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Influence of temperatures and diets on predation rate and olfactory behavior of the predatory bug, *Macrolophus pygmaeus*

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Abstract

The generalist predator, *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae), is considered as a promising biological control agent for managing pest populations in greenhouses. Nevertheless, the influences of various factors, including the type of prey, environmental conditions, and predator sex, on its predation behavior remain to be fully researched. This study explored the effects of these factors on the predation rate of *M. pygmaeus* and its herbivory behavior on tomato plants. According to the results, the predation rate varied significantly depending on the type of prey, with a higher rate observed when fed on eggs of tomato leaf miner (TLM), *Tuta absoluta* Meyrick (Lepidoptera, Gelechiidae) than those eating *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and *Artemia* cysts. The interaction between prey type and temperature had a significant impact, with the highest rate observed when feeding on TLM eggs at 30°C. Female predators exhibited a higher predation rate at 30°C compared to 25°C and 20°C. Irrespective of the presence or absence of animal prey, the generalist predator did not reveal significant phytophagy, as its herbivory behavior did not cause significant damage to the tomato plants. Olfactory responses of *M. pygmaeus* to volatile cues emanating from different food sources indicated no strong attraction to specific prey based on volatile chemical cues. Overall, the findings suggest that the temperature needs to be elevated to 30°C in greenhouses for better control of TLM by the predator.

Keywords: biocontrol program, greenhouse pests, egg, allelochemicals, tomato

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تاثیر دما و رژیم غذایی روی نرخ شکارگری و پاسخ‌های بویایی *Macrolophus pygmaeus*

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چکیده

سن شکارگر *Macrolophus pygmaeus* Rumber (Hemiptera: Pentatomidae) به عنوان یک عامل کنترل بیولوژیکی امیدوارکننده برای مدیریت جمعیت آفات در گلخانه‌ها در نظر گرفته می‌شود. با این حال، تأثیر عوامل مختلف، از جمله نوع طعمه، شرایط محیطی و جنسیت شکارگر، بر رفتار این شکارگر عمومی تا کنون مورد مطالعه قرار نگرفته بود. این مطالعه تأثیر این عوامل بر نرخ شکار *M. pygmaeus* و رفتار گیاهخواری آن روی گیاهان گوجه‌فرنگی، مورد بررسی قرار داده است. نتایج نشان داد که نرخ شکار بسته به نوع طعمه به طور قابل توجهی متفاوت بود، به طوری‌که نرخ بالاتری از تغذیه روی تخم‌های مینوز برگ گوجه‌فرنگی *Tuta absoluta* Meyrick (Lepidoptera, Gelechiidae) (TLM)، در مقایسه با *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) و کیست‌های آرتمیا مشاهده شد. تعامل بین نوع طعمه و دما تأثیر قابل توجهی داشت به صورتی‌که بالاترین نرخ شکارهنگام تغذیه از تخم‌های TLM در دمای ۳۰ درجه سلیسیوس مشاهده شد. مطالعه نشان داد که شکارگران ماده در دمای ۳۰ درجه سلیسیوس نرخ شکار بالاتری نسبت به دماهای ۲۵ و ۲۰ درجه سلیسیوس از خود نشان دادند. صرف نظر از وجود یا عدم وجود طعمه حیوانی، این شکارگر ، گیاه‌خواری قابل توجهی نشان نداد، زیرا رفتار گیاهخواری آن آسیب قابل توجهی به گیاهان گوجه‌فرنگی وارد نکرد. این مطالعه همچنین با استفاده از دستگاه لوله Y شکل، پاسخ‌های بویایی *M. pygmaeus* را به نشانه‌های فرار حاصل از منابع غذایی مختلف بررسی کرد. نتایج نشان داد که این شکارگر بر اساس نشانه‌های شیمیایی فرار، ترجیح قوی برای طعمه خاصی از خود نشان نداد. به طور کلی، یافته‌ها نشان می‌دهد که این گونه شکارگر می‌تواند به انواع طعمه و شرایط مختلف محیطی سازگار شود.

کلید واژه‌ها: برنامه‌های کنترل زیستی، آفات گلخانه‌ای، تخم، اللوکمیکال‌ها، گوجه‌فرنگی

دبیر تخصصی: دکتر سید علی صفوی

Introduction

Tomato, a crop of great economic and nutritional importance worldwide, is enjoyed fresh and as a raw material for food processing industries alike. Nevertheless, the cultivation of this valuable crop is currently facing a severe crisis due to the invasive tomato leaf miner (TLM), *Tuta absoluta* Meyrick (Lepidoptera, Gelechiidae), which is causing widespread damage to tomato crops and other solanaceous plants globally (Urbaneja et al., 2009). Native to the Americas, this pest has rapidly spread to more regions, leading to significant economic losses for outdoor as well as greenhouse tomato farmers. TLM presence is pervasive throughout the entire growing cycle, from seedling to harvest, affecting not only fresh market crops but also those destined for processing. The moth has high reproduction and several generations per year. Larval feeding can be particularly devastating, causing significant losses by attacking leaves, flowers, stems, and fruit. In extreme cases, the damage caused by this infestation can reach up to 80-100% yield loss, making it a major economic concern for the agricultural sector (Urbaneja et al., 2009; Gebremariam, 2015). The larvae's tendency to feed within mines, coupled with the absence of a clear threshold for action, complicates effective chemical pest control. Further, instances of resistance to chemicals employed against TLM have been documented (Guedes et al., 2019). Conversely, biological control methods utilizing natural predators and other natural enemies are critical components of comprehensive integrated pest management strategies, offering a vital alternative to chemical control measures (Salehi et al., 2016).

The predatory bug, *Macrolophus pygmaeus* Rambur (Hem.: Miridae) has been extensively documented as a polyphagous, versatile, and efficacious predator capable of preying on a wide range of crop pests, including whiteflies, thrips, aphids, mites, and larvae of lepidopteran pests in both outdoor and controlled indoor environments (Safari-Monjezi et al., 2021). As such, this biological control agent is identified as a viable commercial solution for managing pest populations in greenhouse settings. Further, *M. pygmaeus* is known as important natural enemy of TLM in tomato crops (Urbaneja et al., 2009). Its establishment in European tomato greenhouses has led to significant improvements in suppressing TLM populations (Urbaneja et al., 2012; Biondi et al., 2018). Eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and cysts of Artemia can be employed as alternative diet for rearing of the predator (Vandekerkhove et al., 2009; Soares et al., 2025).

