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Laboratory evaluation of the feeding behavior of the generalist predatory mirid bug *Tupiocoris cucurbitaceus* (Hemiptera: Miridae) for the biological control of *Phthorimaea absoluta* (Lepidoptera: Gelechiidae)

Evaluación en laboratorio del comportamiento alimenticio del mírido depredador generalista *Tupiocoris cucurbitaceus* (Hemiptera: Miridae) para el control biológico de *Phthorimaea absoluta* (Lepidoptera: Gelechiidae)

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Abstract

The use of predatory insects has gained interest for reliable and environmentally safe pest management to control the South American tomato leafminer, *Phthorimaea absoluta* (Meyrick) (Lepidoptera: Gelechiidae), a pest of tomato crops worldwide. Based on video tracking using EthoVision® software and static feeding multiple-choice tests, we report the prey-searching behavior and feeding preference of the Neotropical mirid bug *Tupiocoris cucurbitaceus* Spinola (Hemiptera: Miridae), a biological control agent of *P. absoluta* when presented with its eggs and other two prey species. *T. cucurbitaceus* exhibits generalist feeding behavior; the nymphs initially showed a preference for *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) nymphs but consumed more *P. absoluta* and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs after 24 h. *T. cucurbitaceus* males preferred *T. vaporariorum* throughout the experiment while females showed no preference for any prey. Furthermore, they did not cause significant damage to the leaves. The findings emphasize the importance of evaluating the simultaneous offer of multiple prey types to understand the effectiveness of biocontrol agents in the field. Overall, the research contributes valuable insights into the feeding habits of *T. cucurbitaceus*, supporting its potential as a biological control agent for *P. absoluta* in tomato crops.

Keywords
diet breadth • mirid bug • *Trialeurodes vaporariorum* • *Ephestia kuehniella* • biocontrol

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Resumen

El uso de insectos depredadores ha ganado interés para el manejo confiable y ambientalmente seguro de la polilla del tomate, *Phthorimaea absoluta* (Meyrick) (Lepidoptera: Gelechiidae), una plaga de este cultivo en todo el mundo. Por medio de un estudio con el programa EthoVision® y de ensayos de opción múltiple, reportamos el comportamiento de búsqueda de presas y la preferencia alimentaria del mírido neotropical *Tupiocoris cucurbitaceus* Spinola (Hemiptera: Miridae), un agente de control biológico de *P. absoluta*, cuando se le presentan huevos de la plaga y simultáneamente otras dos especies de presas. *T. cucurbitaceus* exhibe un comportamiento alimentario generalista. Las ninfas del depredador mostraron inicialmente preferencia por las ninfas de *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) pero consumieron más huevos de *P. absoluta* y *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) al cabo de 24 h. Los machos de *T. cucurbitaceus* prefirieron a *T. vaporariorum* mientras que las hembras no mostraron preferencia por ningún tipo de presa. Además, los individuos no causaron daños directos a las hojas. Los hallazgos enfatizan la importancia de evaluar la oferta de diferentes presas para conocer la efectividad de los agentes de control biológico en el campo. La investigación aporta valiosos conocimientos sobre los hábitos alimentarios de *T. cucurbitaceus*, respaldando su potencial como agente de control biológico para *P. absoluta*.

Palabras clave
amplitud de dieta • chinche depredadora • *Trialeurodes vaporariorum* • *Ephestia kuehniella* • biocontrol

Introduction

The use of generalist arthropod predators for biological control has historically received less attention compared to parasitoids and entomopathogens, given assumed negative effects. Among those effects, the most reported are omnivory, attack of non-target species, competition, and intraguild predation on other natural enemy species present in crops (21, 32). However, important biocontrol successes have been achieved with mite and hemipteran predators since they can feed on a variety of prey and plant resources, ensuring their survival and reproduction and enhancing their establishment (39, 42). For instance, in Spain, biocontrol against the sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is accomplished by releasing predatory mirid bugs and mites in protected sweet pepper, *Capsicum annuum* L. (Solanales: Solanaceae) crops (9, 33).

A crucial aspect when planning the use of generalist predators as biocontrol agents is to determine the range of species on which they effectively feed, *i.e.*, the diet breadth. Although most predators usually feed on various species, they may exhibit a degree of acceptance for different prey based on certain characteristics, such as nutritional quality and stage of development, which ultimately can affect their success as biocontrol agents (16). Two life traits especially defined to assess diet breadth of a predatory species are food-searching behavior and preference. The former implies predator engagement in the activity of prospecting in the environment, the recognition and acceptance of some prey. Factors such as developmental stage, sex, age, starvation, and available prey species influence food-searching behavior. Preference for food is determined by different prey's morphological, physiological, and behavioral traits to obtain enough nutrients and avoid toxic or indigestible food (3, 14, 31).

Miridae bugs (Hemiptera) are important predatory insect species. Most of them show high preying rates and are capable of finding and colonizing new habitats due to their dispersing capacity (27). Approximately 20 species are currently commercialized worldwide as biocontrol agents against whiteflies, eggs and small larvae of lepidopterans, among other horticultural pests (41). Particularly for the biological control of the South American tomato pinworm, *Phthorimaea absoluta* (Meyrick) (Lepidoptera: Gelechiidae), a Neotropical pest that has invaded the European, African, and Asian continents, several mirid species are main predatory agents (4, 7, 9, 10, 19, 23, 28, 38, 40).

Tupiocoris cucurbitaceus Spinola (Hemiptera: Miridae) has been reported in the Americas preying mainly on whiteflies (17, 29). High predation rates and pest kill rates were later examined for this predatory bug over a range of prey species: the whiteflies *B. tabaci* and *Trialeurodes vaporarioum* (Westwood) (Hemiptera: Aleyrodidae), the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), and eggs of three lepidopteran species, the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), the Angoumois grain moth, *Sitrotroga cereallela* Olivier and *P. absoluta* (Lepidoptera: Gelechiidae) (8, 22, 23, 44). Besides, *E. kuehniella* eggs are also applied under a prey-enrichment technique to allow the establishment of mirids, as for the use of *N. tenuis* to control *P. absoluta* in Spain (40, 41). *T. cucurbitaceus* has been recently developed as a commercial biocontrol agent in Argentina and Uruguay against whiteflies and *P. absoluta.* To improve its establishment, releases are performed along with *E. kuehniella* eggs as supplementary food (1, 18, 35). In this research, we aimed to explore *T. cucurbitaceus* prey-searching behavior and feeding preference when offered simultaneously *P. absoluta* and *E. kuehniella* eggs, and *T. vaporariorum* nymphs. This information could help better assess the performance of this generalist predatory mirid in tomato crops as biocontrol agent of *P. absoluta*.

