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Ephestia kuehniella Zeller eggs as a surrogate host to test feeding preference and intraguild interactions between *Macrolophus pygmaeus* (Rambur) and *Trichogramma achaeae* Nagaraja and Nagarkatti

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ABSTRACT

Mass rearing of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) eggs is a laborious task requiring investment of time, equipment and man hour work. The use of a surrogate prey/host, as *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), easily obtained and maintain, could be an option to undertake experiments. The aim of this study was to test to what extent the eggs of *E. kuehniella* is a suitable surrogate host of *T. absoluta* eggs to test for feeding preference and intraguild interactions between *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) and *Trichogramma achaeae* Nagaraja and Nagarkatti (Hymenoptera: Trichogrammatidae). Our results shows that *E. kuehniella* eggs is a suitable surrogate host to test for feeding preference and intraguild interactions between natural enemies, taking in consideration that i) under single diet, feeding on *T. absoluta* eggs is expected to be twice that on *E. kuehniella*, ii) whether offering *T. absoluta* or *E. kuehniella* under different ratios of parasitized *vs* unparasitized eggs, feeding preference was always toward non-parasitized eggs iii) in conspecific experiments with increasing density of *M. pygmaeus* females, the results show an overall decrease on feeding of 38.7% of *E. kuehniella* compared with *T. absoluta* eggs but experiments with *T. achaeae* show an overall increase in parasitism rate of 78.2% of eggs toward *E. kuehniella* eggs iv) in heterospecific experiments with increasing density of both natural enemies, when using *E. kuehniella* eggs we found an overall decrease on feeding consumption of 30% and an increase of parasitism rate of 65%.

1. Introduction

The release of mass-reared native natural enemies has proven to be a sound strategy to control pest populations in a wide range of crop systems (van Lenteren et al., 2018). This strategy is preferable over the importation and introduction of non-native natural enemies, which may pose major concerns for local biodiversity (IPBES, 2023; Soares et al., 2023). This biological control strategy, also called augmentative biological control, allows to obtain an immediate control of pests in crops with a short production cycle or for control of pests during several generations in crops with a long production cycle (van Lenteren et al., 2018).

Mass rearing of entomophagous insects under laboratory conditions usually requires the simultaneous production of the host or prey species, which doubles the rearing cost (Van Driesche and Bellows, 1996). For such, as a substitute of natural preys, commercial facilities use factious diets. The most common and economical factious diet is the eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Despite being one of the most harmful pests of stored grains, particularly flour (Mamay et al., 2022), their eggs have proven to be a suitable substitute prey for wide range of predator and parasitoid species (e.g., Schanderl et al., 1988; Tavares and Vieira, 1992). In addition to this, the eggs of Mediterranean flour moth are among of the most preferred laboratory host to test for biological, ecological and behavioral traits of a plethora of natural enemies. However, due to his high nutritional quality, it can alter the traits of insect species (Awmack and

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Leather, 2002) and thus not fully mirror biological, ecological and behavioral when tests are performed under natural preys.

Native to South America, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a worldwide pest which already invaded more than 100 countries (EPPO, 2023), imposing major concerns for tomatoes production once it causes considerable fruit quality reduction and economic losses to growers (Biondi et al., 2018). In the Mediterranean area two native natural enemies are currently being used in augmentative biological control programs: *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) and *Trichogramma achaeae* Nagaraja and Nagarkatti (Hymenoptera: Trichogrammatidae) (Desneux et al., 2022). Laboratory and field studies using these native natural enemies, either separately or simultaneously, have shown promising results in controlling *T. absoluta* in protected cultures (Oliveira et al., 2017, 2023; Borges et al., 2023a, 2023b; in press).

Very recently we undertook a study aiming to assess prey preference and intraguild interactions between *M. pygmaeus* and *T. achaeae* viewing their use in biological control programs. A limitation to the speed and fluidity of the experiments was the amount of labor and multi-tasks needed to have all the biological material for the experiments (see methodological details in Borges et al., 2023a, 2023b). Producing large quantities of *T. absoluta* eggs is an extremely laborious task requiring large investment of time, equipment and man hour work. Tomato plants must be continuously grown for lepidopteran larvae feeding and egg laying substrate for adult. The leafminer eggs are sticky to adhere to the tomato plant and must be individually collected for use in the experiments. In fact, *T. absoluta* egg collection was the most limiting factor to speed up the experiments. The use of an alternative prey/host easily obtained could be an option to be considered. In view of this, we planned a laboratory experiment aiming to test to what extent the eggs of *E. kuehniella* can be a suitable surrogate host of *T. absoluta* eggs to test for feeding preference and intraguild interactions between *M. pygmaeus* and *T. achaeae*. For such, we i) assessed prey consumption of *M. pygmaeus* fed on single diets of *E. kuehniella* eggs unparasitized or parasitized by *T. achaeae*, ii) compared prey consumption and feeding preference of *M. pygmaeus* when provided with mixed diets of *E. kuehniella* eggs both unparasitized and parasitized, (iii) assessed the effect of the density of either *M. pygmaeus* or *T. achaeae* on the number of *E. kuehniella* eggs consumed and/or parasitized (conspecific experiments and. heterospecific experiments) and iv) discuss the results obtained with the ones published in a recent paper by Borges et al. (2023b) in which *T. absoluta* eggs were used as prey.

2. Methodology

2.1. Biological material

Ephestia kuehniella eggs and *T. achaeae* parasitoids were supplied by a facility of the Biotechnology Centre of Azores (University of the Azores, Portugal). Methodological procedures to maintain laboratory populations of *T. absoluta* are described in Borges et al. (2023a; 2023b). Laboratory stock cultures and the experiments described below were carried out at 25±1 °C, 75 ± 5% relative humidity and 16L:8D photoperiod.