The predation behavior of the generalist predators, including *M. pygmaeus*, is affected by a range of factors, including prey type and environmental conditions. Understanding these factors is critical for optimizing the efficiency of biological pest control agents (de Matos Dias et al., 2018). For instance, it has been demonstrated that light, temperature, and host plants significantly influenced the predation rate of *M. pygmaeus* (Perdikis et al., 2002). As a zoophytophagous predator, *M. pygmaeus* exhibits a dual behavior, feeding on both the prey and plant tissue. While this behavior can provide benefits such as enhanced survival and population maintenance in the absence of prey, it also poses the risk of damaging the host plants (Silva et al., 2022; Zhang et al., 2022). Thus, examining the extent of zoophytophagy as well as the impact of variables such as the prey availability, the prey and predator densities, along environmental conditions on this behavior in predators such as *M. pygmaeus* are essential for effective integration into pest control strategies. Further, the generalist predators

were observed to utilize kairomone to track down their prey, thereby boosting their biocontrol efficacy. For instance, research indicated that tomato plants release herbivore-induced plant volatiles, influencing the predation behavior of *M. pygmaeus* (Zhang et al., 2022). This behavior is affected by a range of factors, including the type of food source (Dickens, 1999). The aim of this study was to determine how temperature variations and different egg diets impact the predatory efficiency and olfactory behavior of the mirid bug *M. pygmaeus*.

Materials and Methods

Insect rearing

One kilogram of sterilized wheat flour was mixed with 300 g of wheat bran and 3 g of active dry yeast, with the mixture placed in metal trays (60 cm × 40 cm × 12 cm) as food source for rearing of *E. kuehniella* larvae. The trays were then inoculated with 0.3 g of fresh *E. kuehniella* eggs, which were evenly distributed across the surface. The trays were covered with a black cotton cloth and placed in a controlled growth chamber with a temperature of 25°C ± 5°C and a relative humidity of 65± 10% under a light cycle of 16:8 hours (light: dark) (Tahernia et al., 2020). Adult insects emerged after 45 days where the trays containing moths were transferred to a growth chamber and eggs were collected daily. These eggs were utilized to establish a colony of the predator species *M. pygmaeus* as well as for subsequent experiments involving herbivory, predation, and olfactometry experiments.

The initial population of *M. pygmaeus* was obtained from the biological control research department's greenhouse at the Iranian Research Institute of Plant Protection (IRIPP), Tehran, Iran. This colony was maintained in a 24 m² unit inside the research greenhouse, housed in net cages (160 cm × 120 cm × 100 cm) at temperature 25 ± 1°C and relative humidity (65 ± 5%), with natural light provided by plants, specifically tobacco (*Nicotiana tabacum* L.). The insects were fed a diet of Artemia, *Artemia parthenogenetica* Bowen and Sterling cysts (provided from Center of Biological Control in the Iranian Research Institute of Plant Protection) along with *E. kuehniella* eggs at a ratio of 50:50 (Malkeshi et al., 2017).

For TLM rearing, the initial population was collected from tomato greenhouses in Varamin district together with a research greenhouse unit of the biological control research department of IRIPP. The TLM moths were housed in cages (150 cm × 100 cm). The Fallet variety tomato plants served as the host plant. When the adult insects of F2 emerged, a new cage with the same dimensions was installed to facilitate oviposition. Fresh tomato plants from the initial colony were placed in this new cage, with new pots added to the colony to promote reproduction. This approach allowed for the collection of eggs within a shorter period while ensuring the availability of healthy tomatoes for adult insects (Salehi et al., 2016; Tamoli et al., 2016).

Predation of *M. pygmaeus*

Prior to conducting the experiments, a cohort of 5th instar nymphs was collected from the colony using an aspirator and placed in plastic containers (10 × 10 ×10 cm) containing fresh tomato leaves and a standardized diet consisting of *E. kuehniella* eggs, wheat flour, and Artemia cysts. Fifteen nymphs were included in each container, placed in a germinator with controlled temperature conditions of 25°C ± 1°C, relative humidity of 65% ± 5%, and the photoperiod 16:8 (light: darkness). After 24 hours, one-day-old adults were isolated and fed in the germinator for an additional 24 hours without the presence of insect prey (*E. keuhuniella* eggs and Artemia cysts). Subsequently, the adults were starved by keeping them in the germinator with fresh tomato leaves for 24 hours. For performing

the predatory experiments, small glass Petri dishes (diameter 5.6 cm) were washed and placed in the germinator for 24 hours. The bottom of each dish was covered with green cardboard paper for convenient viewing, and healthy tomato leaves were placed inside microtubes containing water to maintain leaf freshness. Depending on the experiment design, either 80 eggs of *E. keuhniella*, Artemia cysts or 100 eggs of TLM were placed separately on each leaf in each dish. Three-days-old male and female adult predators, which had been kept without animal feeding for 24 hours in the germinator, were employed at a density of one male or female per dish with 20 replicates per sex. The experiments were undertaken at different temperatures (20, 25, and 30°C) for 24 hours in growth chamber under controlled temperature conditions as mentioned previously. The predation rate was measured through counting consumed eggs or cysts, identified by their altered coloration (Urbaneja et al., 2009).

Herbivory of *Macrolophus pygmaeus*

To inspect the herbivory behavior of the predator species, experiments were carried out on the amount of herbivory under different conditions. After a 24-hour period of starvation, 3-days-old adult predators were placed at densities of 1, 3, and 6 individuals in the presence and absence of animal prey on tomato bushes inside plastic cylinders (37 cm tall, 12 cm opening diameter) with mesh covers for ventilation. The experiments were undertaken in a greenhouse environment with controlled temperature conditions as mentioned previously. Each treatment was replicated 10 times over a 72-hour period, along which time necrotic rings were recorded every 24 hours (Urbaneja et al., 2009).