MATERIALS AND METHODS

Plant and insect materials

Tomato plants, *Solanum lycopersicon* L. (Solanales: Solanaceae), Elpida variety Enza Zaden, The Netherlands, were cultivated at the Centro de Estudios Parasitológicos y de Vectores (CEPAVE, CONICET-UNLP-Asociado CICPBA). Tomato seedlings were individually transplanted to 1 l-plastic pot, watered daily, and kept free of insect pests by protecting them inside 60 x 40 x 40 cm (length x width x height) white voile cages (BioQuip Inc., USA). Plants used in *P. absoluta*, *T. cucurbitaceus*, and *T. vaporariorum* colonies and experiments had 4-5 expanded leaves.

Tupiocoris cucurbitaceus colony was initiated with individuals collected from organic tomato crops in farms located near the La Plata Horticultural Belt (N Buenos Aires province, Argentina). Insects were maintained in a controlled environment walk-in rearing room at 25 ± 2°C, 60 ± 10% RH, 14:10 L:D. Identifications of *T. cucurbitaceus* were confirmed by taxonomists at La Plata Museum (Entomology Department, School of Natural Sciences and Museum, National University of La Plata). To obtain individuals of *T. cucurbitaceus* of known age for the tests, 30 cohorts were reared in a white voile cage along with a potted tomato plant (as described above). *Ephestia kuehniella* eggs provided by Brometán SRL (Argentina) and commercial bee pollen were spread *ad libitum* on the leaves as food, and distiller water was provided in soaked sponge pieces inside containers. Oviposition on leaves was allowed for 24 h, and later the plant was placed in a new cage to start a cohort and replaced with a fresh one. Once nymphs emerged, they were fed as adults until they reached the developmental stages needed for the trials (4-5th instar nymphs or adults) (6). To obtain *P. absoluta* eggs, a colony was initiated by maintaining bouquets of tomato leaves infested with moth larvae collected from the field placed in white voile cages. Once pupae were formed, they were transferred to a new cage until moths emerged. Adults were provided with a honey solution (70%) and allowed to mate. Potted tomato plants were offered daily as oviposition substrate and those replaced were held in clean voile cages (30). A uniform cohort of 24 h-old eggs was used in the experiments. *T. vaporariorum* nymphs used in the feeding preference experiment were obtained from adults captured in tomato crops using a manual aspirator and transported to the laboratory for identification (26). Then, they were released in white voile cages and provided with potted tomato plants, to lay eggs (37). Once they hatched, development was checked and after 15 days revised every 24 h to collect late instar nymphs for the assays.

Prey searching behavior assay

To assess *T. cucurbitaceus* food searching capacity, a three-treatment experiment was set up, considering developmental stage and sex: 1) late (4-5th instars) nymph, 2) 7-d adult coupled female, and 3) 7-d adult coupled male. Individuals were isolated from the cohort and kept starved for 24 h before performing the assays in plastic Petri dishes (diameter: 5 cm, height: 1 cm), provided with small, moistened cotton pieces as water sources.

The experimental unit consisted of a plastic container (diameter: 9 cm, height: 5 cm) with a tomato leaflet on 1-cm layer of water agar (1%) to maintain its turgidity. Three different prey patches were offered simultaneously to one *T. cucurbitaceus* individual: the target patch (TP) containing 30 *P. absoluta* eggs (24 h old) and two non-target patches (NTP) with 30 *E. kuehniella* eggs and 5 *T. vaporariorum* nymphs (late instar), respectively (figure 1). Since we were interested in determining the predatory action of *T. cucurbitaceus* on *P. absoluta*, we considered this species as a TP while the other prey species were treated as NTP. To avoid food depletion during the experiment, the quantity of *P. absoluta* and *E. kuehniella* eggs offered was estimated based on previous diet reports by Burla *et al.* (2014), López *et al.* (2019) and Duarte *et al.* (2022) for *T. cucurbitaceus*, *Macrolophus basicornis* (Stål) and *Engytatus varians* (Distant) (Hemiptera: Miridae), respectively. The number of *T. vaporariorum* nymphs used in the experiment was based on *T. cucurbitaceus* food uptake registered by Burla *et al.* (2014). Patches of prey were carefully deposited on the leaflet using a fine brush with the aid of a stereoscope microscope and placed at equidistant points. The quality of all prey items was checked before starting the trial to discard collapsed eggs or dead whiteflies. Each treatment was replicated 15 times and experimental units were not re-utilized.

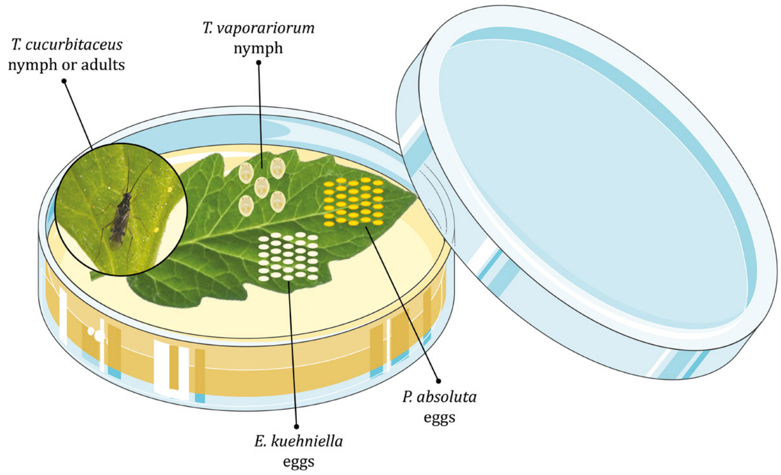


Figure 1. Assembly of the experimental unit to analyze the predation behavior of *Tupiocoris cucurbitaceus* on three types of prey exposed in patches on a fresh tomato leaflet and maintained on a layer of agar-water.