2.2. Prey consumption of *M. pygmaeus* feed on single diets of *E. kuehniella* unparasitized or parasitized by *T. achaeae*

To obtain eggs of *E. kuehniella* parasitized by *T. achaeae*, fresh (<24 h) UV sterilized eggs of *E. kuehniella* were placed in plastic boxes (10 cm diameter x 2 cm height) with *T. achaeae* recently emerged (<24 h). Five days later, parasitized eggs turn black and were collected for use in the experiments. *Ephestia kuehniella* unparasitized eggs used in the experiments were roughly from the same age of parasitized eggs. These same methodological procedures were used for the experiments described in the next sections. Prior to the experiment, *M. pygmaeus* females were only fed on tomato and honey diluted in water for 24 h. In the next day, 9–12 days old predator females were offered 100 parasitized or unparasitized eggs of *E. kuehniella* (N = 15) evenly distributed over a tomato plant leaflet for 24 h. The number of parasitized and unparasitized eggs of *E. kuehniella* that were predated was recorded.

2.3. Feeding preference of *M. pygmaeus* feed on mixed diets of *E. kuehniella* eggs unparasitized or parasitized by *T. achaeae*

9–12 days old females of *M. pygmaeus* were offered 3 different ratios of parasitized/unparasitized eggs: 30/70, 50/50 and 70/30 in a total of 100 eggs (N = 15). Parasitized and unparasitized eggs were mixed and then randomly distributed on the adaxial tomato plant leaflet surface, using a dissection needle. Before the experiments, *M. pygmaeus* females were deprived of animal protein food source feeding only on tomato plant and honey diluted in water, for 24h. During the experiments, females were allowed to feed on the eggs put over a tomato leaflet for 24 h and the number of unparasitized and parasitized eggs consumed was counted. Feeding preference was calculated using the formula of "Manly's preference index" (α) (Manly et al., 1972).

2.4. Effect of intraguild interactions between *M. pygmaeus* and *T. achaeae* on the number of *E. kuehniella* eggs consumed and/or parasitized

Three sets of experiments were performed. A total of 400 unparasitized *E. kuehniella* were offered to different densities of *M. pygmaeus* and/or *T. achaeae* for 24 h (methodology adopted from Northfield et al., 2010). Conspecific experiments with females of *T. achaeae* (Experiment 1) and conspecific experiments with adults of *M. pygmaeus* (Experiment 2) consisted in 4 treatments in which 1, 2, 4 or 6 natural enemies were offered *E. kuehniella* eggs. Heterospecific experiments with predator + parasitoid females (Experiment 3) consisted in 4 treatments in which 1 + 1, 2 + 2, 4 + 4 or 6 + 6 natural enemies were offered *E. kuehniella* eggs.

2.5. Statistical analysis

Prey consumption of *M. pygmaeus* fed on single diets of unparasitized or parasitized eggs of *E. kuehniella* was analysed using a *t*-test.

To contrast feeding preference (Manly index) of *M. pygmaeus* fed on mixed diets of unparasitized and parasitized eggs of *E. kuehniella*, a Wilcoxon's matched-pairs signed rank test was applied to each of the three diets.

To compare the effect of *M. pygmaeus* or *T. achaeae* densities (conspecific intraguild interactions) on the number of *E. kuehniella* eggs consumed or parasitized, a general linear model (GLM) with one-way ANOVA was used, using the Post-Hoc LSD test. To test for the effect of *M. pygmaeus* and *T. achaeae* densities (heterospecific intraguild interactions) on the number of *E. kuehniella* eggs consumed and parasitized, a Generalized Linear Model (GZLM) was used and a Pairwise multi comparisons were performed with P values corrected using Bonferroni test. If significant interactions between independent variables were verified, a univariate general linear model (GLM) with one-way ANOVA was used, instead. Finally, the number of eggs consumed or parasitized in conspecific and heterospecific experiments were contrasted with the predicted values if the interactions were additive and non-interactive, using One-sample *t*-test.

The results obtained in the current experiments, namely prey consumption on single diets and feeding preference under mixed diets, were contrasted with the results published in Borges et al. (2023b), in which *T. absoluta* was used as food resource. For such, we contrasted the independent factors i) host species (eggs of *T. absoluta* or *E. kuehniella*) and ii) host quality (parasitized or unparasitized eggs), by using a Generalized Linear Model (GZLM) was used and a Pairwise multi comparisons were performed with P values corrected using Bonferroni test. If significant interactions between independent variables were verified, a univariate general linear model (GLM) with one-way ANOVA was used, instead.

The effect of intraguild interactions between *M. pygmaeus* and/or *T. achaeae* on the number of eggs consumed and/or parasitized, was also contrasted with the results by Borges et al. (2023b). A univariate general linear model (GLM) with one-way ANOVA was applied to compare the parasitism or egg consumption between host species (eggs of *T. absoluta* vs *E. kuehniella*) at each single natural enemy density.

A Kolmogorov-Smirnov and Levene tests were used to assess normal distribution and homogeneity of variances of data, respectively. Mean values were considered significantly different when $P < 0.05$. All statistical analyses were done using SPSS v. 27 (IBM Corp, 2020).

3. Results

3.1. Voracity of M. pygmaeus fed on single diets E. kuehniella eggs parasitized by T. achaeae or unparasitized

We found a significant difference between the mean number of *E. kuehniella* eggs consumed (parasitized = 5.2 ± 0.9 and unparasitized: 37.3 ± 6.4 (*t*-test = −6.646, df = 28, P = 0.001; Fig. 1).

3.2. Feeding preference of M. pygmaeus fed on mixed diets of E. kuehniella eggs unparasitized or parasitized by T. achaeae

Females of *M. pygmaeus* showed a significant preference for unparasitized eggs (WMPSR; 70:30, Z = −3.408, $P < 0.001$; 50:50, Z = −3·324, $P < 0.001$; 30:70, Z = −3.181, $P < 0.001$) (Fig. 2).