Olfactory trials

In order to explore the predator's attraction for prey based on volatile chemical cues, an olfactory study was carried out using a Y-shaped tube apparatus (Attarzadeh et al. 2019, Mousavi et al. 2022). Healthy tomato leaves, free from any disease or pest symptoms, were applied as the baseline for the experiment. Tomato leaves were separated and placed in the reservoirs of the Y-shaped tube, with one of the baits (*E. kuehniella* eggs, Artemia cysts, TLM eggs, leaves infested with tomato moth larvae, or bee pollen) introduced in an amount of 100 pieces for each treatment. Three-day-old adult male or female *M. pygmaeus* were starved for 24 hours in a germinator with tomato leaves, and then tested in a growth chamber with environmental conditions as mentioned previously. Each treatment was replicated 10 times for each sex, with the attraction to allelochemicals emitted from animal prey and tomato leaves measured. After applying purified and moist air to the reservoirs, individual male and female insects were positioned in the main branch of the Y-shaped tube and allowed to move for 5 minutes before recording their attraction to one of the sub-branches if they traveled a distance of at least 7 cm from the sub-branch (Attarzadeh et al., 2019; Rashedi et al., 2019).

The olfactory test treatments compared the predator attraction to: *E. kuehniella* eggs and tomato leaves; TLM larvae-infested leaves and healthy tomato leaves; TLM egg-infested tomato leaves and healthy tomato leaves; flower pollen and *Artemia* cysts; and *E. kuehniella* eggs and *Artemia* cysts.

Data analyses

For the predation and herbivory (phytophagy symptom percentage) trials, a factorial design (three prey types × three environmental temperatures × two adult sex) was utilized. The study included 10 replicates for each treatment. The data were analyzed via ANOVA, with Tukey's post-hoc test applied to determine differences between means across the treatment groups. Nevertheless, owing to non-normal data

distribution, the phytophagy symptom percentage of *M. pygmaeus* in the absence of prey was compared using the Kruskal-Wallis non-parametric test instead (Zar, 2010). The predator's feeding strategy on various parts of the tomato plant (petiole, stem, and leaflet) and its olfactory responses to different food cues were analyzed using a chi-squared test of independence. The goal was to inspect whether the observed frequencies of feeding on each part of the plant and the observed frequencies of path choices towards different prey species differed significantly from the expected frequencies under the null hypothesis that the predator's feeding and olfactory responses are randomly distributed, with ten replications. All statistical analyses were performed using SAS software, version 9.2.

Results

The findings of ANOVA exploring the main effects of prey type, temperature, and predator sex, as well as their interactions, on the predation rate of the *M. pygmaeus* predator, are reported in Table 1. The results reveal that the predation rate of *M. pygmaeus* on *E. kuehniella* eggs, Artemia cysts, and TLM eggs differs significantly ($F = 57.36$; $df = 2, 29$; $P < 0.001$). The predation rate of *M. pygmaeus* on TLM eggs (25.26%±2.07%) was significantly higher than those on *E. kuehniella* eggs (14.02%±1.27%) and Artemia cysts (12.07%1.06%), as displayed in Figure 1.

The predation rate of *M. pygmaeus* varied significantly across different temperatures (Figure 2). Notably, the predation rate at the highest ambient temperature tested (30°C) was 23.13%±1.85%, which was 2.47 times higher than the rate at the lowest temperature tested (20°C) at 9.35%±0.91%. The predation rate at 25°C (19.11%±1.68%) was around 17.38% lower than those at 30°C.

Table 1. Multi-Factor Analysis of Variance (ANOVA) examining the interactive effects of Prey species (*Tuta absoluta, Ephestia kuehniella,* and Artemia cysts), temperature (20, 25, and 30 °C), and adult sex on predation rate of *Macrolophus pygmaeus*

Source of variation	Degree of freedom	Mean square	F	*P*-value
Prey (P)	2	79.83	57.36	<.0001
Temperature (T)	2	92.41	66.4	<0.0001
Sex (S)	1	534.72	384.19	<0.0001
P×T	4	3.94	2.84	0.0245
P×G	2	9.36	6.73	0.0014
T×G	2	6.29	4.52	0.0115
P×T×G	4	1.46	1.05	0.3791

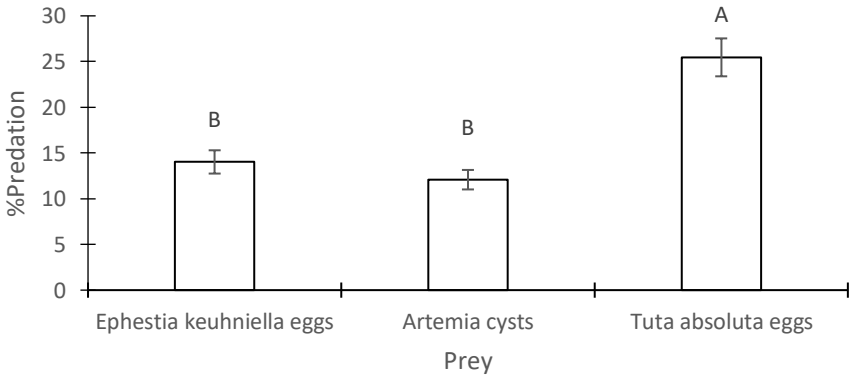


Figure 1. Predation percentages of *Macrolophus pygmaeus* when feeding on various prey types, including eggs of *Tuta absoluta, Ephestia kuehniella,* and Artemia cysts (The same letters indicate non-significant difference-Tukey, P > 0.05). The error bar shows the standard error for each mean.

