Searching behavior and prey preference by the predatory mirid bug Tupiocoris cucurbitaceus, a potential biocontrol agent of tomato crops in Argentina

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Abstract

Generalist arthropod predators usually exhibit a degree for prey acceptance and this behavior can ultimately determine their potential as biocontrol agents. The South American tomato leafminer *Tuta absoluta* is a key pest of tomato crops worldwide. Biological control has gained interest as a reliable and environmentally safe pest management technique, and particularly the use of predatory mirid bugs has shown positive results to control *T. absoluta* and other horticultural pests in several countries. In a laboratory study we assessed: 1) prey searching behavior and 2) feeding preference of late nymphs and adults of both sexes of *Tupiocoris cucurbitaceus*, a mirid bug present in Argentinian tomato crops with the aim of deepen its status as a biocontrol candidate of *T. absoluta.* Predatory individuals were offered simultaneously three patches of prey: the target patch (TP) with *T. absoluta* eggs and two non-target patches (NTP) with *Ephestia kuehniella* eggs and *Trialeurodes vaporariorum* late-instar nymphs. First, a 30-min trial was analyzed using EthoVision® software in which we found that mirids equally accepted TP and NTP, and only nymphs preferred to feed on *T. absoluta* eggs. After 24 h, predatory nymphs consumed proportionally more of the three prey offered, but mainly lepidopteran eggs. These results prove that *T. absoluta* eggs can be part of the prey diet of *T. cucurbitaceous* especially for the nymphal stage. Further studies on prey availability in field conditions are needed to use *T. cucurbitaceus* as a biocontrol agent of *T. absoluta* in Argentina.

Key Message

- *Tupiocoris cucurbitaceus* is a spontaneous predatory mirid bug in Argentinian tomato crops.

- *Tuta absoluta* eggs can be part of the diet of *T. cucurbitaceus.*

- The predator preferentially feeds on other prey items, such as *Ephestia kuehniella* eggs and whitefly nymphs.

- Its performance as an augmentative biocontrol agent could be limited under field conditions.

- Conserving its natural populations to enhance biocontrol of *T. absoluta* could be worth investigating.

Introduction

The implementation of biological control requires knowing fundamental aspects of the biology, ecology, and taxonomy of the natural enemies of pests, which provide information for decision-making on their use and/or conservation (van Driesche et al. 2008; Jacas and Urbaneja 2008; Bueno 2009). By definition, predators are species that kill and consume living organisms for survival and reproduction (Price et al. 2011). They require various prey to complete the life cycle, and for that purpose, they must show mobile and highly efficient food searching stages. The use of generalist arthropod predators has been historically dismissed in biological control programs, compared to parasitoids and entomopathogens, due to negative effects, such as polyphagy or omnivory, the attack of non-target species, and competition

and intraguild predation on other natural enemy species present in crops (van Lenteren 2012; van Lenteren et al. 2018a). However, they are recently recognized for their impact on multiple pests, as they may feed on a variety of prey and plant resources ensuring their survival and reproduction, avoiding their extinction, and reaching more stable agroecosystems. Furthermore, and not less important, they can be manipulated by means of conservation of their natural populations in the agroecosystem or by releasing mass-reared individuals through augmentative biocontrol (Symondson et al. 2002; van Lenteren et al. 2018a; Snyder 2019; Paula et al. 2020; van Lenteren et al. 2021). For instance, in Spain chemical control was replaced by biological control through releasing predatory mirid bugs and acari in protected sweet pepper crops against whiteflies and thrips (Sánchez and Lacasa 2008; Urbaneja et al. 2009; Calvo et al. 2012; Pérez-Hedo and Urbaneja 2015).

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 breath. Although most predators usually feed on several species, they may exhibit a degree of acceptance for different prey based on certain particularities, such as their nutritional quality, behavior, morphology, and stage of development, and ultimately, this affects the success of these biocontrollers (Enkegaard et al. 2001). Two characteristics that have been especially pointed out to assess the diet breath of a predatory species are searching behavior and food preference. The former implies the predator´s activity of prospecting in the environment and the acceptance of some prey. Factors such as the developmental stage, sex, age, starvation, the prey species found and the availability of alternative prey influence searching behavior. On the other hand, food preference is determined by morphological, physiological, and behavioral traits of prey to obtain enough nutrients and avoid toxic or indigestible food (Bell 1990; Parajulee et al. 1994; Donnelly and Phillips 2001; Schowalter 2006; Price et al. 2011). In addition, omnivorous predators need to feed upon other sources than animal prey, such as pollen, nectar, plant fluids or tissues to persist in the environment which must be carefully evaluated as well (Coll and Guershon 2002).