3.3. Effect of intraguild interactions between M. pygmaeus and T. achaeae on the number of E. kuehniella eggs consumed and/or parasitized under laboratorial conditions

3.3.1. Conspecific experiments

We found significant differences on the number of eggs of *E. kuehniella* consumed by *M. pygmaeus* (GLM test: Z = 11.423, df = 3, $P < 0.001$). Prey consumption significantly increased with predator density, despite no differences between the treatments of two, four or six *M. pygmaeus* (Fig. 3). The number of eggs consumed is lower than that predicted for additive and non-interactive scenarios (the expected values of eggs consumed/parasitized in each treatment was obtained by the sum of the consumed/parasitized eggs by one individual alone), especially in the treatments with four and six *M. pygmaeus* (2 *M. pygmaeus*: t = −0.589, df = 14, p = 0.565; 4 *M. pygmaeus*: t = −4.678, df = 14, $p < 0.001$; 6 *M. pygmaeus*: t = −5.911, df = 14, $p < 0.001$ Fig. 3).

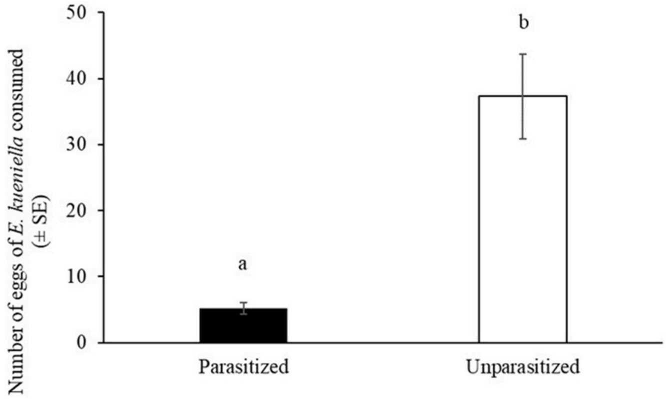

Fig. 1. Mean number of eggs consumed (±SE) by females of *M. pygmaeus* fed on single diets of eggs of *E. kuehniella* parasitized by *T. achaeae* or unparasitized. Different letters indicate significant differences (*t*-test; $P < 0.05$).

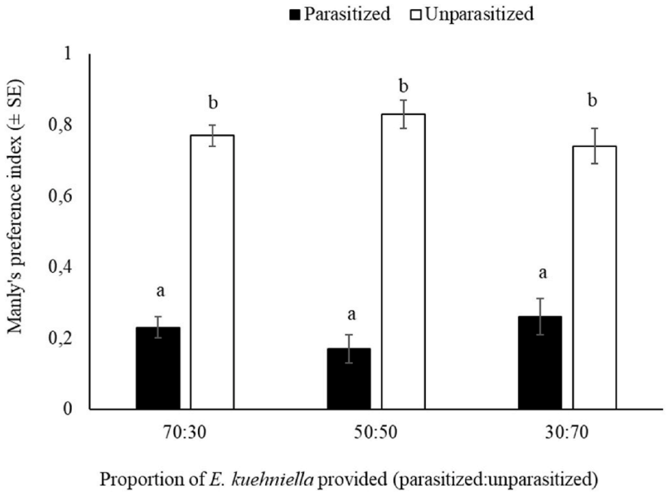

Fig. 2. Prey preference of *M. pygmaeus* fed on three different ratios of *E. kuehniella* eggs (70 parasitized:30 unparasitized, 50:50 or 30:70). Different letters on each prey ratio indicate significant differences (Wilcoxon WMPSR test; $P < 0.05$).

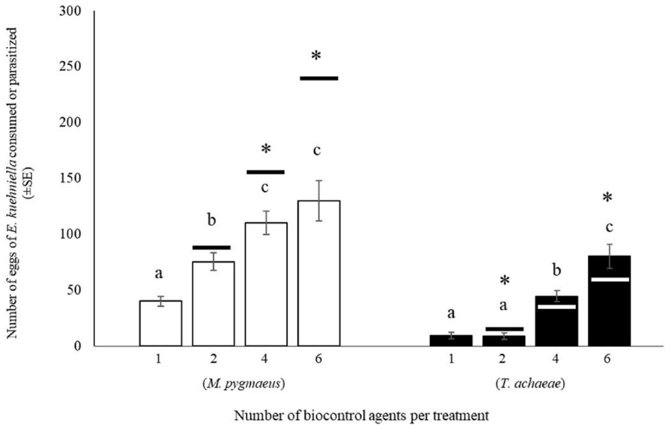

Fig. 3. Number of *E. kuehniella* eggs consumed by *M. pygmaeus* (white histograms) and parasitized by *T. achaeae* (black histograms) in function of the increased number of conspecifics provided. Different letters in each panel indicate significant differences between treatments (GLM; $P < 0.05$). Black or white lines over the bars depicted the number of eggs consumed or parasitized predicted for additive and non-interactive between conspecifics and the asterisks stands for statistically significant.

There was a significant difference in the number of eggs of *E. kuehniella* parasitized by *T. achaeae* (GLM test: Z = 28.014, df = 3, $p < 0.001$). 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 *E. kuehniella* eggs parasitized by *T. achaeae* is significant higher than the expected parasitism predicted for additive and non-interactive scenarios, especially in the treatments with 2 *T. achaeae* and significant lower in the treatments with 6 *T. achaeae* (2 *T. achaeae*: t = −3.161, df = 14, p = 0.007; 4 *T. achaeae*: t = 1.153, df = 14, p = 0.174; 6 *T. achaeae*: t = 2.259, df = 14, p = 0.04 Fig. 3).