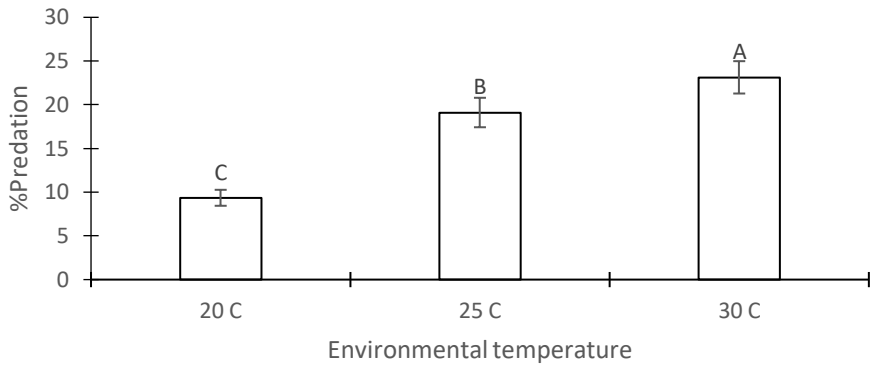


Figure 2. Predation percentages of *Macrolophus pygmaeus* on *Ephestia kuehniella* and Artemia cysts at different environmental temperatures (The same letters indicate non-significant difference -Tukey, P > 0.05). The error bar shows the standard error for each mean.

The predation rate of *M. pygmaeus* females was significantly higher, at 27.42%±1.47%, compared to males, which had a predation rate of 7.04%±0.44%, representing a 3.85-fold increment (F = 384.19; df=1; P < 0.001) (Figure 3). The interaction between prey type and environmental temperature revealed a significant impact on predation rates (F = 2.84; df = 4; P =0.0245), with the highest rate of predation observed in the combination of TLM eggs at 30°C (33.45%±4.07%) and the lowest in the combination of *E. kuehniella* eggs at 20°C (7.01%±1.38%). Thus, the predation rate was 4.77 times higher when feeding on TLM eggs at 30°C compared to feeding on *E. kuehniella* at the 20°C (Figure 4).

The interaction between environmental temperature and insect sex had a significant influence on predation rates (F =4.52; df =2, 29, P =0.0115), with females exhibiting a higher predation rate at 30°C (36.73%±2.65%) compared to 25°C (30.31%±2.558%) and 20°C (15.44%±1.39%). The males indicated a similar trend, with a significantly higher predation rates at 30°C (9.75%±0.81%) and 25°C (8.09%±0.79%) compared to 20°C (3.75%0.39%) (Figure 5).

The findings of our study revealed a statistically significant interaction between prey type and insect sex, with profound implications for predation rates (F =6.73; df =2, 29, P =0.0014). Notably, the combination of TLM eggs and female adults yielded the highest predation rate (40.49%±3.02%), whereas the combination of *E. kuehniella* eggs (4.85%±0.61%) and Artemia cysts (5.58%±0.60) with male bugs manifested the lowest predation rate. A striking difference was noted in predation rates between female and male bugs, with females displaying an 8.34-fold increase in predation when feeding on TLM eggs compared to *E. kuehniella* eggs and Artemia cysts (Figure 6).

The results of the chi-square test, carried out on the olfactometric data collected from leaves infected with prey of different ages consumed by *M. pygmaeus*, are outlined in Table 2. The findings suggest that there was no statistically significant preference for odors across all tested conditions, as indicated by the non-significant chi-square test results.

In the presence of prey, no damage arising from the herbivory by the predator *M. pygmaeus* was observed on tomato plants. In the absence of prey, a Kruskal-Wallis non-parametric test demonstrated that the partial damage did not differ significantly from the control treatment (df=2; H-value=1.5; *P*-value=0.472).

Table 3 indicates the damage percentage caused by *M. pygmaeus* inflicted on different parts of the tomato plant in the absence of prey. A non-parametric chi-square test exhibited that the phytophagy damage of the bug was significantly higher in the petiole compared to other parts of the plant, including the stem and leaf.

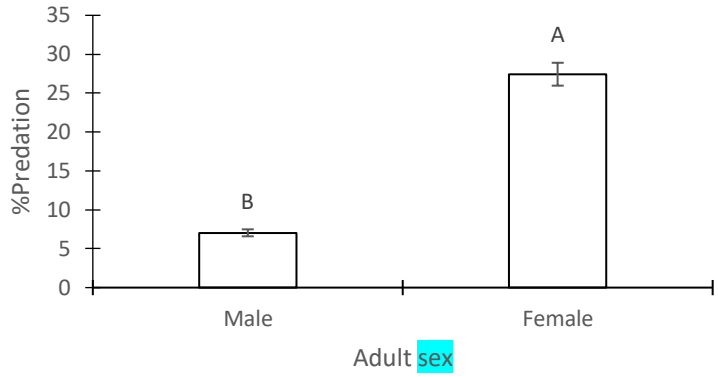


Figure 3. Predation percentages of *Macrolophus pygmaeus* male and female adults (The same letters indicate non-significant difference -Tukey, P > 0.05). The error bar shows the standard error for each mean.

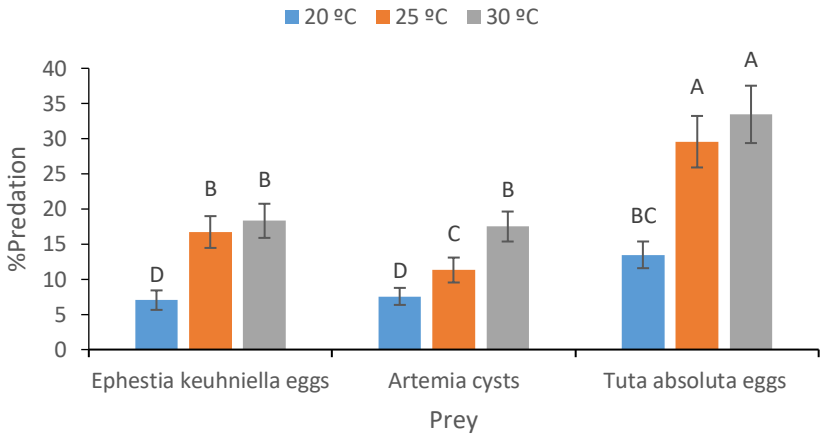


Figure 4. The mean comparison of *Macrolophus pygmaeus* predation percentages on different prey species at three environmental temperatures (The same letters indicate non-significant difference -Tukey, P > 0.05). The error bar shows the standard error for each mean.