Miridae bugs (Hemiptera) are an important group of predatory insect species. Most of them show high preying rates and are able to find and colonize new habitats due to their dispersing capacity. Their polyphagous feeding habit allows them to exploit a variety of resources and rapidly increase their populations (Messelink et al. 2014; Salas Gervassio et al. 2017). The use of these insects as biocontrol agents against whiteflies, eggs, and small larvae of lepidopterans, among other horticultural pests, has increased and around 20 species are currently commercialized (van Lenteren et al. 2018a). Other positive traits for these insects as biological control agents are mentioned, as they can be mass-reared relatively easily and are released in low numbers (through inoculative releasing). Nymphs and adults belonging to genera *Campiloneuropsis*, *Dicyphus*, *Engytatus*, *Macrolophus*, *Nesidiocoris* and *Tupiocoris* are main biological control agents reported for Europe and South America (Castañé et al. 2004; Urbaneja et al. 2009; Bueno et al. 2012; Calvo et al. 2012; López et al. 2012; Ingegno et al. 2013; Silva et al. 2016; López et al. 2019). They can eventually show zoophytophagous feeding habits, especially those belonging to the Subfamily Bryocorinae, tribe Dicyphini, that are commonly associated with plant species of Caryophyllales, Fabales, Fagales, Rosales and Solanales, where they find prey, and can cause damage to crops under certain circumstances (Cassis and Schuh 2012). Zoophytophagy has been well studied for

Campiloneuropsis infumatus Carvalho, two *Dicyphus* species, *Macrolophus basicornis* Stal and *M. pygmaeus* (Rambur), and *Nesidiocoris tenuis* Reuter (Sánchez and Lacasa 2008; Calvo et al. 2009; Arnó et al. 2010; Castañé et al. 2011; Silva et al. 2016).

Horticulture is a main agroproductive activity in Argentina and plant protection mainly relies on chemical control. In recent years, biological control has gained interest, by importing arthropods and microorganisms to be used in commercial biological control strategies or by developing protocols for conserving spontaneous natural enemies already present in agroecosystems. Besides, hundreds of natural enemies are being studied to determine their potential as biocontrol agents (Greco et al. 2020). Particularly for tomato crops, the whiteflies *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and the South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) affect open and protected crops year-round (Polack 2020). Whitefly species have a cosmopolitan distribution and cause direct and indirect losses to crops by sap-feeding and also vectoring plant diseases (Byrne et al. 1990). *Tuta absoluta* instead is a key pest of tomato crops in the Neotropics which has invaded the European, African, and Asian continents last decade and has quarantine status for Australia and North American countries (Desneux et al. 2011; Tonnang et al. 2015; Ponti et al. 2015). Larvae damage plants by mining leaves, stems, and fruits, and occasionally may lead to rotting of fruits. Intensive application of insecticides causes pests´ resistant populations, eliminates their natural enemies and increases production costs, making crops dependent on this technology (Lietti et al. 2005; Roditakis et al. 2015).

The mirid bug *Tupiocoris cucurbitaceous* Spinola (Hemiptera: Miridae) has been reported for the Americas preying mainly on whiteflies (Ferreira and Henry 2011; Montiel Cáceres et al. *ms* in revision). Its populations can be found on a range of plant families, such as Asteraceae, Cucurbitaceae, Geraniaceae and Solanaceae (Carpintero and Carvalho 1993) and it was also registered feeding on tobacco *Nicotiana tabacum* L. (López et al. 2012). Regarding *T. absoluta* biological control, advances done in Europe and Brazil for other mirids show promissory use of *T. cucurbitaceus* for such purpose, such as periodic commercial releasing of *N. tenuis* (Calvo et al. 2012; Urbaneja et al. 2012) and trials to study the potential of *C. infumatus*, *Engytatus varians* (Distant) and *M. basicornis* (Bottega et al. 2017; Bueno et al. 2013; Silva et al. 2016; van Lenteren et al. 2018b). There exist previous laboratory reports of the preying rate and preference of *T. cucurbitaceus* when offered *T. vaporarioum* and *T. absoluta* (Lopez et al. 2012; Cagnotti et al. 2016; López et al. 2019), and it has recently been developed as a commercial biocontrol agent in Argentina and Uruguay (Basso and Cibilis-Stewart 2020; Greco and Rocca 2020). Despite its high predation and pest kill rate examined over a range of prey species (Cagnotti et al. 2016; López et al. 2019; van Lenteren et al. 2021), studies devoted to elucidate searching behavior and feeding preference of this mirid have not been analyzed yet. Thus, to continue studying its potential as a biocontrol agent of *T. absoluta* in tomato crops in Argentina, we aimed to explore in the laboratory: 1) prey searching behavior, and 2) feeding preference of late instar nymphs and adults of *T. cucurbitaceus* when offered simultaneously to different prey items. Additionally, we report phytophagous behavior of this mirid on tomato leaves.

Materials And Methods

Plant and insect materials

Tomato plants (*Solanum lycopersicon* L., Elpida variety Enza Zaden, The Netherlands) were cultivated in a greenhouse at the CEPAVE (CONICET-UNLP-Asociado CICPBA). Tomato seedlings were individually transplanted to 1 L-plastic pots, watered daily, and kept free of pesticide applications by protecting them inside 60 x 40 x 40 cm (length x width x height) white voile cages (BioQuip, Inc, USA) to prevent damage by insect pests. Plants used in colonies and experiments had 4–5 expanded leaves.