3.3.2. Heterospecific experiments

We found a significant interaction between factors i) feeding mode (parasitism *vs* predation and ii) natural enemy densities (1 + 1, 2 + 2, 4 + 4 or 6 + 6) in the parasitism/consumption of *E. kuehniella* eggs (Wald Chi-Square = 51.048, df = 3, $P < 0.001$). There was a significant difference between the number of eggs parasitized (21.57 ± 2.72) and the number of eggs consumed (98.98 ± 8.61) (Wald Chi-Square $= 1842.017$, $df = 1$, $P \leq 0.0001$). We also found a significant increase in the overall parasitism and consumption with the increase of guild abundance (Wald Chi-Square $= 1066.639$, $df = 3$, $p \leq 0.0001$, 1 + 1: 21.27 ± 3.20, 2 + 2: 38.57 ± 5.35, 4 + 4: 78.57 ± 11.04, 6 + 6: 102.70 ± 15.11). Pairwise comparation shows significant differences between all treatments (Fig. 4). The number of eggs of *E. kuehniella* parasitized by *T. archaeae* was significantly lower than predicted for additive and non-interactive scenario (1 + 1: $t = 3.724$, $df = 14$, $p = 0.001$; 2 + 2: $t = 4.882$, $df = 14$, $p < 0.001$; 6 + 6: $t = -4.576$, $df = 14$, $p < 0.001$). For *M. pygmaeus*, on the other hand, the results show an additive and non-interactive scenario (1 + 1: $t = -0.229$, $df = 14$, $p = 0.822$; 2 + 2: $t = 0.147$, $df = 14$, $p = 0.885$; 6 + 6: $t = 1.071$, $df = 14$, $p = 0.302$) (Fig. 4).

4. Discussion

Throughout this section we will discuss and contrast the results obtained in the current with the one published in the study by Borges et al. (2023b), in which *T. absoluta* eggs were used as prey and the methodological procedures were the same.

Prey consumption. Prey consumption by females of *M. pygmaeus* fed on single diets of *E. kuehniella* eggs reveals that the mirid was able to consume more unparasitized than parasitized eggs. Similar results were obtained in previously studies in which consumption decreased when parasitized eggs were provided (Urbaneja et al., 2009; Chailleux et al., 2013; Borges et al., 2023b). However, the mentioned studies were preformed using eggs of *T. absoluta*. Contrasting the results obtained in the present study with those published by Borges et al. (2023b), in which *T. absoluta* eggs was provided, we found no statistically significant interaction between the independent factors i) host species (eggs of *T. absoluta* or *E. kuehniella*) and ii) host quality (parasitized or unparasitized eggs) (Chi-Square $= 2.804$, $df = 1$ $P = 0.094$). The number of eggs of *T. absoluta* consumed was significant higher (Chi-Square $= 12.545$, $df = 1$ $P < 0.001$) and significantly more unparasitized eggs were consumed (Chi-Square $= 77.487$, $df = 1$ $P \leq 0.0001$). Females of *M. pygmaeus* consumed 5.2 ± 0.9 and 37.3 ± 6.3 parasitized and unparasitized eggs of *E. kuehniella*, respectively and 13.6 ± 3.4 and 60.7 ± 5.3 parasitized and unparasitized eggs of *T. absoluta*, respectively. This means that, when fed on parasitized eggs of *E. kuehniella* or *T. absoluta*, the mirid reduce consumption by 86% or 78%, respectively. Consumption on *T. absoluta* eggs was approximately twice than that of *E. kuehniella* and this may be related with relative eggs sizes. Indeed, egg size of *T. absoluta* is about 0.38 mm in length by 0.21 mm in width (Vargas 1970) and 0.57 mm × 0.30 mm for *E. kuehniella* (Richards and Thomson 1932). Given these results, *E. kuehniella* eggs seems a suitable surrogate host to test feeding consumption of unparasitized and parasitized eggs of *T. absoluta*.

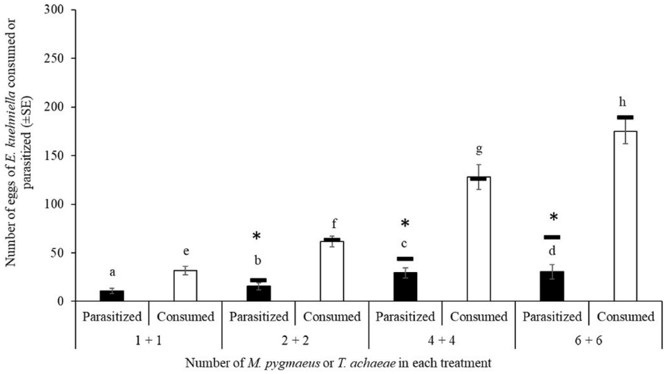

Fig. 4. Number of *E. kuehniella* eggs (±SE) parasitized by *T. achaeae* or consumed by *M. pygmaeus* in treatments in which the number of heterospecifics provided increased. Different letters in each panel indicate significant differences between treatments (GLM, $P < 0.05$). Black lines over the bars depicted the number of consumed eggs predicted for the case of additive and non-interactive between heterospecific and the asterisks stands for statistically significant.

