 Voracity and feeding preference of *M. pygmaeus* fed on mixed diets of *Tuta absoluta* eggs unparasitized or parasitized by *Trichogramma achaeae*

Females of *M. pygmaeus* that were 9–12-day-old were offered parasitized/unparasitized eggs in three ratios: 30:70, 50:50 and 70:30 in a total of 100 eggs. Eggs were spread over the tomato plant leaflet surface. No lepidopteran eggs were provided to females in the previous 24 h of the experiment. Females were allowed to feed for 24 h and then the remaining number of eggs was counted. Feeding preference was calculated using the formula of 'Manly's preference index' (α)³⁴:

$$\alpha = \frac{\ln \frac{(n1-r1)}{n1}}{\ln \frac{(n1-r1)}{n1} + \ln \frac{(n2-r2)}{n2}} \quad (1)$$

where $n1$ and $n2$ are the number of *Tuta absoluta* parasitized or unparasitized eggs provided and $r1$ and $r2$ are the number of *Tuta absoluta* parasitized or unparasitized eggs eaten in 24 h by *M. pygmaeus*.

2.4 Effect of intraguild interactions between *M. pygmaeus* and *Trichogramma achaeae* on the number of *Tuta absoluta* eggs consumed and/or parasitized under laboratory conditions

The methodology was adopted from Northfield *et al.*³⁵ We carried out a set of three experiments in which 500 unparasitized *Tuta absoluta* eggs were offered to different numbers of *M. pygmaeus* and/or *Trichogramma achaeae* for 24 h. Experiment 1 (conspecific tests with parasitoid females) and Experiment 2 (conspecific tests with predatory adult females) consisted in four treatments in which 1, 2, 4 or 6 natural enemies were offered *Tuta absoluta* eggs. Experiment 3 (heterospecific treatments with predator + parasitoid females) consisted in four treatments in which 1 + 1, 2 + 2, 4 + 4 or 6 + 6 natural enemies were offered to *Tuta absoluta* eggs.

To provide for the very large number of *Tuta absoluta* eggs needed for this experiment (500 eggs × 15 replicates × 12 treatments), we placed 250 fresh eggs (< 24 h) on one leaflet and 250 eggs that had been kept at 5 °C for a maximum of 10 days on another leaflet. Treatments were replicated 15 times. As in the previous experiments, *M. pygmaeus* females were only fed on tomato and honey diluted in water before the tests. Tests were performed for 24 h, and the number of eggs eaten were counted. The remaining eggs were kept at 25 °C for 6–7 days until parasitized eggs had turned black, to determine the number of parasitized eggs.

2.5 Effect of intraguild interactions between *M. pygmaeus* and *Trichogramma achaeae* on the number of *Tuta absoluta* eggs consumed and/or parasitized in semi-field conditions

The semi-field experiment was carried out in a biologically managed tomato crop glasshouse. Using sleeve cages, tomato leaves from the mid and high strata of the plant were isolated. Next, 20–30 *Tuta absoluta* adults were introduced inside the sleeve cage to oviposit for 2–3 days. After that time, the lepidopteran was removed and the predators and/or the parasitoids were released and kept for 24 h inside the sleeve cage, according to the respective experimental treatment. A control treatment was performed wherein the sleeve cage was removed, leaving the tomato plant leaf containing *Tuta absoluta* eggs exposed to environmental conditions in the glasshouses, including naturally occurring natural enemies, the most abundant of which were *M. pygmaeus*, *Dicyphus cerastii* Wagner (Hemiptera: Miridae) and *Trichogramma achaeae*.³⁶ To assess the presence of naturally occurring *Tuta absoluta* in the glasshouse during experiments, a random tomato plant leaf was cut daily and brought to the laboratory to check for the presence of *Tuta absoluta* eggs. Very few lepidopteran eggs were found, attesting to the low population density of the pest in the glasshouse. Predators and parasitoids were obtained in the laboratory as previously described. Each treatment was repeated 15 times. At the end of the experiments, the tomato plant leaves were cut and brought back to the laboratory for observation under microstereoscope to determine the number of eggs consumed. The tomato plant leaves of the treatments containing *Trichogramma achaeae* were kept at 25 °C until any parasitized egg turned black, to determine parasitism rate. The experiments started on 24 July 2021, and were completed by 19 August 2021.