Figura 1. Esquema de la unidad experimental para analizar el comportamiento de depredación de *Tupiocoris cucurbitaceus* sobre tres tipos de presas expuestas en parches sobre un folíolo fresco de tomate y mantenidos con una capa de agar-agua.

Prey searching behavior was studied for 30 min using the software EthoVision® XT (Noldus, The Netherlands) which videotapes and analyzes animal activity inside an arena. Various steps were followed to calibrate the recording of *T cucurbitaceus* when visiting the food patches or the time they spent outside the patches (*i.e.*, clean parts of the leaflet). Observations were made between 10 am and 2 pm. Environmental conditions remained similar for all replicates during the trial (25±2°C and 60±10% RH).

Four behavioral descriptors were evaluated: 1) time spent in each of the three food patches or on the clean leaflet, 2) accumulated time of *T. cucurbitaceus* nymphs and adults in movement or non-movement, 3) visit frequency (first visit to TP and NTP), *i.e*., the number of times that the predator entered the patch, and recurrent visit or re-visits to TP, and 4) the maximum number of times predators alternated among patches. Given our interest in mirid behavior concerning that prey, only revisits to the TP (*i.e.*, with *P. absoluta*) were considered. Thus, we set out the trial to analyze whether *T. cucurbitaceus*, after choosing TP as the first option, decided to revisit more frequently, *i.e.*, whether the predator preferred that food, or not.

Feeding preference assay

The feeding preference of *T. cucurbitaceous* nymphs, females, and males at 30 min and then for 24 h was evaluated by registering the number of preyed lepidopteran eggs or whitefly nymphs (figure 1, page 100). The number of preys consumed at 30 min was counted by removing predators from the experimental unit and keeping in labelled Eppendorf tubes, then restored to its unit. Later, all units were placed in a rearing chamber (I501PF, SEMEDIX, Argentina) at controlled temperature, relative humidity, and photoperiod conditions (25 ± 1°C, 65 ± 5% RH, 14:10 L:D) to check prey consumption at 24 h, without food replacement. After the end of the trial, the experimental units were checked to record preyed food using a stereoscopic microscope (Nikon SMZ1270) to observe and count the remains of eggs and nymphs caused by the stylets of the mirid. The occurrence of phytophagous behavior was checked by observing the presence of feeding punctures in leaflets (38). Preference was observed at 30 min since we aimed to discern whether the starvation period could influence the first food election for the predator. Instead, consumption at 24 h could bring information on prey choice when prey density decreased.

Statistical analysis

The time spent by individuals in the clean leaflet or prey patches was analyzed with a Generalized Linear Mixed Model (GLMM) using lme4 package and glmer function (2) with the type of patches and developmental stage, *i.e.*, late nymph, female and male adults as fixed factors and the individual as a random factor. The accumulated time of *T. cucurbitaceus* nymphs and adults in movement or non-movement was measured as the proportion of the time in movement / the total time of the experiment (1800 s) (response variable) and analyzed using Beta regression with betareg package (12), being the stage of predatory individuals (nymphs, female, and male adults of *T. cucurbitaceus*) the predictive factor. The frequency of the first visit to TP or NTP was analyzed using 2 x 2 contingency tables with Fisher's exact test for each developmental stage and sex of *T. cucurbitaceus* separately. Then, since the study was aimed to evaluate the feeding preference of this predator on *P. absoluta*, compared with other two possible prey items, we evaluated the frequency of those individuals who revisited the TP after visiting that TP as its first choice using 2 x 2 contingency tables with Fisher's exact test. The maximum number of food patch alternations was analyzed by Kruskal-Wallis test because the data was not normal. Then, Dunn's test checked for significant differences among factor levels with a p-value adjusted by the Benjamini-Hochberg method for multiple comparisons.

Considering the feeding preference assay, the proportions of prey eaten at 30 min were compared using Manly's Alpha index without prey replacement (25), as follows:

$$\alpha_i = \frac{ri}{ni}\left(\frac{1}{\sum_1^m \frac{rj}{nj}}\right)$$

where

αi = Manly's Alpha index for prey i

ri, rj = Proportion of prey type *i*

j in the diet (i and j = 1, 2,…, *m*)