Feeding preference. Independently of the ratio of prey provided, *M. pygmaeus* females always displayed a feeding preference for unparasitized eggs. Under mixed diets, about 80% of the eggs consumed were unparasitized. Feeding preference toward unparasitized hosts occurs on other predator species. For instance, *M. pygmaeus* prefer to consume *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) over *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) when immature stages and adults of *B. tabaci* and *E. mundus* were offered (Malo et al., 2012). In Malo's (2012) study, parasitized and unparasitized preys were provided in a ratio of 50:50. However, preference responses are affected by the relative abundances of the preys (Murdoch, 1969; Cock, 1978; Sherratt and Harvey, 1993). Our methodological procedures guards against this possibility because predators were supplied with different ratios of unparasitized and parasitized eggs. Contrasting our results with feeding preference determined by Borges et al. (2023b), we found a non-significant interaction between the factors i) host species (eggs of *T. absoluta* or *E. kuehniella*) and ii) proportion of prey provided (70:30, 50:50 and 30:70) (Chi-Square $= 5.150$, $df = 2$, $P = 0.076$), no significant difference in the Manly index for *T. absoluta* and *E. kuehniella* eggs (Chi-Square $= 3.236$, $df = 1$, $P = 0.072$) and for proportion of prey provided (Chi-Square $= 1.553$, $df = 2$, $P = 0.460$). Mean values of Manly index for *T. absoluta* (parasitized: 0.160 ± 0.03 and unparasitized: 0.840 ± 0.03) and *E. kuehniella* (parasitized: 0.218 ± 0.024 and unparasitized: 0.782 ± 0.024) eggs. For diet ratios, mean values were [70:30 (parasitized: 0.152 ± 0.024 and unparasitized: 0.833 ± 0.024), 50:50 (0.196 ± 0.039 and 0.833 ± 0.024) and 30:70 (0.2 06 ± 0.032 and 0.84 ± 0.032)]. In short, independently of the ratio of prey and its quality (parasitized vs unparasitized), we found no differences in the feeding preferences and thus *E. kuehniella* seems a suitable surrogate host to test for feeding preference.

Conspecific experiments. The results of the conspecific experiments reveal: i) that despite a steady increase in prey consumption with predator density, no significant differences in treatments with two, fourth and six *M. pygmaeus* females, and ii) a substantial alteration on the amount of prey consumed predicted by an additive scenario. Contrasting our results on prey consumption with the previous results by Borges et al. (2023b), we found that the number of eggs of *T. absoluta* consumed (152.2 ± 11.7) was significantly higher than *E. kuehniella* (88.8 ± 7.2) (Chi-Square $= 53.116$, $df = 1$, $P < 0.001$) and the number of eggs consumed increases as the number of conspecifics increases in the guild (Wald Chi-Square $= 153.474$, $df = 3$, $P \leq 0.0001$, 1 *M. pygmaeus*: 54.4 ± 4.3, 2: 86.5 ± 7.1, 4: 147.5 ± 11.5, 6: 193.7 ± 17.2), with no significant differences between the first two treatments. However, there was a significant interaction between the independent factors i) host species (eggs of *T. absoluta* or *E. kuehniella*) and ii) *M. pygmaeus* density (Chi-Square $= 23.486$, $df = 3$, $P \leq 0.0001$). Contrasting the mean number of *T. absoluta vs E. kuehniella* eggs consumed by *M. pygmaeus*, to each single conspecific treatment, we found a significant decrease in the consumption of *T. absoluta* eggs, with exception for the density of 2 *M. pygmaeus* females (Fig. 5A, Table 1). Similarly to the experiments with *E. kuehniella* eggs, the number of eggs of *T. aboluta* consumed were lower than that predicted for additive and non-interactive scenarios (2 *M. pygmaeus*: $t = -3.512$, $df = 14$, $p = 0.003$; 4 *M. pygmaeus*: $t = -5.649$, $df = 14$, $p < 0.001$; 6 *M. pygmaeus*: $t = -8.439$, $df = 14$, $p < 0.001$). These results reveal mutual interference as the number of *M. pygmaeus* females increased. For purposes of laboratory experiments, *E. kuehniella* mimics in a similar way the trends in food consumption rates and the antagonistic impacts that the predator has when feeding on *T. absoluta*. However, when using *E. kuehniella* eggs as a surrogate host, we should expect an overall decrease on feeding consumption of 38,7% of eggs, ranging from 23% to 49%, for densities of two, fourth and six

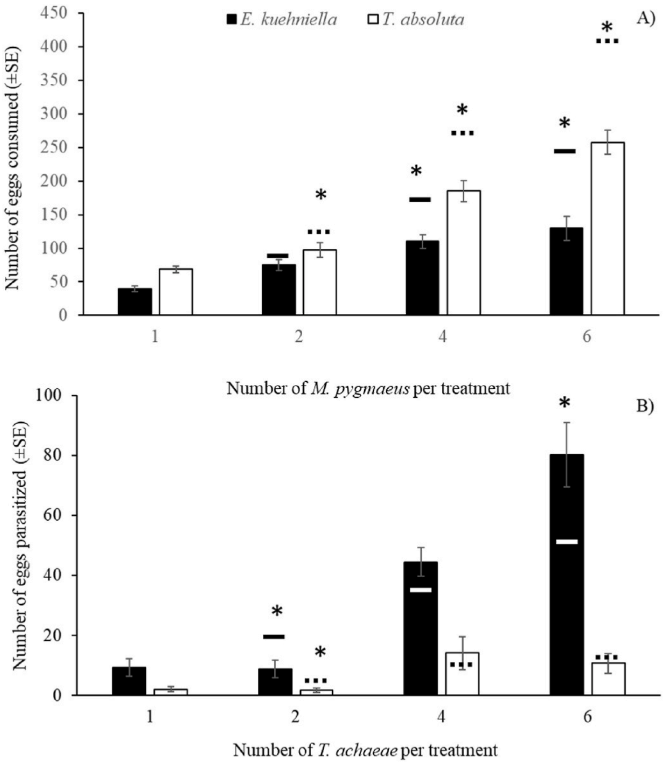

Fig. 5. Number of *E. kuehniella* (black histograms) or *T. absoluta* (white histograms) eggs consumed by *M. pygmaeus* (A) or parasitized by *T. achaeae* (B) in treatments in which an increasing number of conspecifics were provided. Different letters in each panel indicate significant differences between treatments (*t*-test or Kruskall-Wallis test; $P < 0.05$). Black or dashed lines over the bars depicted the number of consumed or parasitized eggs predicted for the case of additive and non-interactive between conspecific and the asterisks stands for statistically significant.

Table 1
Statistical significance between the number of eggs consumed (*E. kuehniella vs T. absoluta*) and number of eggs parasitized (*E. kuehniella vs T. absoluta*) under different biological control agent's densities of *M. pygmaeus* or *T. achaeae*.