2.6 Statistical analysis

Voracity of *M. pygmaeus* fed on single diets of parasitized or unparasitized eggs of *Tuta absoluta* was analyzed using a *t*-test. Univariate general linear model (GLM) with one-way analysis of variance (ANOVA) was used to test the effect of: (i) mixed diets of parasitized and unparasitized eggs on the voracity of *M. pygmaeus*; (ii) the densities of *M. pygmaeus* and/or *Trichogramma achaeae* on the consumption of parasitized and/or unparasitized *Tuta absoluta* eggs; (iii) *in situ* efficacy of *Trichogramma achaeae* and/or *M. pygmaeus* against *Tuta absoluta*. Pairwise multi comparisons were performed and *P* values were corrected using Bonferroni test. When two factors were in consideration, a generalized linear model (GZLM) was performed. Wilcoxon's matched-pairs signed rank test (WMPSR; $P < 0.05$) was used to compare the feeding preference (Manly index).

Normal distribution and homogeneity of variances of data were assessed using the Kolmogorov–Smirnov and the Levene tests, respectively. In cases where data were not distributed normally, the Kruskal–Wallis test ($P < 0.05$), was performed applying the Bonferroni correction. Data were log transformed (consumption +1) when zero values occurred in the data set. Mean values were considered significantly different when $P < 0.05$. All statistical analyses were done using SPSS v27.³⁷

3 RESULTS

3.1 Voracity of *M. pygmaeus* fed on single diets of *Tuta absoluta* eggs unparasitized or parasitized by *Trichogramma achaeae*

There was a significant difference between the voracity of *M. pygmaeus* adults fed on parasitized (13.6 ± 3.5) and

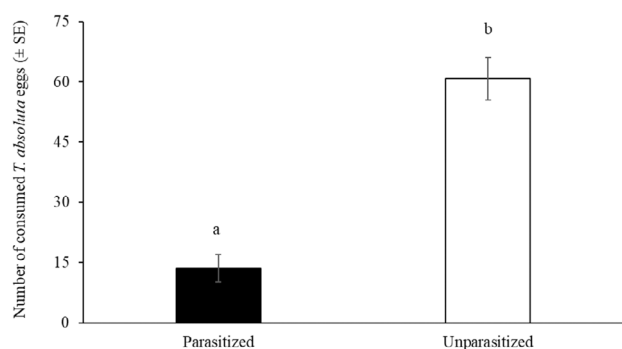


Figure 1. Voracity [mean number of eggs consumed (\pm standard error)] by adults of *Macrolophus pygmaeus* fed on single diets of eggs of *Tuta absoluta* parasitized by *Trichogramma achaeae* or unparasitized. Different letters indicate significant differences (*t*-test; $P < 0.05$).

unparasitized (60.7 ± 5.3) *Tuta absoluta* eggs (*t*-test = -7.311 , $df = 28$, $P = 0.001$; Fig. 1).

3.2 Voracity and feeding preference of *M. pygmaeus* fed on mixed diets of *Tuta absoluta* eggs unparasitized or parasitized by *Trichogramma achaeae*

We found a significant interaction between the proportion of eggs provided that were parasitized, and the proportion of parasitized versus unparasitized eggs consumed (chi-square = 23.032, $df = 2$, $P < 0.001$). Because of this, we analyzed the independent factors separately. Concerning total voracity, we found a significant difference between the three mixed diets (chi-square = 18.248, $df = 2$, $P < 0.001$). When the proportion of parasitized to unparasitized eggs was 70:30, the mean number of eggs consumed was 21.4 ± 2.9 ; for the proportion 50:50, the mean number of eggs consumed was 33.3 ± 4.5 ; and finally for the proportion 30:70, the mean number of consumed eggs was 48.2 ± 4.6 . Pairwise comparison showed a significant difference between all proportions (Fig. 2(A)). Significantly larger numbers of unparasitized eggs were eaten when they were offered in the proportion 30:70 than in the two other proportions (70:30 = 16.6 ± 2.1 unparasitized eggs eaten, 50:50 = 25.8 ± 3.9 and 30:70 = 42.7 ± 4.0 ; chi-square = 24.848, $df = 2$, $P < 0.001$) (Fig. 2(A)), but the number of parasitized eggs eaten did not differ significantly among treatments (70:30 = 4.8 ± 1.0 , 50:50 = 7.5 ± 1.3 and 30:70 = 5.5 ± 1.2 ; chi-square = 2.647, $df = 2$, $P < 0.266$) (Fig. 2(A)).