ni, nj = Proportion of prey type *i* and *j* in the environment

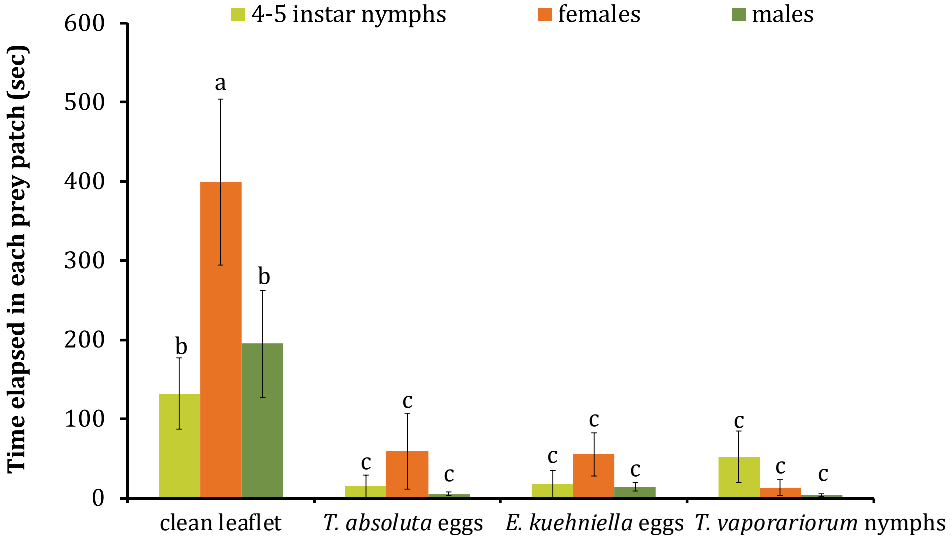
m = number of prey types possible

The Manly's Alpha index varies between 0 and 1, and because in this study three types of prey were offered, values of α = 0.33 indicated no preference, greater than 0.33 a preference, and lower than 0.33 a rejection. The number of prey of each food type consumed at 24 h was calculated as the number of prey alive after 24 h - the initial number of offered prey. Then the proportion of consumed prey (*i.e.*, the number of individuals of each prey type consumed / the number of individuals of that prey type alive after 24 h) was analyzed using a logistic model (binomial family, logit link function), with the individuals (4-5th instar nymphs, females, and males of *T. cucurbitaceus*) and the type of prey (*P. absoluta* eggs, *T. vaporariorum* nymphs, and *E. kuehniella* eggs) as the predictor variables. All analyses were carried out using R software (36).

RESULTS

Prey searching behavior

Predators spent significantly more time on the clean leaflet than in any of the food patches (χ2= 57.44; df= 3; P<0.001), with females spending more time outside of food patches than 4-5th nymphs and males (χ2= 6.5; df= 2; P= 0.04). When present on food patches, all predators spent a similar amount of time on any of the three sources (figure 2).



All bar graphs are represented by mean ± SE. Different letters show significant differences (p-value<0.05).

Las barras indican el promedio ± EE. Letras diferentes indican diferencias significativas (p valor<0,05).

Figure 2. Time (s) elapsed in the different food patches (*P. absoluta* eggs, *E. kuehniella* eggs, and *T. vaporariorum* nymphs) by *T. cucurbitaceus* individuals (4-5th instar nymphs, females, and males) in 30 min.

Figura 2. Tiempo (seg) transcurrido por individuos de *T. cucurbitaceus* (ninfas de 4-5° estadio, hembras y machos) en los diferentes parches de alimento (huevos de *P. absoluta*, huevos de *E. kuehniella* y ninfas de *T. vaporariorum*) en 30 min de observación.

Regarding walking activity, all predatory individuals remained still for almost the 30 min tested, except for more active males ($\chi2= 25.07$; df= 2; $P<0.001$). Given the small size and coloration of these insects -particularly nymphs-, on some occasions, the software was unable to detect activity responses (either moving or not moving). Failure was about 180 s for nymphs, while for adults of both sexes, the error was lower (<50 s).

The frequency of first visits to the TPs (*P. absoluta* eggs) and NTPs (*E. kuehniella* eggs and whitefly nymphs) was similar for all *T. cucurbitaceus* individuals (females: P= 0.08, males: P= 0.13, nymphs: P= 0.5). Mirid nymphs and males returned to the TPs independently of their first visit to that patch (males: P= 0.13, nymphs: P= 0.5). Females did not re-visit TPs (P= 0.04) (figure 3). In addition, males of *T. cucurbitaceus* showed a higher frequency of interchanges among patches than females and nymphs ($\chi2= 15.25$; df= 2; $P<0.001$).

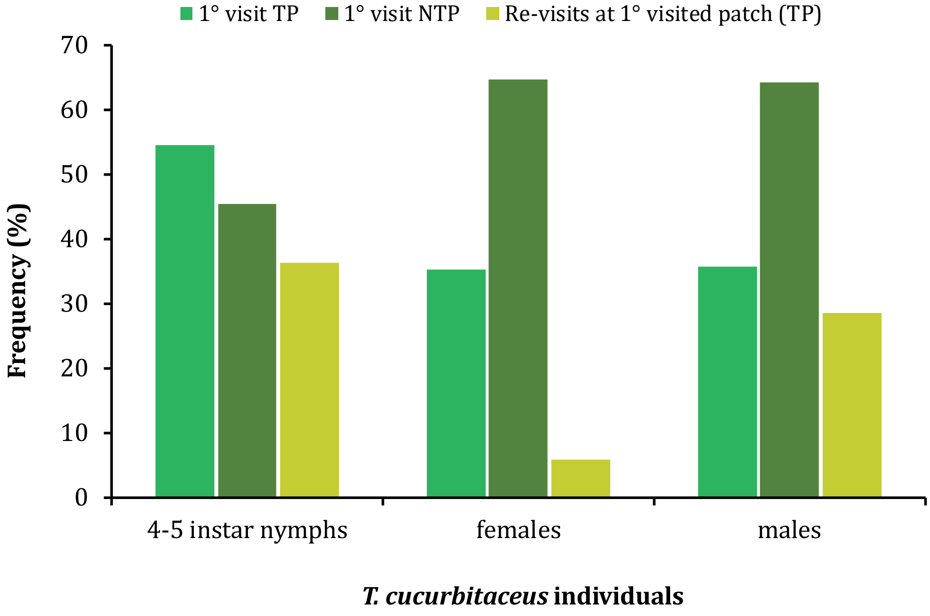


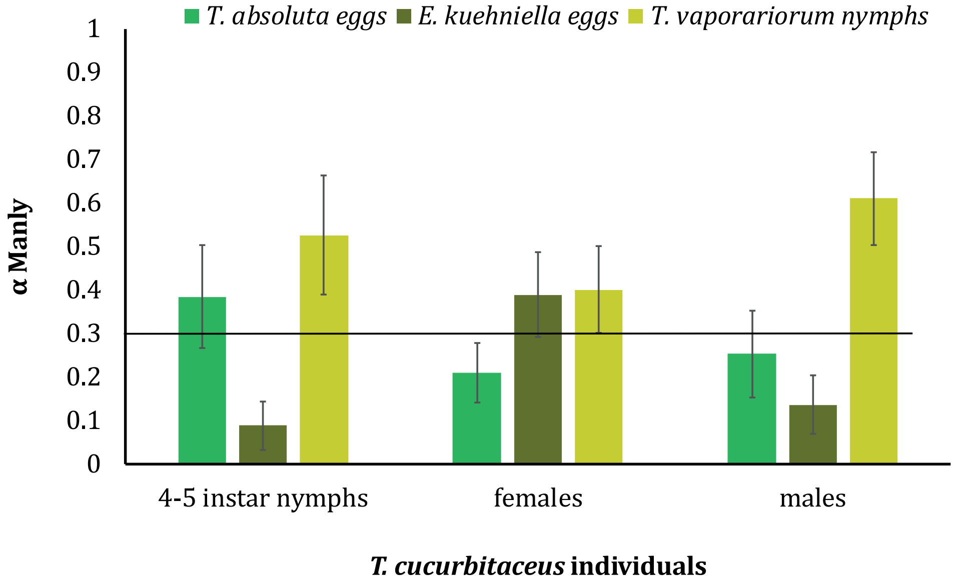
Figure 3. Frequency (%) of first visit to the target patch (*P. absoluta* eggs) and non-target patch (*E. kuehniella* eggs and *T. vaporariorum* nymphs), and re-visits to the target patch when it was the first patch visited by individuals of *T. cucurbitaceus* (4-5th instar nymphs, females, and males) in 30 min. TP: target patch, NTP: non-target patch.