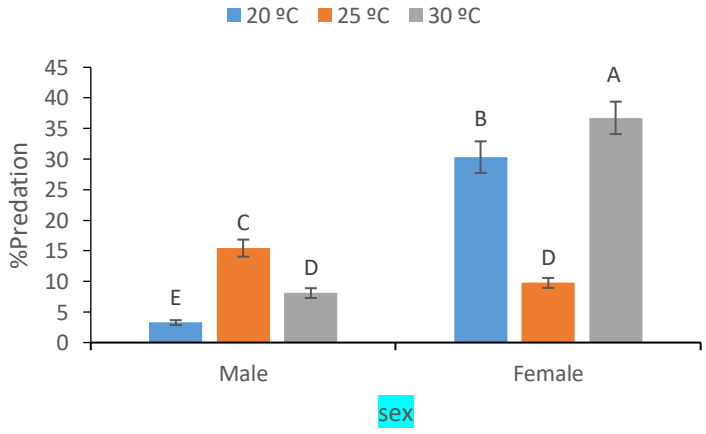


Figure 5. The mean comparison of male and female adults of *Macrolophus pygmaeus* predation percentages at three temperatures (The same letters indicate non-significant difference -Tukey, P > 0.05). The error bar shows the standard error for each mean.

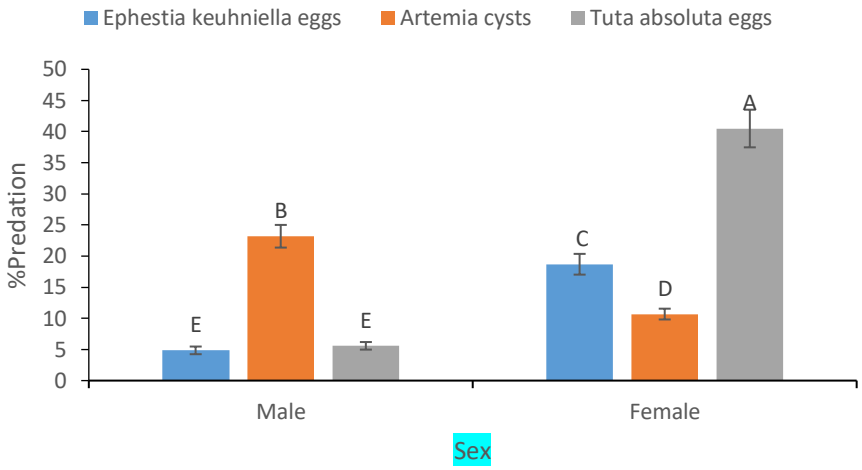


Figure 6. The mean comparison of male and female adults of *Macrolophus pygmaeus* predation percentages on different prey species (The same letters indicate non-significant difference -Tukey, P > 0.05). The error bar shows the standard error for each mean.

Table 2. The results of olfactometery experiments indicating the preference of male and female adults of *Macrolophus pygmaeus* between dual free choices of different treatments

Sex	Treatment	N	Observed	Expected	Chi-Square$_{(df=1)}$	*P*-value
Male	Infested tomato leaf to TLM* eggs	10	7	5	1.6	1
	Non-infested tomato leaf		3	5		
Female	Infested tomato leaf to TLM eggs	10	5	5	0	0.527
	Non-infested tomato leaf		5	5		
Male	Infested tomato leaf to TLM larvae	10	5	5	0	1
	Non-infested tomato leaf		5	5		
Female	Infested tomato leaf to TLM Larvae	10	7	5	1.6	0.206
	Non-infested tomato leaf		3	5		
Male	TLM eggs	10	7	5	1.6	0.206
	Non-infested tomato leaf		3	5		
Female	TLM eggs	10	5	5	0	1
	Non-infested tomato leaf		5	5		
Male	Pollen	10	6	5	0.4	0.527
	Artemia cysts		4	5		
Female	Pollen	10	5	5	0	1
	Artemia cysts		5	5		
Male	Eggs of *Ephestia keuhniella*	10	6	5	0.4	0.527
	Artemia cysts		4	5		
Female	Eggs of *Ephestia keuhniella*	10	6	5		
	Artemia cysts		**4**	**5**		

* TLM: Tomato leaf miner or *Tuta absoluta*

Table 3. Frequency of observed symptom of phytotoxity causing by Macrolophus pygmaeus feeding in different parts of tomato plants

Treatment	N	Observed	Expected	Chi-Square$_{(df=2)}$	P-value
Petiole	9	9	3	18	<0001
Stem		0	3		
Leaflet		0	3		

Discussion

Our findings revealed significant differences in the predation rate of *M. pygmaeus* on various prey types, with a particularly high rate observed on TLM eggs. These findings are in line with results of Borges et al. (2024), who found that prey consumption by female predators was higher on eggs of *T. absoluta* than on *E. kuehniella* eggs. The feeding behavior of insect predators is affected by various factors, including physical properties, allelochemical compounds, and nutritional value of their prey. Physical characteristics such as size, hardness, and digestibility of the prey play a key role in the selection of food sources of the predators (Safaei et al., 2016, Rajabpour et al. 2018; Rajabpour, 2022). Further, allelochemicals can affect the attractiveness and palatability of food to insects. The nutritional value of food is an essential factor, as a diet rich in nitrogen can promote the growth, survival, and fertility of insects (Evans and Schmidt, 1990; Brodeur and Boivin, 2006; Bandani, 2012). Lykouressis et al. (2014) found that the type of prey significantly affected both nymphal development and adult fertility, but not survival. Notably, the duration of the nymphal stage was prolonged when *M. pygmaeus* fed on *T. absoluta* eggs compared with other prey sources. Further, the mean fertility of *M. pygmaeus* females was highest when they were fed *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) nymphs, followed by *Macrosiphum euphorbiae* Thomas (Hemiptera: Aphididae) and *E. kuehniella* eggs, while it was lower when fed TLM eggs. *M. pygmaeus* exhibited a higher predation rate on TLM eggs compared to *E. keuhniella* eggs and Artemia cysts. This may be attributed to the size of the prey, as *M. pygmaeus* is more likely to detect and consume smaller preys (Sharifian et al., 2015). The size of the prey affects the amount of consumption by the predator, with smaller prey items being consumed in greater quantities to fulfill nutritional needs (Fantinouet al., 2009). In addition, the nutritional value of food can impact the biological activities of insects, with a diet rich in nitrogen supporting the survival and population growth of *M. pygmaeus* (Bandani, 2012). These findings suggest that an herbivorous-carnivorous diet may be appropriate for *M. pygmaeus* in situations where animal prey is scarce. Further, the ability of *M. pygmaeus* to control pests can be a valuable feature for lowering control costs in integrated pest management strategies. Our findings are in accordance with previous studies, such as Perdikis and Lykouressis (2000), which reported that *M. pygmaeus* exhibits varying predation rates on different prey species, with *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) being the most susceptible followed by *Myzus persicae* Sulzer, *Macrosiphum euphorbiae* Thomas, *Aphis gossypii* Glover (Hemiptera: Aphididae), and *Tetranychus urticae* Koch. (Acari, Tetranychiidae). The varying feeding habits on different prey species result in distinct biological characteristics in the predator, highlighting the significance of considering the diversity of prey in understanding predator-prey dynamics.