All insect colonies were initiated with individuals collected in organic horticultural crops located nearby the La Plata Horticultural Belt (N Buenos Aires province, Argentina) and maintained at controlled temperature, relative humidity, and photoperiod conditions (25 ± 2°C, 70 ± 10% RH, 14:10 L:D) in a walk-in rearing room at the CEPAVE. *Tupiocoris cucurbitaceus* rearing was carried out by adapting a protocol used for another predatory bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) (Bueno 2009). Adults of *T. cucurbitaceus* were sampled in the field by tapping tomato leaves in a 45 x 20 x 5 cm (length x width x height) white plastic tray (Colombraro, Argentina) and then recovered by picking them with a manual insect aspirator. Collected mirids were transported to the laboratory, identified using a stereoscopic microscope (SM 1270, Nikon, Japan) and reared in white voile cages (as described above), fed with sterile *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs (Brometán SRL, Argentina), commercial pollen and water *ad libitum*, and provided with tomato plants to allow oviposition. Once the nymphs emerged, they were transferred to three different cages to avoid cannibalism, considering their developmental stage: 1st instar nymphs (Cage 1), 2nd-3rd instars nymphs (Cage 2) and 4th-5th instars nymphs (Cage 3). Adults were picked from Cage 3 and conditioned in new voile cages to initiate the rearing cycle. *Tupiocoris cucurbitaceus* colony was checked every 48 h. *Tuta absoluta* rearing was performed following Nieves et al. (2015) protocol. Leaves with presence of *T. absoluta* larvae were dislodged from tomato crops and transported to the laboratory, kept fresh in jars with water and placed in 40 x 40 x 60 cm (length x width x height) white voile cages (BioQuip, Inc, USA) until the formation of pupae which were collected as they dropped in a tray containing sterilized sand. Pupae were transferred to new voile cages (same dimensions as described above) until moths emerged. Adults were provided with a honey solution (70%) and allowed mating. Every 3 days, potted tomato plants were offered as substrate of oviposition and those replaced were held in clean voile cages to obtain the larvae which were fed with fresh potted tomato plants until they pupate and initiate a new cycle. To produce *T. vaporariorum* nymphs used in the feeding preference experiment, a modified rearing protocol (Scopes and Biggerstaff 1971) was performed. Whitefly adults were captured in tomato crops using a manual aspirator and transported to the laboratory for identification. Then, they were released in 40 x 40 x 60 cm (length x width x height) white voile cages and provided with potted tomato plants, to lay eggs. Once they hatched, they were checked weekly to observe their development and after 20 days revised every 24 h to collect late instar nymphs which were used for the assays.

Searching Behavior Assay

For the experiment, three treatments were considered taking into account *T. cucurbitaceus* developmental stage and sex: 1) 4th to 5th- instar nymph, 2) 7 d- old adult female, and 3) 7-d old adult male. Individuals were isolated from the colony and kept starved for 24 h prior to performing the assays in plastic Petri dishes (diameter: 5 cm), provided with a cotton boll as water source.

The experimental unit consisted of a plastic Petri dish (diameter: 9 cm) with a tomato leaflet placed on 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 *T. absoluta* eggs (24–48 h old) and two non-target patches (NTP) with 30 *E. kuehniella* eggs and 5 *T. vaporariorum* nymphs (late instar). 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 remaining space of the leaflet free of food was considered “leaflet”. Each treatment was replicated 15 times and experimental units were not re-utilized.

Prey searching behavior was studied during 30 min using the software EthoVision® XT (Noldus, The Netherlands) which videotapes the activities and movements of animals inside an arena. Various steps were followed to calibrate the recording of mirids when visiting the food patches. Four behavioral descriptors were evaluated: 1) the time (s) spent in each patch and in the leaflet, 2) the accumulated time (s) of mirids in movement or non-movement, 3) the frequency of visits (first visit and recurrent visit or re-visits) to TP and NTP, and 4) the maximum number of times predators interchanged patches. The first descriptor was analyzed using a 2-way ANOVA with permutation test since normality and homoscedasticity assumptions were not accomplished. Predictive factors were the developmental stage and sex of *T. cucurbitaceus* (4-5th nymphs, 7 d-old females and males of mirids) and the patch (*T. absoluta* eggs, *E. kuehniella* eggs, *T. vaporariorum* nymphs, or the leaflet (no prey)). The accumulated time in movement or non-movement (response variable) were analyzed using 2-way ANOVA with permutation test. In this case, the predictive factors were the type of predatory individuals (nymphs, female, and male adults of *T. cucurbitaceus*) and their behavior (movement or non-movement). The frequency of first and re-visits to TP and NTP was analyzed, separately, for developmental stage and sex of *T. cucurbitaceus* using log-linear models for 3 (4-5th nymphs, females, and males) x 2 (TP and NTP) contingency tables. Odd ratios (OR) were estimated to quantify the likelihood of an event in relation to another. Then, other analysis was performed to evaluate separately for *T. cucurbitaceus* nymphs, and 7 d-old adults of both sexes the number of replicates in which the individual revisited the TP after visiting that patch the first time, using 2 x 2 contingency tables. The maximum number of interchanges among patches was analyzed by means of the Kruskal-Wallis test because data did not fit the assumption of normality. Then, a Dunn's test was performed to check for significant differences among factor levels with a p-value adjusted by the Benjamini-Hochberg method for multiple comparisons. All analyses were carried out using R software (R Core Team, 2020).