Figura 3. Frecuencia (%) de primera visita al parche blanco (huevos de *P. absoluta*) y parche no blanco (huevos de *E. kuehniella* y ninfas de moscas blancas), y revisitas al parche blanco cuando este fue el primer parche visitado por individuos de *T. cucurbitaceus* (ninfas de 4-5° estadio, hembras y machos) en 30 min de observación.

Feeding preference

During the 30 min trial, all *T. cucurbitaceus* individuals tested consumed more *T. vaporariorum* nymphs, while predatory nymphs also fed on *P. absoluta* eggs and females did on *E. kuehniella* eggs. Besides, *T. cucurbitaceus* nymphs and males rejected feeding on *E. kuehniella* eggs (figure 4, page 104). After 24 h trial, *T. cucurbitaceus* nymphs ate more eggs of *P. absoluta* than females and males. Mirid nymphs also consumed a greater proportion of *E. kuehniella* eggs and *T. vaporariorum* nymphs than females and males except for the latter which mainly fed on whitefly nymphs (table 1, page 104; figure 5, page 105).

At the beginning of the experiment, all 24 h starved nymphs and adults of *T. cucurbitaceus* practiced phytophagy on the leaflet immediately after being placed in the experimental unit even in the presence of prey. However, no direct leaf injury was registered after feeding.



The black line indicates indifference (α= 0.33). All bars indicate mean ± SE.

La línea negra indica indiferencia (α= 0,33). Las barras indican el promedio ± EE.

Figure 4. Preference Index (Manly's α) of 4-5th instars nymphs, females, and males of *T. cucurbitaceus* for *P. absoluta* and *E. kuehniella* eggs, and *T. vaporariorum* nymphs, after the first 30 m of the trial.

Figura 4. Índice de preferencia (α de Manly) de las ninfas de 4-5° estadio, hembras y machos de *T. cucurbitaceus* por huevos de *P. absoluta* y *E. kuehniella* y ninfas de *T. vaporariorum* luego de 30 min de iniciado el ensayo.

Table 1. Results of the ANOVA of the logistic model (binomial family) to analyze the proportion of different prey eaten by *T. cucurbitaceus* (4-5 instar nymphs, females, and males) after 24 h.

Tabla 1. Resultados del ANOVA del modelo logístico (familia binomial) para analizar la proporción consumida de las diferentes presas por *T. cucurbitaceus* (ninfas de 4-5° estadio, hembras y machos) al cabo de 24 h.

	df	deviance	resid. df	resid. deviance	P-*value*
Null model			140	1547.48	
Prey	2	9.53	138	1537.95	0.008
Individuals	2	793.63	136	744.32	<0.001
Prey x Individuals	4	86.96	132	657.37	<0.001

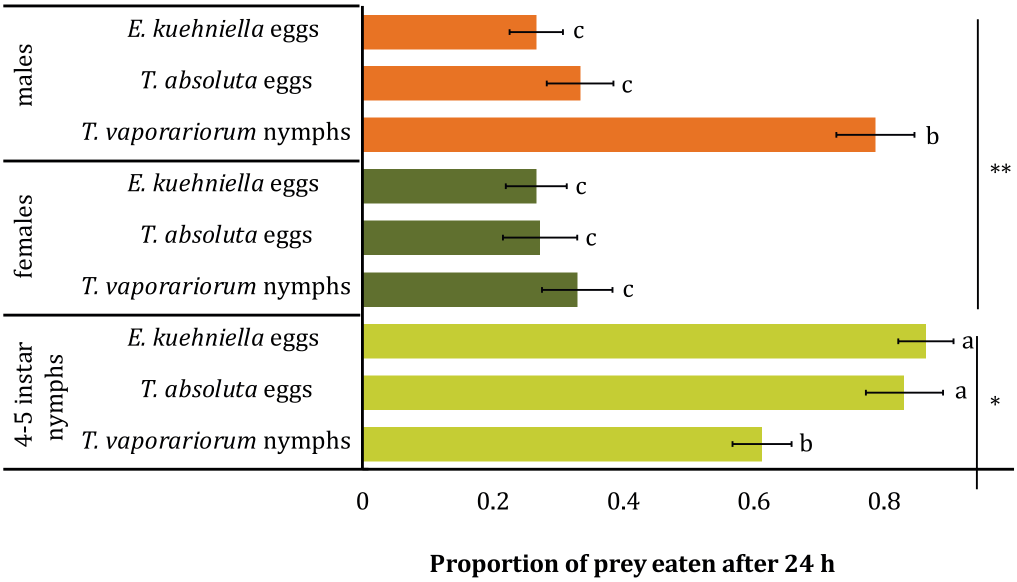


Figure 5. Proportion (mean) of each prey item eaten by *T. cucurbitaceus* individuals (4-5th instars nymphs, females, and males) in 24 h.

Figura 5. Proporción (promedio) de cada ítem de presa consumido por individuos de *T. cucurbitaceus* (ninfas de 4-5° estadio, hembras y machos) en 24 h.

All bar graphs indicate mean ± SE. Asterisks denote significant differences between individuals (late instar nymphs, females, and males), and different letters show significant differences among prey items (p-value<0.05).