Regarding feeding preference, *M. pygmaeus* showed a significant preference for unparasitized eggs (WMPSP; 70:30, $Z = -2.803$, $P = 0.005$; 50:50, $Z = -2.344$, $P < 0.019$; 30:70, $Z = -3.408$, $P < 0.001$) (Fig. 2(B)).

3.3 Effect of intraguild interactions between *M. pygmaeus* and *Trichogramma achaeae* on the number of *Tuta absoluta* eggs consumed and/or parasitized under laboratorial conditions

3.3.1 Conspecific experiments

We found significant differences in the number of eggs of *Tuta absoluta* eaten by *M. pygmaeus* between the four treatments (ANOVA: $F = 39.083$, $df = 3$, $P < 0.001$), and voracity significantly increased with predator density. No differences were found between single and two predator treatments, but they differed from the other two treatments (Fig. 3). The number of eggs consumed is lower than predicted for additive and non-interactive scenarios (Fig. 3).

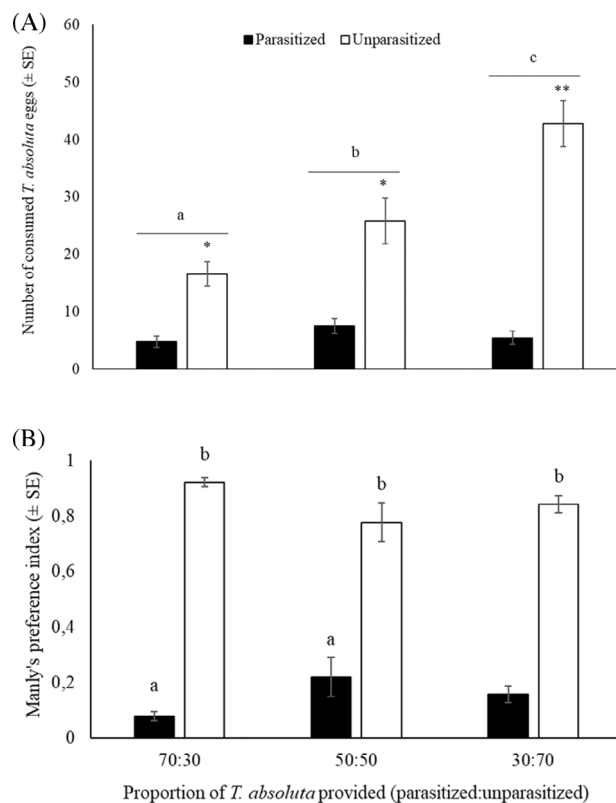


Figure 2. Voracity (A) and Manly's preference index (B) for adults of *Macrolophus pygmaeus* fed on three different ratios of *Tuta absoluta* parasitized and unparasitized eggs (70:30 parasitized/unparasitized, 50:50 parasitized/unparasitized or 30:70 parasitized/unparasitized). For (A) different letters indicate significant differences in total voracity between the three diets, and different number of asterisks indicate a significant difference between total voracity of unparasitized eggs (GZLM; $P < 0.05$). For (B) different letters indicate significant differences (Wilcoxon WMPSP; $P < 0.05$).

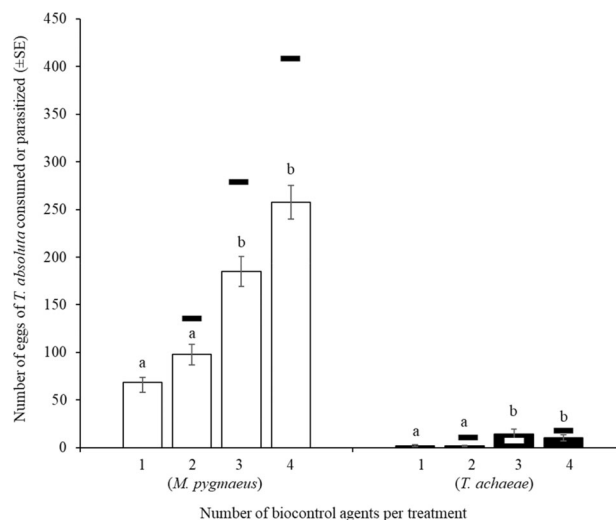


Figure 3. Number of *Tuta absoluta* eggs consumed by *Macrolophus pygmaeus* (white histograms) and number of *Tuta absoluta* eggs parasitized by *Trichogramma achaeae* (black histograms) in treatments in which the number of conspecifics was provided. Different letters in each panel indicate significant differences between treatments (GLM; $P < 0.05$). Black lines over the bars depicted the number of eggs consumed or parasitized predicted for the case of additive and non-interactive between conspecifics.