The results revealed that the predation activity of *M. pygmaeus* is temperature-dependent, with a significant increase in predation rate observed as the temperature rose from 20 to 30 ℃. Temperature fluctuations have a profound impact on the physical and chemical reactions within the insect body, directly influencing hunting behavior. Specifically, elevated temperatures boost metabolic activity, leading to increased hunting to acquire energy, whereas diminished temperatures result in reduced predation rates owing to lower energy demands. This association is well-documented (Harrison et al., 2012). In greenhouses employing biological control agents, maintaining favorable temperature conditions is critical to ensure the growth and reproductive success of crops (such as tomato) and the physiological well-being of insects. However, temperature fluctuations can either beneficially promote feeding from animal prey or harmfully affect pest control costs, depending on whether they encourage increased nutrition and reduced pest populations or compromise establishment, protection, and support costs. Our findings are in line with previous research, which has demonstrated that *M. pygmaeus* is capable of completing its life cycle across a wide temperature range, including 15-30°C on tomato, with optimal development occurring at 30°C (Perdikis and Lykouressis, 2000). Temperature thresholds for *M. pygmaeus* development ranged from 6.92°C to 9.29°C across life stages, with thermal constants (degree-days) varying from 98 to 541 (Perdikis and Lykouressis, 2002). This temperature tolerance suggests that *M. pygmaeus* may be well-adapted to thrive under various environmental conditions, potentially expanding its ecological niche and contributing to its success as a biological control agent. Comparison of the functional response of this bug to TLM eggs at 20°C and 25°C revealed that both the attack rate (a) and handling time (T_h) diminished with increasing temperature, leading to a significant rise in the maximum predation rate by *M. pygmaeus* at 25°C compared to 20°C (Yiacoumi et al., 2024), in accordance with the findings of our research. Furthermore, Sylla et al. (2016) noted that prey type significantly affected the nymphal development and adult fertility of *M. pygmaeus*, but not its survival, with the slowest development and the lowest fertility found when fed TLM eggs. Nevertheless, fertility was higher when the predator fed on *B. tabaci* nymphs, *Macrosiphum euphorbiae* Thomas aphids, or *E. kuehniella* eggs compared to TLM eggs as food.

The results indicate a striking difference in predation behavior between male and female *M. pygmaeus*, with females demonstrating a significantly higher predation rate compared to males. The production of eggs by females requires a significant amount of energy, which is reflected in their greater body mass compared to males (Bandani, 2012). Moreover, this sex-specific difference in weight is affected by nutritional strategy, as seen in species where males are responsible for foraging for both sexes and subsequently accumulate more fat reserves (Bandani, 2012). In *M. pygmaeus* adults, females are larger than males, with both sexes engaging in food searching behavior. Thus, the hunting rate of the tested prey under different temperature conditions was higher in female *M. pygmaeus* than in males. The relationship between feeding frequency and egg-laying capacity is a characteristic of predatory insects (Bandani, 2012), where increased predation by females can result in increased energy reserves, causing a greater number of eggs laid and ultimately an increment in the population's next generation. Further, the energy requirements of adult insects depend on their age, sex, and reproductive status, with young adults requiring more energy to complete the hardening processes of their

cuticle, muscles, and reproductive system. In the context of *M. pygmaeus*, this signifies that females require more energy to support their reproductive activities, which is reflected in their greater body size and feeding behavior compared to males. Similar to our findings, Borges et al. (2024) found that female's prey consumption on eggs of *T. absoluta* was higher than that of *E. kuehniella*, presumably owing to the comparative size/amount of biomass of the eggs.

Our data suggest that *M. pygmaeus* does not exhibit a statistically significant preference for any of the tested odors, as the chi-square test results did not present any significant associations. Chemical signals play a key role in mediating relationships between species, allowing the sender species and receiver species to manage their interactions as well as behaviors in response to the composition and concentration of chemical constituents (Price et al., 2011). Predators employ chemical cues for host location through two primary mechanisms. The first involves settlement, where the predator releases a chemical signal to attract its prey. The second and more common method involves active search, where the predator employs chemical messengers in three distinct phases. In the initial phase, the predator applies volatile compounds as synomones to locate suitable habitats for searching. In the subsequent phase, it directs volatile substances from the prey towards itself. Ultimately, in the third phase, chemical compounds from both the host plant and prey get mixed to induce predation (Price et al., 2011). The Miridae family of predators is characterized by their generalist predation behavior, making them a popular choice for biological control in tomato greenhouses owing to their ability to suppress populations of multiple pests, including different life stages of whiteflies, nymphs, eggs, as well as first and second instars (Wheeler, 2001). No attraction to host by *M. pygmaeus* among the olfactory treatments implies that it does not utilize specialized allelochemicals for prey tracking. As a result, our expectation for effective biological control of its prey, such as TLM, needs to be tempered by the absence of attraction between the plant host and diverse prey species.