Las barras indican el promedio ±EE. Los asteriscos muestran diferencias significativas entre individuos (ninfas de 4-5° estadio, hembras y machos), y letras diferentes indican diferencias significativas entre ítems de presas (p valor<0,05).

DISCUSSION

In this study, we present novel knowledge on the feeding habits of *T. cucurbitaceus*, a biocontrol agent of *P. absoluta* and whiteflies. The results confirmed the generalist feeding behavior of *T. cucurbitaceus* since the different developmental stages tested did not show clear patterns for food search. Mirid males searched for and consumed more *T. vaporariorum*. *Tupiocoris cucurbitaceus* nymphs also initially preferred whiteflies nymphs, but after 24 h consumed more lepidopteran eggs. Notably, *T. cucurbitaceus* females avoided *P. absoluta* eggs when first presented and ate all prey items in a similar proportion. As a result, the depletion of the mostly consumed prey across the experiment (observed in the 24 h trial) could force them to choose other available prey. This may indicate that, in the field, *T. cucurbitaceus* will consume the more abundant prey species. Likewise, Jaworski *et al.* (2013) showed that *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) was able to switch feeding from *B. tabaci* to *P. absoluta* depending on their relative numbers. These results stress the importance of evaluating simultaneous prey offers to corroborate the in-field effectiveness of entomophagous biocontrol agents. Other studies proved that in a single-prey system, *T. cucurbitaceus* showed a greater consumption rate of *P. absoluta* eggs than those fed on *B. tabaci* and *M. persicae* nymphs, and *T. urticae* adults (23). Urbaneja *et al.* (2009) also found high consumption of *P. absoluta* eggs by other two mirids, *Macrolophus pygmaeus* Rambur and *Nesidiocoris tenuis* Reuter.

Zoophytophagy is an important aspect to consider when using predators as biological control agents (11, 34). Interestingly, starved 4-5th nymphs and adults of *T. cucurbitaceus* consumed plant tissue before feeding on prey but this behavior did not result in noticeably injury to the leaflet. Similarly, non-damaging feeding habit was also reported previously for this mirid species tested on tobacco *Nicotiana tabacum* L. and tomato plants without adding any prey (8). However, the potential plant injury caused by *T. cucurbitaceus* should be more thoroughly evaluated to discard effects on the crop yield.

In sum, meticulous studies on diet breadth of generalist predators should avoid failures in biocontrol programs (42). Currently, two mirid species, *M. pygmaeus* and *N. tenuis*, have proved to be successful in control programs in Europe against the tomato moth *P. absoluta* (13). In Brazil, several studies showed other hemipteran species such as *Macrolophus basicornis* (Stal), *Engytatus varians* (Distant), *Campyloneuropsis infumatus* (Carvalho), (Hemiptera: Miridae), and *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) as promissory biological control agents of this pest (5, 7, 38, 43). Notably, *T. cucurbitaceus* is a dominant predatory species in northern Buenos Aires horticultural crops, co-occurring with *T. vaporariorum* and *P. absoluta* populations (29) allowing strategies for its augmentation and conservation to improve pest control. This study and others (22, 23, 24, 44) highlight the value of native beneficial fauna and the importance of preserving their natural presence in crops to contribute to IPM programs. In that context, we are currently assessing the potential of other entomophagous insects of *P. absoluta* as biological control agents, including intraguild predation interaction studies.

Conclusions

Results confirmed the generalist feeding behavior of *T. cucurbitaceus* since the different developmental stages tested in the laboratory did not show clear patterns when searching for the prey items offered. This finding could indicate that, in the field, this predatory species will consume the more abundant prey species, evidencing the importance of evaluating simultaneous prey offers to corroborate biocontrol effectiveness under crop conditions. Since we observed modest leaf consumption by *T. cucurbitaceus* in laboratory trials, the potential plant injury should be more thoroughly evaluated to discard effects on crop yield. We are currently assessing the potential of other entomophagous insects of *P. absoluta* as biological control agents, including intraguild predation interaction studies.

References

1. Basso, C.; Cibils-Stewart, X. 2020. Foundations and developments of pest management in Uruguay: a review of the 20 lessons and challenges. Agrociencia Uruguay. 24(2): 409. https://doi.org/10.31285/AGRO.24.409
2. Bates, D.; Mächler, M.; Bolker, B.; Walker, S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67(1): 1-48. https://doi:10.18637/jss.v067.i01
3. Bell, W. J. 1990. Searching behavior patterns in insects. Annual Review of Entomology. 35: 447-467. https://doi.org/10.1146/annurev.en.35.010190.002311
4. 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: 239-258. https://doi.org/10.1146/annurev-ento-031616-034933
5. Bottega, D. B.; Souza, B. H. S.; Rodrigues, N. E. L.; Eduardo, W. I.; Barbosa, J. C.; Boiça Júnior, L. A. 2017. Resistant and susceptible tomato genotypes have direct and indirect effects on *Podisus nigrispinus* preying on *Tuta absoluta* larvae. Biological Control. 106: 27-34. https://doi.org/10.1016/j.biocontrol.2016.12.0066
6. Bueno, V. H. P. 2009. Biological pest control: Mass production and quality control. 2nd. Editora da UFLA, Lavras, Brazil.
7. Bueno, V. H. P.; van Lenteren, J. C.; Calixto, L. A. M. Jr.; Montes, F.; Silva, D.; Santiago, L. D.; Pérez, L. M. 2013. New records of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) predation by Brazilian Hemipteran predatory bugs. Journal of Applied Entomology 137: 29-34. https://doi.org/10.1111/jen.12017
8. Burla, J. P.; Grille, G.; Lorenzo, M. E.; Franco, J.; Bonato, O.; Basso, C. 2014. Effect of different diets on the development, mortality, survival, food uptake and fecundity of *Tupiocoris cucurbitaceus* (Hemiptera: Miridae). Florida Entomology 97(4): 1816-1824.
9. Calvo, F. J.; Bolckmans, K.; Belda, J. E. 2012. Biological control-based IPM in sweet pepper greenhouses using *Amblyseius swirskii* (Acari: Phytoseiidae). Biocontrol Science and Technology. 22: 1398-1416. https: //doi.org/10.1080/09583157.2012.731494
10. Castañé, C.; Alomar, O.; Goula, M.; Gabarra, R. 2004. Colonization of tomato greenhouses by the predatory mirid bugs *Macrolophus c*aliginosus and *Dicyphus tamaninii*. Biological Control 30: 591–597. https://doi.org/10.1016/j.biocontrol.2004.02.012
11. Castañé, C.; Arnó, J.; Gabarra, R.; Alomar, O. 2011. Plant damage to vegetable crops by zoophytophagous mirid predators. Biological Control 59: 22-29.https://doi.org/10.1016/j.biocontrol.2011.03.007