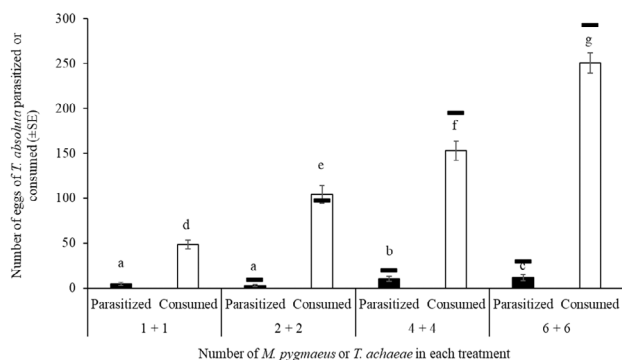


Figure 4. Number of *Tuta absoluta* eggs parasitized by *Trichogramma achaeae* or consumed (\pm standard error) by *Macrolophus pygmaeus* in treatments in which the number of heterospecifics provided increased. Different letters in each panel indicate significant differences between treatments ($P < 0.05$). Black lines over the bars depicted the number of consumed eggs predicted for the case of additive and non-interactive between conspecifics.

There was a significant difference in the number of eggs of *Tuta absoluta* parasitized by *Trichogramma achaeae* between the four treatments (Kruskal–Wallis test: $Z = 13.423$, $df = 3$, $P = 0.004$), and parasitism significantly increased with parasitoid density. No differences were found between treatments with single and two parasitoids, but these two treatments differed significantly from the treatments with four and six parasitoids (Fig. 3). The number of *Tuta absoluta* eggs parasitized by *Trichogramma achaeae* and the expected parasitism predicted for additive and non-interactive scenarios, are similar (Fig. 3).

3.3.2 Heterospecific experiments

We found a significant interaction between factors (feeding mode: parasitism by *Trichogramma achaeae* versus predation by *M. pygmaeus*, and natural enemy densities: 1 + 1, 2 + 2, 4 + 4 or 6 + 6) in the parasitism/consumption of *Tuta absoluta* eggs (Wald chi-square = 56.699, $df = 3$, $P < 0.001$). There was a significant difference between the number of eggs parasitized (7.52 ± 1.29) and the number of eggs consumed (139.10 ± 10.57) (Wald chi-square = 5011.389, $df = 1$, $P \leq 0.0001$). We also found a significant increase in the overall parasitism and consumption with the increase in heterospecific abundance of *Trichogramma*

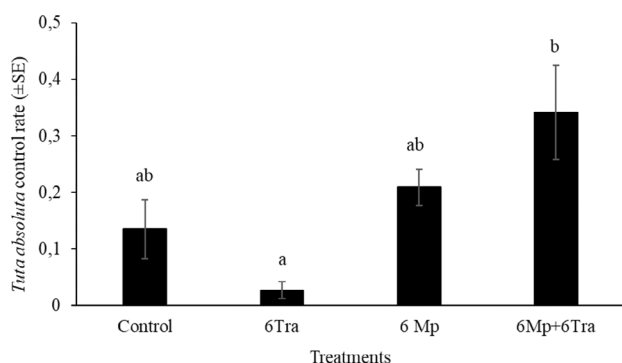


Figure 5. *In situ* impact of biological control agents against *Tuta absoluta* (proportion of *Tuta absoluta* eggs consumed by *Macrolophus pygmaeus* and parasitized by *Trichogramma achaeae*), after field release of conspecific or heterospecific guilds of *Trichogramma achaeae* and *M. pygmaeus*. Different letters in the panel indicate significant differences between treatments (Kruskal–Wallis test; $P < 0.05$).