Feeding Preference Assay

The same experimental units were used to evaluate the feeding preference by *T. cucurbitaceous* nymphs, females, and males at 30 min by registering the number of preyed lepidopteran eggs or whitefly nymphs. Then, 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. Since mirids have a piercing-sucking feeding habit, the remains of preyed food can be easily recognized. Damage of leaflets due to mirid piercing of plant tissues was also checked to determine the occurrence of phytophagous behavior. Proportions of prey eaten at 30 min were compared by means of Manly's Alpha index without prey replacement (Manly 1974), as follows:

$$\alpha_i = \frac{ri}{ni}\left(\frac{1}{\sum {}^{rj}/_{nj}}\right)$$

where α*i* = Manly's Alpha index for prey i

ri, rj = Proportion of prey type *i* and *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 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 proportion of total preys eaten by *T. cucurbitaceus* (4-5th instar nymphs, females, and males) after 24 h of the experiment was analyzed using a logistic model (binomial family, logit link function), with the individuals (4-5th instar nymphs, females, and males) and the type of prey as the predictor variables.

Results

Searching behavior

Mirids spent more time in the clean leaflet than in the patches with prey, with females spending significantly more time in the leaflet than in the patches with prey (Table 1, Fig. 1).

Table 1
Results of two factor ANOVA with permutation test to analyze the time spending for *T. cucurbitaceus* individuals (late instar nymphs, females, and males) in different prey patches (*T. absoluta* eggs, *E. kuehniella* eggs, and *T. vaporariorum* nymphs) and in the leaflet.

	df	F	p-value
Individual (4–5 instar nymphs, females, and males)	2	3.97	0.02
Prey patch	3	17.99	< 0.001
Interaction	6	2.1	0.03
Error	163		
Number of iterations = 5000			

In relation to the accumulated time in movement and non-movement, individuals stood still significantly more time than in movement, and in particular, females remain still longer than nymphs (Table 2; Fig. 2). It is necessary to clarify that, due to the small size and coloration of these insects —particularly the nymphs—, the program sometimes failed to detect their activity (either moving or not moving). In the case of nymphs, this lack of detection was about 180 s, while for adults of both sexes the error was much lower (< 50 s).

Table 2
Results of the two-factor ANOVA with permutation test to analyze the accumulated time (s) in movement and non-movement of *T. cucurbitaceus* individuals (4-5th instar nymphs, females, and males).

	df	F	p-value
Individuals (4–5 instar nymphs, females, and males)	2	6.95	< 0.001
Activity (movement and non-movement)	1	8283.25	< 0.001
Interaction	2	7.15	0.001
Error	82		
Number of iterations = 5000			

The frequency of first visits to the TP (*T. absoluta* eggs) and NTPs (*E. kuehniella* eggs and whitefly nymphs) was similar for all *T. cucurbitaceus* individuals (Table 3; Fig. 3). For 4-5th mirid instar nymphs, the frequency of re-visits to the TP and NTPs was independent of the patch visited for the first time ($\chi2 = 0.41$; df = 2; p-value = 0.52). The same occurred for females ($\chi2 = 2.30$; df = 2; p-value = 0.13) and males ($\chi2 = 0.10$; df = 2; p-value = 0.76) (Fig. 3). Although no significant statistical differences were found, when analyzing the odd ratios, the frequency of total re-visits to the NTPs (*E. kuehniella* eggs and *T. vaporariorum* nymphs) by *T. cucurbitaceus* nymphs and adults of both sexes was 4.8 times greater than

that to the TP (*T. absoluta* eggs), and males re-visited the target patch 2.5 times more often than females (Table 4).

Table 3
Results of the log-linear model to analyze the frequency of the first visit of individuals of *T. cucurbitaceus* (4-5th instar nymphs, females, and males) to the target patch (*T. absoluta* eggs) and non-target (*E. kuehniella* eggs and *T. vaporariorum* nymphs). The model takes as reference the females and the non-target patch.

	Estimate	SE	p-value
Intercept	2.33	0.27	< 0.001
Males	-0.19	0.36	0.59
4-5th instar nymphs	-0.35	0.38	0.36
Target patch	-0.42	0.31	0.17

Table 4
Results of the log-linear model to analyze the frequency of re-visits of *T. cucurbitaceus* individuals (4-5th instar nymphs, females, and males) to the target patch (*T. absoluta* eggs) and non-target (*E. kuehniella* eggs and *T. vaporariorum* nymphs). The model takes as reference the females and the non-target patch.

	Estimate	SE	p-value
Intercept	3.87	0.14	< 0.001
Males	0.70	0.17	< 0.001
4–5 instar nymphs	0.04	0.20	0.84
Target patch	-1.57	0.35	< 0.001
Males*target patch	0.92	0.39	0.02
4–5 instar nymphs*target patch	0.65	0.44	0.13

In addition, males of *T. cucurbitaceus* showed a higher frequency of interchanges among patches than females and 4-5th instar nymphs ($\chi 2 = 15.25$; df = 2; p-value < 0.001; Fig. 4).

Feeding Preference

After the first 30 min of the trial, a clear preference was observed for 4-5th instar nymphs and males of *T. cucurbitaceus* to feed on *T. vaporariorum* nymphs. In addition, 4-5th instar nymphs of the predator also

showed a preference for *T. absoluta* eggs, while females showed the same preference for *T. vaporariorum* nymphs and *E. kuehniella* eggs (Fig. 5).