12. Cribari-Neto, F.; Zeileis, A. 2010. Beta Regression in R. Journal of Statistical Software. 34(2): 1-24. https://doi:10.18637/jss.v034.i02
13. Desneux, N.; Han, P.; Mansour, R. Arnó, J.; Brévault, T.; Campos, M.; Chailleux, A.; Guedes, R.; KarimiJ, J.; Kouassi, A.; Lavoir, A.; Luna, M.; Perez-Hedo, M.; Urbaneja, A.; Verheggen, F.; Zappalà, L.; Abbes, K.; Ali, A.; Bayram, Y.; Cantor, F.; Cuthbertson, A.; De Vis, R.; Erler, F.; Firake, D.; Haddi; K.; Hajjar, M.; Ismoilov, K.; Jaworski, C.; Kenis, M.; Liu, H.; Madadi, H.; Martin, T.; Mazih, A.; Messelink, G.; Mohamed, S.; Nofemela, R.; Oke, A.; Ramos, C.; Ricupero, M.; Roditakis, E.; Shashank, P.; Wan, F.; Wang, M.; Wang, S.; Zhang, Y.; Biondi, A. 2021. Integrated pest management of *Tuta absoluta*: practical implementations across different world regions. Journal of Pest Science. 95: 17-39. https://doi.org/10.1007/s10340-021-01442-8
14. Donnelly, B. A.; Phillips, T. W. 2001. Functional response of *Xylocoris flavipes* (Hemiptera: Anthocoridae): effects of prey species and habitat. Environmental Entomology. 30: 617-624. https://doi.org/10.1603/0046-225X-30.3.617
15. Duarte Martínez, L.; Martínez Rivero, M. A.; Bueno, V. H. P.; Collatz, J. 2022. Predation behaviour and prey preference of two neotropical mirids against two key lepidopteran pests in tomato. International Journal of Tropical Insect Science 42(1): 815-825 https://doi.org/10.1007/s42690-021-00605-5
16. Enkegaard, A.; Brødsgaard, H. F.; Hansen, D. L. 2001. *Macrolophus caliginosus*: functional response to whiteflies and preference and switching capacity between whiteflies and spider mites. Entomologia Experimentalis et Applicata 101: 81-88 https://doi.org/10.1046/j.1570-7458.2001.00893.x
17. Ferreira, P. S. F.; Henry, T. J. 2011. Synopsis and keys to the tribes, genera, and species of Miridae (Hemiptera: Heteroptera) of Minas Gerais, Brazil. Part I: Bryocorinae. Zootaxa. 2920: 1-41. https://doi.org/10.11646/zootaxa.2920.1.1
18. Greco, N.; Rocca, M. 2020. Depredadores. In: Polack, L. A.; Lecuona, R. E.; López, S. N. (eds.). Control biológico de plagas en horticultura: experiencias argentinas de las últimas tres décadas, Ciudad Autónoma de Buenos Aires: Ediciones INTA.
19. Ingegno, B. L. B.; Ferracini, C.; Gallinotti, D.; Alma, A.; Tavella, L. 2013. Evaluation of the effectiveness of *Dicyphus errans* (Wolff) as predator of *Tuta absoluta* (Meyrick). Biological Control. 67: 246-252. https://doi.org/10.1016/j.biocontrol.2013.08.002
20. Jaworski, C. C.; Bompard, A.; Genies, L.; Amiens-Desneux, E.; Desneux, N. 2013. Preference and prey switching in a generalist predator attacking local and invasive alien pests. PLoS ONE. 8(12): e82231. https://doi.org/10.1371/journal.pone.0082231
21. Koul, O.; Dhaliwal, G. S. 2003. Predators and Parasitoids. Taylor & Francis. London.
22. López, S. N.; Arce Rojas, F.; Villalba, V.; Cagnotti, C. 2012. Biology of *Tupiocoris cucurbitaceus* (Hemiptera: Miridae), a predator of the greenhouse whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) in tomato crops in Argentina. Biocontrol Science and Technology. 22(10): 1107-1117. https://doi.org/10.1080/09583157.2012.705260
23. López, S. N.; Orozco Muñoz, A.; Andorno, A. V.; Cuello, E. M.; Cagnotti, C. 2019. Predatory capacity of *Tupiocoris cucurbitaceus* (Hemiptera: Miridae) on several pests of tomato. Bulletin of Insectology 72: 201-205.
24. Luft, E.; Luna, M. G.; Galise, G.; Speranza, S.; Virla, E. 2015. Mortalidad natural de huevos de la polilla del tomate, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) en Argentina e Italia, y primera mención de *Encarsia porteri* (Mercet) (Hymenoptera: Aphelinidae) afectando sus poblaciones. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 47(2): 219-229.
25. Manly, B. F. J. 1974. A model for certain types of selection experiments. Biometrics. 30: 281-294. https://doi.org/10.2307/2529649
26. Martin, J. H. 1987. An identification guide to common whitefly pest species of the world (Homoptera Aleyrodidae). Tropical Pest Management 33(4): 298–322. http://dx.doi.org/10.1080/09670878709371174
27. Messelink, G.; Bloemhard, C. M. J.; Hoogerbrugge, H.; van Schelt, J.; Ingegno, B. L.; Tavella, L. 2014. Evaluation of mirid predatory bugs and release strategy for aphid control in sweet pepper. Journal of Applied Entomology 139(5): 333–341 https://doi.org/10.1111/jen.12170
28. Mollá, O.; Biondi, A.; Alonso-Valiente, M.; Urbaneja, A. 2014. A comparative life history study of two mirid bugs preying on *Tuta absoluta* and *Ephestia kuehniella* eggs on tomato crops: implications for biological control. BioControl 59: 175–183. https://doi.org/10.1007/s10526-013-9553-8
29. Montiel Cáceres, R.; Salas Gervassio, N. G.; Minghetti, E.; Dellapé, P.; Luna, M. G.; Rocca, M. 2023. Heteropteran bugs assemblage associated with organic tomato farms: knowledge for pest management. Neotropical Entomology 52: 251–262 https://doi.org/10.1007/s13744-022-01007-z
30. Nieves, E.; Pereyra, P. C.; Luna, M. G.; Medone, P.; Sánchez, N. E. 2015. Laboratory population parameters and field impact of the larval endoparasitoid *Pseudapanteles dingus* (Hymenoptera: Braconidae) on its host *Tuta absoluta* (Lepidoptera: Gelechiidae) in tomato crops in Argentina. Journal of Economic Entomology 108: 1553–1559. https://doi.org/10.1093/jee/tov115