achaeae and *M. pygmaeus* (Wald chi-square = 413.474, $df = 3$, $P \leq 0.0001$, 1 + 1: 26.87 ± 4.78 , 2 + 2: 53.53 ± 10.46 , 4 + 4: 81.67 ± 14.10 , 6 + 6: 131.11 ± 22.55). No significant differences were found between treatment 1 (1 female of *M. pygmaeus* + 1 female of *Trichogramma achaeae*) and treatment 2 (2 females of *M. pygmaeus* + 2 females of *Trichogramma achaeae*), but treatments 3 and 4 (4 females of *M. pygmaeus* + 4 females of *Trichogramma achaeae* and 6 females of *M. pygmaeus* + 6 females of *Trichogramma achaeae*, respectively) differ between them. Pairwise comparisons only show the absence of a significant difference between the number of eggs parasitized by *Trichogramma achaeae* in the treatments 1 and 2 (Fig. 4). At higher densities, the number of eggs of *Tuta absoluta* consumed by *M. pygmaeus* and parasitized by *Trichogramma achaeae* was lower than that predicted for additive and non-interactive scenarios (Fig. 4).

3.4 Effect of intraguild interactions between *M. pygmaeus* and *Trichogramma achaeae* on the number of *Tuta absoluta* eggs consumed and/or parasitized under field conditions

We found a significant difference between the rate of parasitism and consumption between experimental treatments (Kruskal–Wallis test: $Z = 6.018$, $df = 3$, $P < 0.001$). Pairwise comparisons only showed the absence of a significant difference between the following treatments: conspecific treatment with six females of *Trichogramma achaeae* and the heterospecific treatment (Fig. 5).

4 DISCUSSION

We evaluated the voracity of *M. pygmaeus* females on *Tuta absoluta* eggs unparasitized or parasitized by *Trichogramma achaeae*. Predatory females were able to consume a high number of unparasitized eggs but consumed significantly fewer parasitized eggs. Urbaneja et al.²⁸ observed identical values, but Chailleux et al.,³⁸ recorded a much lower mean number of eggs for an experimental period of 12 h; an individual predator consumed roughly ten unparasitized eggs and only roughly two parasitized eggs. These results provide evidence that intraguild predation occurs on *Trichogramma achaeae* and suggests that releases of *Trichogramma achaeae* against *Tuta absoluta* in tomatoes may slightly decrease the level of pest control when the predatory mirids are present. However, only in choice test experiments it is possible to assess the extent to which decreases in level of pest control may occur.

On mixed diets of unparasitized and parasitized eggs, the predator showed an overall decrease in voracity, even when 70% of the diet was composed by unparasitized eggs. Voracity decreased as the proportion of parasitized eggs increased. Independently of the mixed diet provided, no significant differences were found in the voracity of *M. pygmaeus* on parasitized eggs. Overall, fewer eggs were consumed when parasitized and unparasitized eggs were offered as a mixed diet than as single diets. These results highlight that, under mixed diets, even including *ad libitum* unparasitized eggs of *Tuta absoluta*, females of *M. pygmaeus* also consumed eggs parasitized by *Trichogramma achaeae*. The consumption of oophagous parasitoids, including *Trichogramma* spp. by predators is common³⁹ even for *Trichogramma achaeae*.³⁸

Macrolophus pygmaeus engages in intraguild predation on parasitized eggs of *Tuta absoluta*.³⁸ The occurrence of intraguild predation was also demonstrated in our study; however, *M. pygmaeus* preferred to consume unparasitized eggs of *Tuta absoluta*. Feeding preference toward unparasitized prey occurs in other

phytophagous species. For instance, Malo *et al.*⁴⁰ conducted a choice test in which an equal number of unparasitized nymphs or adults of *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) versus nymphs or adults parasitized by the parasitoid *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) were provided to *M. pygmaeus*. These authors found that *M. pygmaeus* consumed more unparasitized than parasitized preys. Preference response, however, is strongly affected by the relative abundances of the prey offered^{11–13} and, in contrast to the aforementioned study, our methodological procedures in which we provided to the predator varying ratios of parasitized/unparasitized eggs (30:70, 50:50 and 70:30 in a total of 100 eggs), guards against this possibility.