After 24 hours, 4-5th instar nymphs of *T. cucurbitaceus* ate a higher proportion of all prey than females and males. Whiteflies were more consumed by *T. cucurbitaceus* adult males and less preferred by 4-5th nymphs (Table 5; Fig. 6).

Table 5
Results of the logistic model (binomial family) to analyze the proportion of different prey eaten by *T. cucurbitaceus* (4–5 instar nymphs, females, and males). The model takes as reference the *E. kuehniella* eggs and females.

	Estimate	SE	z	P-value
Intercept	0.3102	0.06808	4.556	< 0.001
T. vaporariorum nymphs	0.08945	0.1842	0.485	0.63
T. absoluta eggs	0.008054	0.09639	0.084	0.93
Males of *T. cucurbitaceus*	5.834E-17	0.09943	0	1
4–5 instar nymphs of *T. cucurbitaceus*	1.688	0.1525	11.072	< 0.001
T. vaporariorum nymphs*Males of *T. cucurbitaceus*	1.145	0.3392	3.377	< 0.001
T. absoluta eggs*Males of *T. cucurbitaceus*	0.08726	0.1418	0.615	0.54
T. vaporariorum nymphs**T. cucurbitaceus* nymphs	-1.138	0.3168	-3.591	< 0.001
T. absoluta eggs**T. cucurbitaceus* nymphs	-0.2279	0.21	-1.095	0.27

Feeding observations of T. cucurbitaceus on tomato leaves

It was observed at the initiation of the experiment that all 24 h-starved nymphs and adults of *T. cucurbitaceus* performed phytophagy immediately after being placed in the experimental unit and in the presence of prey before starting zoophagy.

Discussion

In the present study we report novel knowledge on the zoophytophagous mirid *T. cucurbitaceus*, a predatory biocontrol agent that is currently commercialized in Argentina and Uruguay against the South American tomato pinworm *T. absoluta*, a key pest worldwide, regarding its searching behavior and feeding preference. Previous laboratory studies aimed to determine its predatory capacity reported a daily consumption rate of 30 *T. vaporariorum* nymphs for females and a lower number for nymphs and male of the predator (8 to 10 nymphs respectively). This mirid actively consumed eggs of a lepidopteran species commonly used for mass rearing, *Sitrotroga cerealella* Olivier (Lepidoptera: Gelechiidae) (an average of 30 eggs for females and about a half for nymphs and males of *T. cucurbitaceus*) (López et al.

2012). López et al. (2019) found a consumption of 27 and 72 *T. absoluta* eggs per day for early, late nymphs and male adults of *T. cucurbitaceus* meanwhile females can prey up to 147 eggs. Besides, all stages are able to feed on small *T. absoluta* prey larvae (3 to 5 individuals), rejecting older larvae, and van Lenteren et al. (2021) showed that *T. cucurbitaceus* has a high *T. absoluta* reduction capacity and a great potential for practical application in crop production conditions. Other research demonstrated similar predation capacity on *T. absoluta* and other pests for several mirid species in South America and Europe (Urbaneja et al. 2009; Bueno et al. 2012, 2013; Ingegno et al. 2013, 2019). Besides, *T. cucurbitaceus* is a dominant species in the hemipteran community inhabiting N Buenos Aires horticultural crops, coincidently with *T. vaporariorum* and *T. absoluta* populations (Montiel Cáceres et al. ms in revision) what allows planning augmentation and conservation strategies of this natural enemy to improve pest control. To cope with that, information on its breath diet is necessary to evaluate the efficiency in controlling the target pests and avoid failures in biocontrol programs.

Results of this study revealed that, in general, all individuals of the predatory bug *T. cucurbitaceus* did not immediately feed in prey patches once released in the arena, and instead, they consumed plant tissue. Similarly, in a study done by Duarte Martínez et al. (2022) they found that mirids *M. basicornis* and *Engytatus varians* (Distant) offered *T. absoluta* and *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae) eggs as prey rested on the tomato leaflet without feeding up to 1800 s. This behavior can be explained since insects generally remain nearly motionless and often hidden while scanning with chemosensory receptors or tactile organs before beginning the search for food, and in that manner avoiding being exposed to potential predators (Bell 1990). It would be interesting to carry out complementary studies related to better knowing *T. cucurbitaceus* searching behavior activities, as for example, the handling and consumption time, the period elapsed to clean the mouthparts, etc.

When considering the first visit and revisits of simultaneously offer of different prey patches, to both *T. cucurbitaceus* 4-5th instar nymphs and adults, they did not follow a clear pattern of search, since they chose the three types of prey indistinctly; although there seems to be a tendency for females and males to visit the non-target (whiteflies and *E. kuehniella* eggs) patches first. Moreover, their first food choice did not affect subsequent choices; however, mirid males revisited the *T. absoluta* patch more than nymphs and females. A predator's search behavior is often related to the type of food it consumes (Huey and Pianka 1981). It is known that the more generalist predators seek a specific type of prey less frequently compared to the more specialized ones, since the former can feed on a wide range of other prey (Coll and Guershon 2002). In addition, several biotic and abiotic, external and internal, environmental factors can influence the searching behavior of insects. For example, resource quality affects search duration, searching speed, capture rate, and scanning. The quality of the prey may also directly affect the course of search, and in this case the insect's ability is pointed out as a major trait to sample among patches and to use this information to determine if the current patch is still more profitable than some other patch in the habitat (Bell 1990). Another factor is hunger, which may play an important role on the searching behavior exhibited by insects (Heimpel and Hough-Goldstein 1994; Henaut et al. 2002). Generally, an intensive search path is observed after prey capture (Evans 1976; Heimpel and Hough-Goldstein 1994); however, it has been observed for some heteropteran predators that they did not systematically adopt an intensive