31. Parajulee, M. N.; Phillips, T. W.; Hogg, D. B. 1994. Functional response of *Lyctocoris campestris* (F.) adults: effects of predator sex, prey species, and experimental habitat. Biological Control. 4: 80-87.
32. Paula, D. P.; Andow, D. A.; Barratt, B. I. P.; Pfannenstiel, R.; Gerard, P.; Todd, J.; Zaviezo, T.; Luna, M.; Cédola, C.; Loomans, A.; Howe, A.; Day, M.; Ehlers, C.; Green, C.; Arpaia, S.; Yano, E.; Lövei, G.; Hinomoto, N.; Fontes, E.; Pires, C.; Togni, P.; Nechols, J.; Eubanks, M.; van Lenteren, J. 2021. Integrating adverse effect analysis into environmental risk assessment for exotic generalist arthropod biological control agents: A three-tiered framework. BioControl. 66: 113-139. https://doi.org/10.1007/s10526-020-10053-8
33. Pérez-Hedo, M.; Urbaneja, A. 2015. Prospects for predatory mirid bugs as biocontrol agents of aphids in sweet peppers. Journal of Pest Science. 88: 65-73. https://doi.org/10.1007/s10340-014-0587-1
34. Pérez-Hedo, M.; Rambla, J. L.; Granell, A.; Urbaneja, A. 2017. Biological activity and specificity of Miridae-induced plant volatiles. BioControl. 63: 203-213. https://doi.org/10.1007/s10526-017-9854-4
35. Polack, L. A.; López, S. N.; Silvestre, C.; Viscarret, M.; Andorno, A.; del Pino, M.; Peruzzi, G.; Gómez, J.; Iezzi, A. 2017. Control biológico en tomate con el mírido *Tupiocoris cucurbitaceus*. Comunicación INTA.
36. R Core Team. 2020. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Austria. https://www.Rproject.org/
37. Scopes, N. E. A.; Biggerstaff, S. M. 1971. The production, handling and distribution of the whitefly *Trialeurodes vaporariorum* and its parasite *Encarsia formosa* for use in biological control programmes in glasshouses. Plant Pathology 20(3): 111-116. https://doi.org/10.1111/j.1365-3059.1971.tb00525.x
38. Silva, D. B.; Bueno, V. J. P.; Montes, F. C.; van Lenteren, J. C. 2016. Population growth of three mirid predatory bugs feeding on eggs and larvae of *Tuta absoluta* on tomato. Biological Control. 61: 545-553. https://doi.org/10.1007/s10526-016-9736-1
39. Symondson, W. O. C.; Sunderland, K. D.; Greenstone, M. H. 2002. Can generalist predators be effective biocontrol agents? Annual Review of Entomology. 47: 561-594. https://doi.org/10.1146/annurev.ento.47.091201.145240
40. 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: 292-296. https://doi.org/10.1111/j.1439-0418.2008.01319.x
41. van Lenteren, J. C.; Tommasini, M. G. 2003. Mass production, storage, shipment and release of natural enemies. In: van Lenteren, J. C. (ed). Quality control and production of biological control agents. Theory and testing procedures. CABI Publishing, Wallingford. UK.
42. van Lenteren, J. C.; Bolckmans, K.; Köhl, J.; Ravensberg, W. J.; Urbaneja, A. 2018a. Biological control using invertebrates and microorganisms: plenty of new opportunities. Biological Control. 63: 29-59. https://doi.org/10.1007/s10526-017-9801-4
43. van Lenteren, J. C.; Bueno, V. H. P.; Calvo, F. J.; Calixto, A. M.; Montes, F. C. 2018b. Comparative effectiveness and injury to tomato plants of three neotropical mirid predators of *Tuta absoluta* (Lepidoptera: Gelechiidae). Journal of Economic Entomology. 111: 1080-1086. https://doi.org/10.1093/jee/toy057
44. van Lenteren, J. C.; Lanzoni, A.; Hemerik, L. Bueno, V.; Bajonero Cuervo, J.; Biondi, A.; Burgio, G.; Calvo, F.; de Jong, P.; López, S.; Luna, M.; Montes, F.; Nieves, E.; Aigbedion-Atalor, P.; Riquelme Virgala, M.; Sánchez, N.; Urbaneja, A. 2021. The pest kill rate of thirteen natural enemies as aggregate evaluation criterion of their biological control potential of Tuta absoluta. Science Report. 11(1): 10756. https://doi.org/10.1038/s41598-021-90034-8

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EXPERIMENTAL ETHICS

Permits for insect collections were obtained from Dirección de Flora y Fauna, Ministerio de Desarrollo Agrario de la provincia de Buenos Aires. Natural resources involved in this study are the exclusive property of the Buenos Aires province, Argentina.

CONFLICTS OF INTEREST

This manuscript and the authors of the manuscript are not involved in any potential conflicts of interest, including financial interests and relationships and affiliations.