	Conspecific experiments: biological control agent's densities (*M. pygmaeus* or *T. achaeae*)	GLM (Z)	df	p
Number of eggs consumed	**1**	16.989	1	<0.001
	2	2601	1	0.118
	4	15.180	1	<0.001
	6	23.733	1	<0.001
Number of eggs parasitized	**1**	5.08	1	0.032
	2	5.262		0.03
	4	15.847		<0.001
	6	36.133		<0.001

M. pygmaeus females, respectively.

Contrasting the results of conspecific experiments with *T. achaeae* with the one published in Borges et al. (2023b), we found that number of eggs of *E. kuehniella* parasitized (35.7 ± 4.9) was significantly higher than *T. absoluta* (7.1 ± 1.7) (Chi-Square = 65.97, df = 1, $P < 0.001$) and the number of eggs consumed increases as the number of conspecifics increases in the guild (Wald Chi-Square = 92.855, df = 3, $P \leq 0.0001$, 1 *M. pygmaeus*: 5.6 ± 1.7, 2: 5.3 ± 1.6, 4: 29.3 ± 4.6, 6: 45.4 ± 8.4), with no significant differences in the first two treatments. However, there was a significant interaction between the independent factors i) host species (eggs of *T. absoluta* or *E. kuehniella*) and ii) *M. pygmaeus* density (Chi-Square = 52.616, df = 3, $P < 0.001$). Contrasting the mean number of *T. absoluta vs E. kuehniella* eggs parasitized by *T. achaeae* under each single conspecific treatment, we found a significant increase on the parasitism rate on *E. kuehniella* eggs, mainly for the densities of 4 and 6 *T. achaeae* (Fig. 5b–Table 1). The effects of the use of multiple *T. achaeae* are similar when using either *T. absoluta* or E. *kuehniella*, except for the density of 6 parasitoids (2 *T. achaeae*: t = −3.518, df = 14, p = 0.007; 4 *T. achaeae*: t = 1.005, df = 14, p = 0.332; 6 *T. achaeae*: t = −0.514, df = 14, p = 0.616). Those results suggest that *E. kuehniella* elicit a stronger competition effect when using six parasitoids, leading to an increase parasitism rate and this may be due to higher host quality compared to *T. absoluta* eggs. Recent results show differences in suitability between these two hosts (Borges et al., 2023a, 2024). For purposes of laboratory experiments, *E. kuehniella* leads to i) a similar decreasing trend in the parasitism rate and ii) a synergetic impact contrary to the apparent additive effect when using *T. absoluta* eggs. Moreover, when using *E. kuehniella* eggs as a surrogate host for laboratory experiments, we should expect an overall increase of 78,2% on parasitism rate, ranging from 68% to 86%.

Heterospecific experiments. The outcome of interactions between natural enemies can be (i) additive/non-interactive, (ii) antagonistic 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. The results of our heterospecific experiments shows that, as the number of biocontrol agents increased i) the number of eggs consumed and parasitized significantly increased but ii) the number of egg consumption by *M. pygmaeus* do not reveal a substantial alteration from that predicted by the additive scenario and iii) the parasitism by *T. achaeae* was decreased in a rate of that predicted for the additive scenario. Comparing our results with Borges et al. (2023b), we found that consumption on eggs of *T. absoluta* (139.1 ± 10.6) was significantly higher than *E. kuehniella* (98.9 ± 8.6) (Chi-Square = 36.43, df = 1, $P < 0.001$) and the consumption increases as the number of conspecifics increases (Chi-Square = 379.221, df = 3, $P < 0.001$, 1 *M. pygmaeus* + 1 *T. achaeae*: 40.2 ± 3.6, 2 + 2: 82.9 ± 6.8, 4 + 4: 140.3 ± 8.2, 6 + 6: 212.7 ± 10.9), with significant differences between all treatments. However, there was a significant interaction between the independent factors i) host species (eggs of *T. absoluta* or *E. kuehniella*) and ii) *M. pygmaeus* density (Chi-Square = 11.408, df = 3, P = 0.01). Contrasting the mean number of eggs consumed by *M. pygmaeus* under each single heterospecific treatment, we found a significant higher consume on *E. kuehniella* eggs, with exception for the density of 4 *M. pygmaeus* + 4 *T. achaeae* (Fig. 6A, Table 2). Concerning to the mean number of eggs parasitized by *T. achaeae* under each single heterospecific treatment, we found a higher parasitism rate on *E. kuehniella* eggs, with exception for the density of 1 *M. pygmaeus* + 1 *T. achaeae* (Fig. 6B–Table 2). Under multi specific guilds, there was an antagonistic effect on *T. achaeae*, independently of the host provided; *T. absoluta* or *E. kuehniella* (for *T. aboluta*, 1 + 1: t = −6.08, df = 14, $p < 0.001$; 2 + 2: t = 3.265, df = 14, p = 0.006; 6 + 6: t = 5.084, df = 14, $p < 0.001$). For *M. pygmaeus*, on the other hand, the effect switched from an additive to an antagonistic effect, except for the density 1 *M. pygmaeus* + 1 *T. achaeae* (1 + 1: t = −0.664, df = 14, p = 0.517; 2 + 2: t = −3.836, df = 14, p = 0.002; 6 + 6: t = −3.681, df = 14, p = 0.002). These results indicate that, contrarily to *M. pygmaeus* female, *T. achaeae* are affected by intraguild interactions. Similar study, using *T. absoluta* as prey, showed that *T. achaeae* contributed to control of pest 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 (Chailleux et al., 2013). These comparative results suggest that when using *E. kuehniella* eggs as a surrogate host to test intraguild interactions between *M. pygmaeus* or *T. achaeae*, we should expect an overall decrease on feeding consumption of 30% and an increase of parasitism rate of 65%.