search path after prey ingestion. For example, Lamine et al. (2005) observed that satiated 5th instar and adult of the predatory bug *Deraeocoris lutescens* Schilling (Heteroptera: Miridae) adopted an “intermediate” search path, linked to a possible heterogeneous satiety state of individuals in relation to the occurrence of the last meal. Therefore, the choice of the *T. cucurbitaceus* female could be related to the quality of the resource and the need for high-quality prey to satisfy their reproductive needs, while for males this would not be a necessary requirement, so their choice was indistinct and probably adopted that “intermediate” search path. The lack of a clear first choice for a particular type of prey by *T. cucurbitaceus* could be related to the generalist habit of this predator and, once they "taste" different food, there seems to be a tendency for them to choose to re-visit the non-target patch, perhaps due to the quality of the resource as seen in the preference analysis.

Both mirid nymphs and adults initially preferred to feed on whiteflies, although females also chose *E. kuehniella* eggs and nymphs chose *T. absoluta* eggs, while adults rejected the latter. However, when their preferred prey became depleted, all of them used *T. absoluta* eggs as an alternative food. In addition to prey quality, its own nutritional requirements can affect predator searching behavior (Biesinger and Haefner 2005). Key nutrients such as amino acids and sterols would seem to be much more reliable as predictors of a predator's capability to serve as a faithful consumer of a target prey (Cohen and Brummett 1997). Cohen and Brummett (1997) shown that the generalist predators *Crysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and *Geocoris punctipes* (Say) (Hemiptera: Geocoridae) could not maintain itself for any protracted period feeding only whiteflies because the relative sparseness of methionine in the prey, and they would be forced to find alternative prey (such as lepidopteran eggs) that are rich in methionine to support its needs. In relation to *T. absoluta* as prey, Duarte Martínez et al. (2022) found that the mirid *E. varians* had a strong preference for *N. elegantalis* when offered together with *T. absoluta*, while such a preference was not evident for *M. basicornis*, and this could be associated with their nutritional requirements and the quality of the eggs. Additionally, Mollá et al. (2014) and Sylla et al. (2016) showed that *M. pygmaeus* had slower nymphal development and lower fertility when fed *T. absoluta* eggs compared to *E. kuehniella* eggs, *B. tabaci* nymphs, and *Macrosiphum euphorbiae* (Thomas) aphids. Here again, there is evidence that the eggs of *T. absoluta* probably have a low nutritional quality for mirids, and for this reason these predators need to feed on better quality prey to increase their performance in the crop. However, Jaworski et al. (2013) and Urbaneja et al. (2009) observed that the preference of *M. pygmaeus* and *N. tenuis* towards *T. absoluta* eggs was especially evident in the nymphal stages, probably due to their small size. Similarly, we found that 4-5th instar nymphs preyed more on lepidopteran eggs than on whitefly nymphs. Moreover, Jaworski et al. (2013) showed that the food preference of the mirid *M. pigmaeus* can change towards a diet of eggs and larvae of *T. absoluta* when the latter is found in greater proportion than the whitefly; that is, the predator consumes the prey that is in a higher density.

As we mentioned above, *T. cucurbitaceus* is currently commercialized by a biofactory in Argentina for the control of whiteflies and *T. absoluta* in greenhouse tomato; therefore, an important aspect that must be considered is that the mass rearing of this bug is maintained with *E. kuehniella* eggs, which offer them quality food. Other mirids commercialized by biofactories, mainly in Europe, are reared in a similar way. A

common practice when these biological control agents are released, for example *N. tenuis* in Spain, is the enrichment of prey using *E. kuehniella* eggs in tomato seedlings and crops to favor their subsequent establishment (Calvo et al. 2012). A similar methodology is proposed for the release of *T. cucurbitaceus* in tomato crops in Argentina (Polack et al. 2017). This type of practice should be evaluated with food preference studies under field conditions in order to determine if this natural enemy is able to change the diet and reduce the biological control of the target pest.

Zoophytophagy is an important aspect to consider when using this predator as a biological control agent (Wheeler 2001; Castañé et al. 2011; Pérez-Hedo et al. 2017). Gillespie and McGregor (2000) proposed three models for explaining zoophytophagy of mirids: 1) species that switch between plant and prey feeding—when decreased prey availability predators are forced to increase phytophagy—, 2) species that feed on plant as a requirement to supplement prey food—plant provides resources, such as water, which are necessary for the digestion process, so the amount of plant feeding increases with increased prey feeding—, and 3) species that feed on plants to gather a critical resource required for predation—i.e. the amount of plant feeding is independent of the amount of prey feeding—. Calvo et al. (2009) found that *N. tenuis* appears to follow the first model because damage to tomato decreased in response to greater availability of *B. tabaci*. This behavior differs from that found by Gillespie and McGregor (2000) for *Dicyphus hesperus* Knight for which damage to tomato increased in response to increased prey availability due to the need to obtain water from the plant for external digestion of prey. In contrast, Lucas and Alomar (2001) registered that *Dyciphus tamaninii* Wagner seems to damage tomato fruits independently of prey availability. Our behavioral studies revealed that *T. cucurbitaceus* performs phytophagy on tomato leaves, after being fasted and even in the presence of prey, although no direct leaf damage was observed after feeding. Therefore, this behavior should be studied in greater depth for this species to determine its potential damage to the crop.