In the conspecific experiments, as the number of *M. pygmaeus* females increased, both the number of eggs consumed as well as mutual interference between mirids increased. In comparison with the expected number of eggs consumed in the absence of mutual interference among mirids, decreases of 29.0%, 32.8% and 37.6% in egg consumption were recorded for the treatments with two, four and six predators, respectively. For *Trichogramma achaeae*, mutual interference seems to occur in an unpredictable manner. That is, if we consider the number of parasitized eggs predicted for an additive scenario, 58.1% and 13.9% fewer parasitized eggs were recorded for the treatments with two and six parasitoids, respectively, but 70.2% more parasitized eggs were recorded for the treatment with four parasitoids. From an applied point of view, our results indicate that augmentative biological control programs against *Tuta absoluta* using monospecific releases should always take into consideration the density of eggs of *Tuta absoluta*.

Efficacy of biological control programs may be enhanced by using a combination of different natural enemies. Simultaneous use of *M. pygmaeus* and *Trichogramma achaeae* against *Tuta absoluta*, however, may lead to increased, reduced, or similar efficacy. The results of our heterospecific experiments revealed that fewer *Tuta absoluta* eggs were parasitized or consumed than expected from conspecific treatments, that is, the rate of parasitism was decreased from a minimum of 46.7% to a maximum of 71.6% of that predicted for the additive scenario. The number of eggs consumed by females of *M. pygmaeus* was decreased from that predicted for the additive scenario, by 21.7% and 14.4% for the treatments with eight (4 *M. pygmaeus* + 4 *Trichogramma achaeae*) and 12 (6 *M. pygmaeus* + 6 *Trichogramma achaeae*) biological control agents, respectively, but was slightly increased by 6.8% in the treatment with four (2 *M. pygmaeus* + 2 *Trichogramma achaeae*) biological control agents. If we combine the results of the four heterospecific treatments, there was an overall decrease in mortality of *Tuta absoluta* eggs from the rate predicted for the additive scenario, and this decrease intensifies as the number of natural enemies increases; by 0.4%, 24.0% and 64.9% for the treatments with four, eight and 12 biological control agents, respectively. All of these results indicate a possible occurrence of antagonistic interactions, given that the rate of parasitism by females of *Trichogramma achaeae* and the consumption by females of *M. pygmaeus* were lower than predicted for non-interactive scenario. Previous results have similarly shown that *Trichogramma achaeae*, from south of France, contributed to control of *Tuta absoluta* compared to what could be achieved when only the mirid predator *M. pygmaeus* was present, but without full additive effects of the two natural enemies together.³⁸

Our results do not clarify the mechanisms underlying the alteration in parasitism and consumption of *Tuta absoluta* eggs. However, it is likely that foraging activity of the mirids reduced the

availability of *Tuta absoluta* eggs for the parasitoids, and that the mirids also preyed on eggs parasitized by *Trichogramma achaeae*. In a no-choice experiment, Chailleux *et al.*³⁸ noted that *M. pygmaeus* fed about ten-fold less *Trichogramma achaeae* parasitized *Tuta absoluta* eggs (parasitized for more than 4–5 days) than unparasitized eggs. In the same study, authors found that feeding rates on *Tuta absoluta* eggs parasitized for 0–3 days, were similar to unparasitized eggs. A laboratory study in which *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) was exposed to *Tuta absoluta* eggs, then *Dolichogenidea gelechiidivoris* (Marsh) (Hymenoptera: Braconidae) was exposed to hatching larvae, revealed that their combined efficacy significantly reduced *Tuta absoluta* adult eclosion compared with when either biological control agent acted alone.⁴¹

The results obtained in the experiment under semi-field conditions, coupled with the results of laboratory experiments, confirm that *Trichogramma achaeae* may significantly increase the level of control of the pest over what could be achieved when only *M. pygmaeus* is present in tomatoes. The results obtained in a similar field study shows a better control of *Tuta absoluta*, by just over 20%, when *N. tenuis* and *Trichogramma achaeae* were both present, despite the occurrence of intraguild predation against the parasitoid.³²

Importantly, other eco-friendly alternatives to chemical pesticides, such as the use of microbial and/or botanical pesticides alone or in combination with releases of *Trichogramma* parasitoids or predatory mirid bugs,^{42,43} could also be evaluated in the near future to be implemented in sustainable pest management programs of *Tuta absoluta* in Portugal. In this context, a recent study shows the economic and financial commercial viability of a continuous mass production of *M. pygmaeus*.⁴⁴

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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