The findings of this study suggest that *M. pygmaeus*, a predator that preys on tomato plants, does not cause damage to its host plant when prey is present, while inflicting non-significant damage in the absence of prey, with the petiole being a particularly vulnerable area. Many generalist predators feed on plant-based food sources, such as nectar and pollen, or consume plants themselves along certain stages of their life cycle. The feeding behavior of these insects plays a substantial role in shaping predator-prey dynamics. As a result, understanding the effects of plant consumption on omnivores is critical for developing effective biological control strategies (Portillo et al., 2012). The primary objective of *M. pygmaeus* feeding on plant tissue is to access water and solutes within plant vessels. To achieve this goal, the insect naturally selects plant tissues with minimal trichomes and lowered hardness to expedite its access to these resources. Further, young plant parts tend to have higher nitrogen and salt contents (Bandani, 2012; Portillo, 2012; Rajabpour, 2022), which may be attractive to the insect. Unlike other predators that require accessible nectar to fulfill their energy needs, mirid bugs can obtain carbohydrates by directly consuming plant tissues. This ability to feed on leaves and stems can be regarded as a substitute for nectar, allowing mirids to be less dependent on external nectar sources (Portillo et al., 2012). The presence of necrotic rings on the stem suggests that the insect's feeding behavior may have a localized impact on plant health. Further, some studies have indicated that *M. pygmaeus* feeding on some host plants, such as eggplant, not only did not

cause much damage, but also induced resistance in those plants to pests (Farina et al., 2023). The inflicted injury by *M. pygmaeus* was not only statistically non-significant on tomato plants, but also induced plant resistance to pests (Pappas et al., 2015) and deterred herbivores from plants previously fed upon by predator (Zhang et al., 2019). Eventually, it has also been shown that *M. pygmaeus* herbivory significantly affects their predation behavior on insect prey (Lykouressis et al., 2014).

In conclusion, our study revealed that *M. pygmaeus* exhibited significantly higher predation rates on *T. absoluta* eggs compared to *E. kuehniella* eggs or Artemia cysts, with peak efficiency at 30°C. Female predators consistently outperformed males across all temperatures and prey types. Crucially, *M. pygmaeus* caused negligible damage to tomato plants irrespective of prey availability, confirming its safety as a zoophytophagous agent. Nevertheless, olfactory assays revealed no significant attraction to prey-specific volatiles, suggesting that prey location relies on mechanisms beyond olfactory cues. These findings establish *M. pygmaeus* as an effective, temperature-responsive, and plant-safe biological control agent against *T. absoluta* in greenhouse systems, especially when female populations are optimized at warmer temperatures.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT 4.0 in order to in the writing process to improve the readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

References

Attarzadeh, M., Rajabpour, A., Farkhari, M., & Rasekh, A. (2019). Interactions between *Orius albidipennis* and *Aphidius colemani* (Hymenoptera: Braconidae) for the control of *Aphis gossypii* on greenhouse cucumber. *Journal of Crop Protection*, 8(1), 21-31.

Bandani, A. (2012). Physiology of insects: digestion, excretion, symbiotic microorganisms, metabolism. Tehran University Press, Tehran.

Biondi, A., Guedes, R. N. 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. *Annual review of entomology*, *63*(1), 239-258.

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*, *52*(2), 31. https://doi.org/10.1007/s12600-024-01130-0

Brodeur, J., & Boivin, G. (2006). Trophic and guild interactions in biological control. Springer, New York.

de Matos Dias, D., de Campos, C. B., & Guimarães Rodrigues, F. H. (2018). Behavioural ecology in a predator-prey system. *Mammalian Biology*, *92*(1), 30-36.

Dickens, J. C. (1999). Predator–prey interactions: olfactory adaptations of generalist and specialist predators. *Agricultural and forest entomology*, *1*(1), 47-54.

Evans, D.L., & Schmidt, J.O. (1990). Insect defenses: adaptive mechanisms and strategies of prey and predators. Suny Press.

Fantinou, A.A., Perdikis, D.C., Labropoulos, P.D., & Maselou, D.A. (2009). Preference and consumption of *Macrolophus pygmaeus* preying on mixed instar assemblages of *Myzus persicae*. *Biological Control*, *51*(1), 76-80.

Farina, A., Massimino Cocuzza, G. E., Suma, P., & Rapisarda, C. (2023). Can *Macrolophus pygmaeus* (Hemiptera: Miridae) Mitigate the Damage Caused to Plants by *Bemisia tabaci* (Hemiptera: Aleyrodidae)?. *Insects*, *14*(2), 164.

Gebremariam, G. (2015). *Tuta absoluta*: A global looming challenge in tomato production, Review Paper. *Journal of Biological and Agricultural Health*, *5*(14), 57-62.

Guedes, R. N. C., Roditakis, E., Campos, M. R., Haddi, K., Bielza, P., Siqueira, H. A. A., ... & Nauen, R. (2019). Insecticide resistance in the tomato pinworm *Tuta absoluta*: patterns, spread, mechanisms, management and outlook. *Journal of Pest Science*, *92*(4), 1329-1342.

Harrison, J.F., Woods, H.A., & Roberts, S.P. (2012). Ecological and environmental physiology of insects. OUP Oxford. UK.

Lykouressis, D., Perdikis, D., & Charalampous, P. (2014). Plant food effects on prey consumption by the omnivorous predator *Macrolophus pygmaeus*. *Phytoparasitica*, *42*, 303-309.

Malkeshi, S.H., Talaei-Hassanloui, R., Mohaghegh, J., & Allahyari, H. (2017). Predation rate and prey preference of *Nesidiocoris tenuis* on *Ephestia kuehniella* and *Tuta absoluta* eggs in laboratory. *BioControl in Plant Protection*, *5*(1), 31-43.

Mousavi, K., Rajabpour, A., Parizipour, M.H.G., & Yarahmadi, F. (2022). Biological and molecular characterization of *Cladosporium* sp. and *Acremonium zeylanicum* as biocontrol agents of *Aphis fabae* in a tri-trophic system. *Entomologia Experimentalis et Applicata*, *170*(10), 877-886.

Pappas, M. L., Steppuhn, A., Geuss, D., Topalidou, N., Zografou, A., Sabelis, M. W., & Broufas, G. D. (2015). Beyond predation: the zoophytophagous predator *Macrolophus pygmaeus* induces tomato resistance against spider mites. *PLoS One*, *10*(5), e0127251.