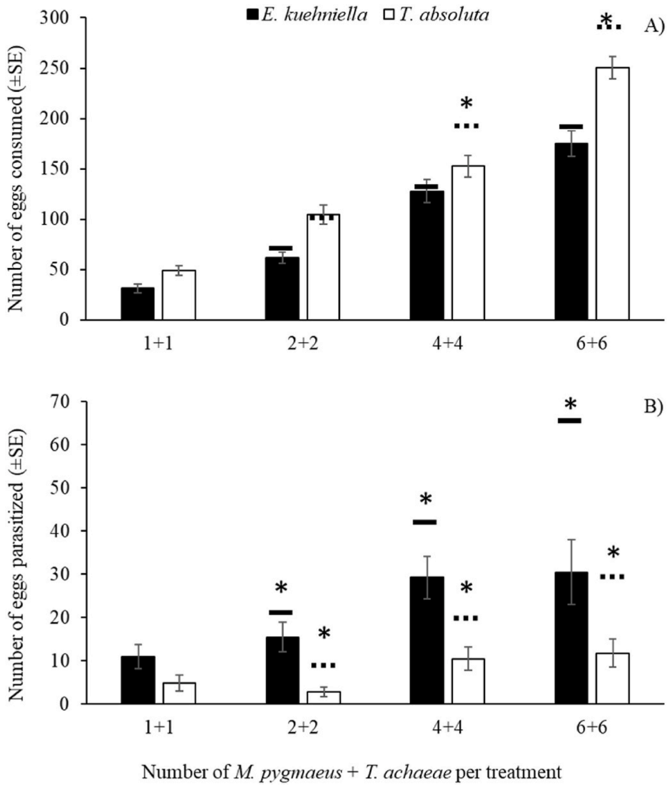

Fig. 6. Number of *E. kuehniella* (black histograms) or *T. absoluta* (white histograms) eggs consumed (±SE) by *M. pygmaeus* (A) or parasitized *T. achaeae* (B) in treatments in which an increasing number of heterospecific were provided (*t*-test or Kruskall-Wallis test; $P < 0.05$). Black or dashed lines over the bars depicted the number of consumed or parasitized eggs predicted for the case of additive and non-interactive between heterospecific and the asterisks stands for statistically significant.

Table 2

Statistical significance between the number of eggs consumed (*E. kuehniella* vs *T. absoluta*) and number of eggs parasitized (*E. kuehniella* vs *T. absoluta*), under different biological control agent's densities of *M. pygmaeus* and *T. achaeae*.

	Heterospecific experiments: biological control agent's densities (*M. pygmaeus* + *T. achaeae*)	GLM (Z)	df	p
Number of eggs consumed	1 + 1	6.389	1	0.017
	2 + 2	13.492	1	0.001
	4 + 4	2.300	1	0.141
	6 + 6	18.774	1	<0.001
Number of eggs parasitized	1 + 1	3.161	1	0.086
	2 + 2	11.444	1	0.002
	4 + 4	10.458	1	0.003
	6 + 6	4.821	1	0.037

In conclusion, when prey was changed, we found some differences in the values of feeding preference and intraguild interactions and this may result from egg size and/or suitability and/or suitability of host eggs to *T. achaeae*. However, we found the trends in terms of feeding preference and intraguild interactions were the same independently if prey and the magnitude of this changes was provided in our study. The use of *E. kuehniella* eggs should not replace laboratory tests on focal prey. However, if such a replacement occurs, and for this system, the magnitude of the changes in the tested parameters are now known.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Availability of data and materials

Not applicable.

CRediT authorship contribution statement

António O. Soares: Writing – original draft, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Ana C. Durão:** Writing – review & editing, Investigation. **Luisa Oliveira:** Writing – review & editing, Methodology, Investigation. **Patrícia Arruda:** Writing – review & editing, Investigation. **Isabel Borges:** Writing – review & editing, Supervision, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

References

Awmack, C.S., Leather, S.R., 2002. Host plant quality and fecundity in herbivorous insects. Annu, Ver, Entomol 47, 817–844. https://doi.org/10.1146/annurev.ento.47.091201.145300.

Biondi, A., Narciso, R., Guedes, C., Wan, F.H., Desneux, N., 2018. Ecology, worldwide spread, and management of the invasive south American tomato pinworm, *Tuta absoluta*: past, present, and future. Annu. Rev. Entomol. 63, 239–258. https://doi.org/10.1146/annurev-ento-031616-034933.

Borges, I., Oliveira, L., Durão, A.C., Arruda, P., Figueiredo, E., Franco, J.C., Lucas, E., Soares, A.O., 2023a. Contrasting phenotypic variability of life-history traits of two feral populations of *Macrolophus pygmaeus* (Hemiptera: Miridae) under two alternative diets. Agronomy 13, 118. https://doi.org/10.3390/agronomy13010118.

Borges, I., Oliveira, L., Durão, A.C., Arruda, P., Soares, A.O., 2023b. Feeding preference and intraguild interactions between the parasitoid *Trichogramma achaeae* and the predator *Macrolophus pygmaeus*, two biological agents of *Tuta absoluta*. Pest Manag. Sci. 79, 4376–4382. https://doi.org/10.1002/ps.7635.

Borges, I., Oliveira, L., Barbosa, F., Figueiredo, E., Franco, J.C., Durão, A.C., Soares, A.O., 2024. Prey consumption and conversion efficiency in females of two feral populations of *Macrolophus pygmaeus*, a biocontrol agent of *Tuta absoluta*. Phytoparasitica. https://doi.org/10.1007/s12600-024-01130-0.

Chailleux, A., Bearez, P., Pizzol, J., Amiens-Desneux, E., Ramirez-Romero, R., Desneux, N., 2013. Potential for combined use of parasitoids and generalist predators for biological control of the key invasive tomato pest *Tuta absoluta*. J. Pest. Sci. 86, 533–541. https://doi.org/10.1007/s10340-013-0498-6.