Currently, horticultural pests in greenhouse crops can be successfully controlled with IPM programs that include the use of natural enemies, particularly generalist predators (van Lenteren et al. 2018a). The mirid species commercialized in Europe, *M. pygmaeus* and *N. tenuis*, have been used successfully in IPM and biological control programs against the tomato moth (Jacobson 2011; Mollá et al. 2011; Urbaneja et al. 2012), and in Brazil there are several species of hemiptera under evaluation as potential biocontrol agents of *T. absoluta*, such as *C. infumatus*, *E. varians*, *M. basicornis* and *Podisus nigrispinus* (Dallas) (Bottega et al. 2017; Bueno et al. 2013; Silva et al. 2016; van Lenteren et al. 2018b). This study, and other done by López et al. (2012, 2019) and van Lenteren et al (2021), confirms that *T. cucurbitaceus* has potential as a biocontrol agent in Argentina, increasing the number of bioproducts available to control horticultural pests. In addition, they highlight the value of native beneficial fauna and the importance of preserving their natural presence in crops, to be used in augmentative releasing or conserving the populations to provide biological control alone or in IPM programs (van Driesche et al. 2008, Desneux et al. 2021).

Statements And Declarations

Ethics approval and consent to participate

This study was carried out with material collected in horticultural farms with the corresponding authorization 'DISPO-2021-962-GDEBA-DPFAAYRNMDAGP' from the Provincial Directorate of Agricultural Control, Food and Natural Resources, Ministry of Agrarian Development of the Argentine Republic. Informed consent was obtained from all individual participants included in the study.

Consent for publication

Informed consent to publish was obtained from all individual participants included in the study.

Availability of data and materials

Not applicable

Competing interests

Not applicable

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Authors' contributions

Margarita Rocca and María Gabriela Luna conceived and designed research. Rocío Montiel Cáceres and Margarita Rocca conducted experiments and analyzed data. María Gabriela Luna and Margarita Rocca wrote the manuscript. All authors read and approved the manuscript.

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Figures

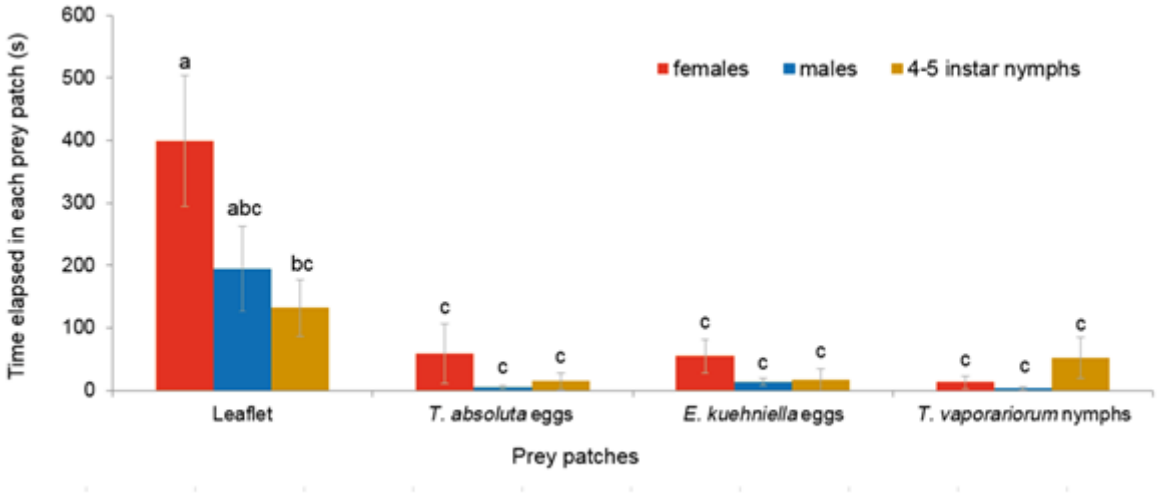

Figure 1

Time (s) elapsed in the different patches (*T. absoluta* eggs, *E. kuehniella* eggs and *T. vaporariorum* nymphs) and on the leaflet with no prey (arena) by *T. cucurbitaceus* individuals (4-5th instar nymphs, females, and males) in 30 min. The bars indicate the standard error. Different letters show significant differences ($p<0.05$).