Perdikis, D. C., & Lykouressis, D. P. (2002). Thermal requirements for development of the polyphagous predator *Macrolophus pygmaeus* (Hemiptera: Miridae). *Environmental Entomology*, *31*(4), 661-667.

Perdikis, D., & Lykouressis, D. (2000). Effects of various items, host plants, and temperatures on the development and survival of *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae). *Biological Control, 17*(1), 55-60.

Portillo, N., Alomar, O., & Wäckers, F. (2012). Nectarivory by the plant-tissue feeding predator *Macrolophus pygmaeus* Rambur (Heteroptera: Miridae): Nutritional redundancy or nutritional benefit? *Journal of Insect Physiology, 58*(3), 397-401.

Price, P.W., Denno, R.F., Eubanks, M.D., Finke, D.L., & Kaplan, I. (2011). Insect ecology: behavior, populations and communities. Cambridge University Press, UK.

Rajabpour, A., Seraj, A.A., & Mehrnia, A. (2018). Developing an artificial diet for rearing *Orius albidipennis* Reuter (Het., Anthocoridae). *Journal of Plant Protection Research, 58*(3), 276–281.

Rajabpour. A. (2022). The Bugs Belong to Family Anthocoridae in Iran (Classification, identification, biology, rearing and use in biocontrol programs). Agricultural Sciences and Natural Resources University Press, Bavi.

Rashedi, A., Rajabpour, A., Rasekh, A., & Zandi-Sohani, N. (2019). Interactions between host plant, Aphis fabae, and its natural enemies, *Orius albidipennis* and *Lysiphlebus fabarum* in a tritrophic system. *Journal of Asia-Pacific Entomology, 22*(3), 847-852.

Safaei, N., Rajabpour, A., & Seraj, A.A. (2016). Evaluation of various diets and oviposition substrates for rearing *Orius albidipennis* Reuter. *Journal of Entomological Society of Iran, 35*(4),29-37.

Safari-Monjezi, Y., Yarahmadi, F., & Zandi-Sohani, N. (2021). Sublethal effects of commonly used insecticides in tomato crop on functional response and biological parameters of *Macrolophus pygmaeus* Rumber (Hemiptera: Miridae). *Journal of Agricultural Science and Technology, 23*(3), 589-602.

Salehi, Z., Yarahmadi, F., Rasekh, A., & Sohani, N. Z. (2016). Functional responses of *Orius albidipennis* Reuter (Hemiptera, Anthocoridae) to *Tuta absoluta* Meyrick (Lepidoptera, Gelechiidae) on two tomato cultivars with different leaf morphological characteristics. *Entomologia Generalis*, *36*(2), 127-136.

Sharifian, I., Sabahi, Q., & Khoshabi, J. (2015). Functional response of Macrolophus pygmaeus (Rambur) and Nesidiocoris tenuis (Reuter) feeding on two different prey species. *Archives of Phytopathology and Plant Protection*, *48*(17-20), 910-920.

Silva, D.B., Hanel, A., Franco, F.P., de Castro Silva-Filho, M., & Bento, J.M.S. (2022). Two in one: the neotropical mirid predator *Macrolophus basicornis* increases pest control by feeding on plants. *Pest Management Science, 78*(8), 3314-3323.

Soares, A. O., Durão, A. C., Oliveira, L., Arruda, P., & Borges, I. (2025). *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. *Crop Protection*, *187*, 106960.

Sylla, S., Brévault, T., Diarra, K., Bearez, P., & Desneux, N. (2016). Life-history traits of *Macrolophus pygmaeus* with different prey foods. *PLoS One*, *11*(11), e0166610.

Tahernia, S., Moayeri, H.R.S., Kavousi, A., Arbab, A., & Davoudi, A. (2020). The influence of photoperiod on two-sex life table parameters of the Mediterranean flour moth, *Ephestia kuehniella* (Lepidoptera: Pyralidae*). Journal of Entomological Society of Iran*, *40*(1): 1-17.

Tamoli, T. E., Seraj, A. A., & Rajabpour, A. (2016). Biological characteristics and population parameters of tomato leaf miner, *Tuta absoluta* (Lep.: Gelechiidae) on potato and tobacco plants under laboratory conditions. *Plant Protection (Scientific Journal of Agriculture), 38*(4), 79-88.

Urbaneja, A., González-Cabrera, J., Arno, J., & Gabarra, R. (2012). Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin. *Pest management science*, *68*(9), 1215-1222.

Urbaneja, A., Montón, H., & Mollá, O. (2009). Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. *Journal of Applied Entomology*, *133*(4): 292-296.

Vandekerkhove, B., Parmentier, L., Van Stappen, G., Grenier, S., Febvay, G., Rey, M., & De Clercq, P. (2009). Artemia cysts as an alternative food for the predatory bug Macrolophus pygmaeus. *Journal of Applied Entomology*, *133*(2), 133-142.

Wheeler, A.G. (2001). *Biology of the plant bugs (Hemiptera: Miridae): pests, predators, opportunists*. Cornell University Press.

Yiacoumi, E., Kouloussis, N. A., & Koveos, D. S. (2024). Effect of sex, age and temperature on the functional response of *Macrolophus pygmaeus* Ramber and *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae) on eggs of *Tuta absoluta*. *Insects*, *15*(7), 485.

Zar, J. H. (2010). Biostatistical analysis pearson prentice-hall. *Upper Saddle River, NJ*.

Zhang, N. X., Andringa, J., Brouwer, J., Alba, J. M., Kortbeek, R. W., Messelink, G. J., & Janssen, A. (2022). The omnivorous predator *Macrolophus pygmaeus* induces production of plant volatiles that attract a specialist predator. *Journal of Pest Science*, *95*(3), 1343-1355.

Zhang, N. X., van Wieringen, D., Messelink, G. J., & Janssen, A. (2019). Herbivores avoid host plants previously exposed to their omnivorous predator *Macrolophus pygmaeus*. *Journal of Pest Science*, *92*(2), 737-745.

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