Cock, M.J.W., 1978. The assessment of preference. J. Anim. Ecol. 47, 805–816. https://www.jstor.org/stable/1937838.

Desneux, N., Han, P., Mansour, R., Arno, J., Brevault, T., Campos, M.R., et al., 2022. Integrated Pest Management of *Tuta absoluta*: practical implementations across different world regions. J. Pest. Sci. 95, 17–39. https://link.springer.com/article/10.1007/s10340-021-01442-8.

EPPO, 2023. *Tuta Absoluta* (GNORAB).

IBM Corp, 2020. IBM SPSS Statistics for Windows, Version 27.0. IBM Corp, Armonk, NY.

IPBES, 2023. In: Roy, H.E., Pauchard, A., Stoett, P., Renard Truong, T., Bacher, S., Galil, B.S., Hulme, P.E., Ikeda, T., Sankaran, K.V., McGeoch, M.A., Meyerson, L.A., Nuñez, M.A., Ordonez, A., Rahlao, S.J., Schwindt, E., Seebens, H., Sheppard, A.W., Vandvik, V. (Eds.), Summary for Policymakers of the Thematic Assessment Report on Invasive Alien Species and Their Control of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. IPBES secretariat, Bonn, Germany. https://doi.org/10.5281/zenodo.7430692.

Malo, S., Arnó, J., Gabarra, R., 2012. Intraguild interactions between the predator *Macrolophus pygmaeus* and the parasitoid *Eretmocerus mundus*, natural enemies of *Bemisia tabaci*. Biocontrol Sci. Technol. 22, 1059–1073. https://doi.org/10.1080/09583157.2012.711469.

Mamay, M., Karakuş, H., Ghramh, H.A., Çıkman, E., 2022. Optimizing diet thickness and egg density for economic mass rearing of *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae): a laboratory host for biological control agents. J. King Saud Univ. Sci. 34, 102276. https://doi.org/10.1016/j.jksus.2022.102276.

Manly, B.F.J., Miller, P., Cook, L.M., 1972. Analysis of a selective predation experiment. Am. Nat. 106, 719–736. https://www.jstor.org/stable/2459502.

Murdoch, W.W., 1969. Switching in general predators: experiments on predator specificity and stability of prey populations. Ecol. Monogr. 4, 335–354. https://doi.org/10.2307/1942352.

Northfield, T.D., Snyder, G.B., Ives, A.R., Snyder, W.E., 2010. Niche saturation reveals resource partitioning among consumers. Ecol. Lett. 13, 338–348. https://doi.org/10.1111/j.1461-0248.2009.01428.x.

Oliveira, L., Durão, A.C., Fontes, J., Roja, I.S., Tavares, J., 2017. Potential of *Trichogramma achaeae* (hymenoptera: Trichogrammatidae) in biological control of Tuta absoluta (Lepidoptera: Gelechiidae) in azorean greenhouse tomato crops. J. Econ. Entomol. 110, 2010–2015. https://doi.org/10.1093/jee/tox197.

Oliveira, L., Borges, I., Silva, D., Durão, A.C., Soares, A.O., 2023. Abundance of *Tuta absoluta* (Meyrick, 1917) and its natural enemies on tomato crops in greenhouses of different production modes (Azores, Portugal) (Lepidoptera: Gelechiidae). SHIL. Rev. lepidopterol. 51, 59–70. https://doi.org/10.57065/shilap.437.

Richards, O.W., Thomson, W.S., 1932. A contribution to the study of the genera Ephestia, GN. (including Strymax, Dyar) and Plodia, GN. (Lepidoptera, Phycitidae), with notes on parasites of the larvae. Trans. R. Entomol. Soc. 80, 169–250. https://doi.org/10.1111/j.1365-2311.1932.tb03306.x.

Schanderl, H., Ferran, A., Garcia, V., 1998. L'élevage de deux coccinelles *Harmonia axyridis* et *Semiadalia undecimnotata* à l'aide d'oeufs d'*Anagasta kuehniella* tués aux rayons ultraviolets. Entomol. Exp. Appl. 49, 235–244. https://doi.org/10.1111/j.1570-7458.1988.tb01184.x.

Sherratt, T.N., Harvey, I.F., 1993. Frequency-dependent food selection by arthropods: a review. Biol. J. Linn. Soc. 48, 167–186. https://doi.org/10.1111/j.1095-8312.1993.tb00885.x.

Soares, A.O., Haelewaters, D., Ameixa, O.M.C.C., Borges, I., Brown, P.M.J., Cardoso, P., de Groot, M.D., Evans, E.W., et al., 2023. A roadmap for ladybird conservation and recovery. Conserv. Biol., e13965 https://doi.org/10.1111/cobi.13965.

Tavares, J., Vieira, V., 1992. Producão em massa de *Ephestia kuehniella* Zeller (Lep., Pyralidae) IV—Técnicas de recolha dos adultos e ovos. Açoreana 7, 461–470.

Urbaneja, A., Montón, H., Mollá, O., 2009. Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. J. Appl. Entomol. 133, 292–296. https://doi.org/10.1111/j.1439-0418.2008.01319.x.

Van Driesche, R.G., Bellows, T.S., 1996. In: Van Driesch, R.G., Bellows, T.S. (Eds.), Biological Control Agents for Plant Pathogens, Biological Control. Springer, US, Boston, MA, pp. 93–101. https://doi.org/10.1007/978-1-4613-1157-7_6, 1996.

van Lenteren, J.C., Bolckmans, K., Köhl, J., Ravensberg, W.J., Urbaneja, A., 2018. Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl 63, 39–59. https://doi.org/10.1007/s10526-017-9801-4.

Vargas, H., 1970. Observaciones sobre la biologia enemigos naturales de las polillas del tomate, Gnorimoschema absoluta (Meyrick). Depto. Agricultura, Universidad del Norte-Arica 1, 75–110.