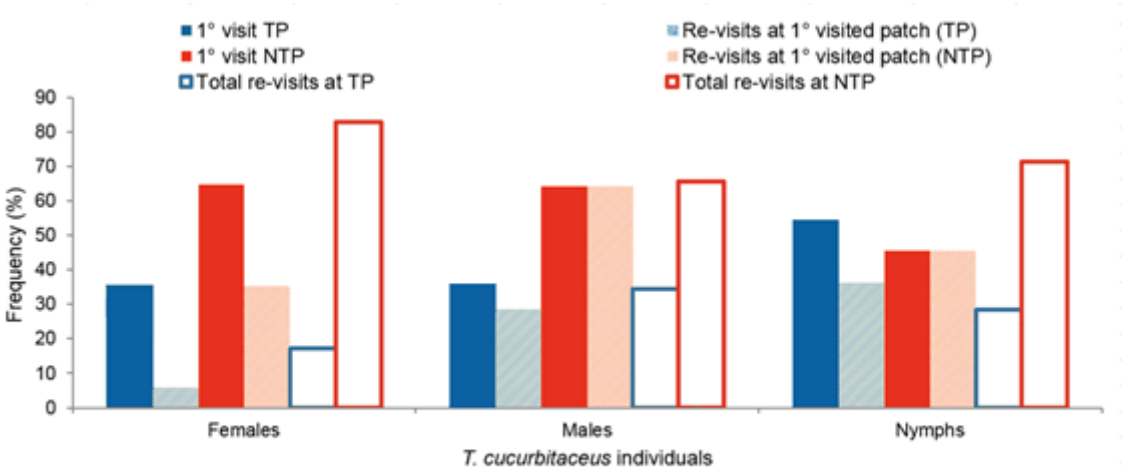

Figure 2

Cumulative time (s) in movement and non-movement of *T. cucurbitaceus* individuals (4-5th instar nymphs, females, and males) in 30 min in an experimental unit with a tomato leaflet and different prey items. The bars indicate the standard error. Different letters show significant differences ($p<0.05$).

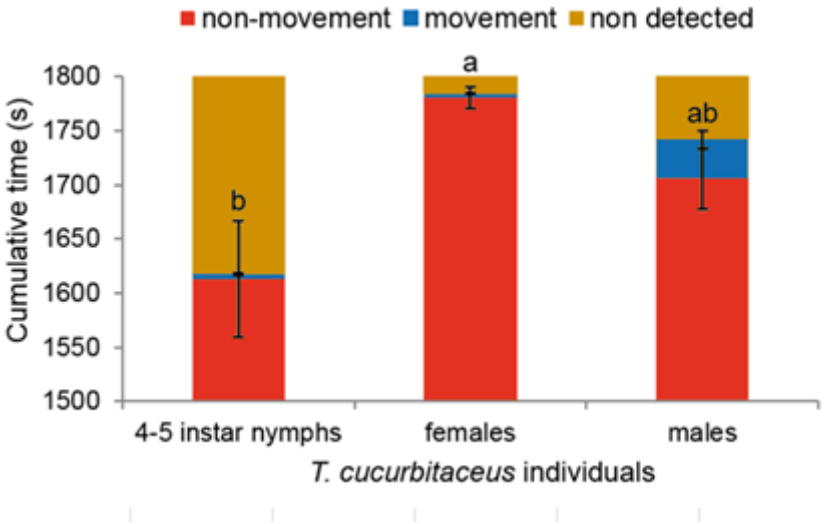

Figure 3

Frequency (%) of first visit, re-visits to the first patch visited, and total revisits to the target patch (*T. absoluta* eggs) and non-target patch (*E. kuehniella* eggs and *T. vaporariorum* nymphs) by individuals of *T. cucurbitaceus* (4-5th instar nymphs, females, and males) in 30 min. TP: target patch, NTP: non-target patch. The bars indicate ±SE. Different letters show significant differences (p-value<0.05).

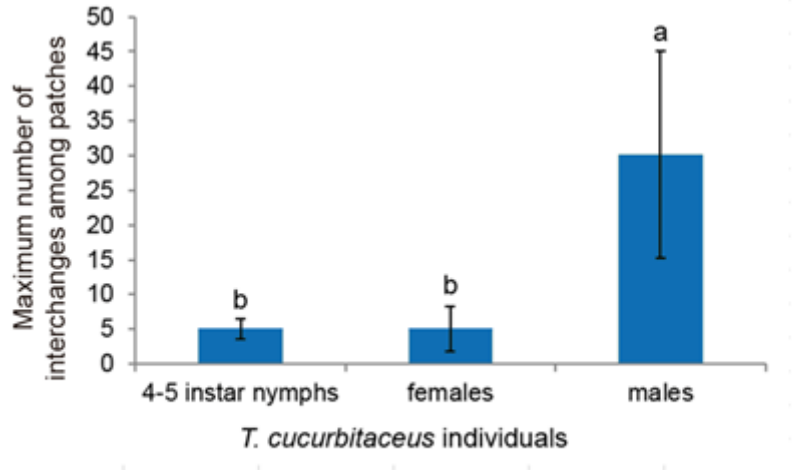

Figure 4

Maximum number of interchanges among patches with *T. absoluta* and *E. kuehniella* eggs or *T. vaporariorum* nymphs, and on the leaflet with no prey (arena), by individuals of *T. cucurbitaceus* (4-5th

instars nymphs, females, and males) in 30 m. The bars indicate ±SE. Different letters show significant differences (p-value<0.05).

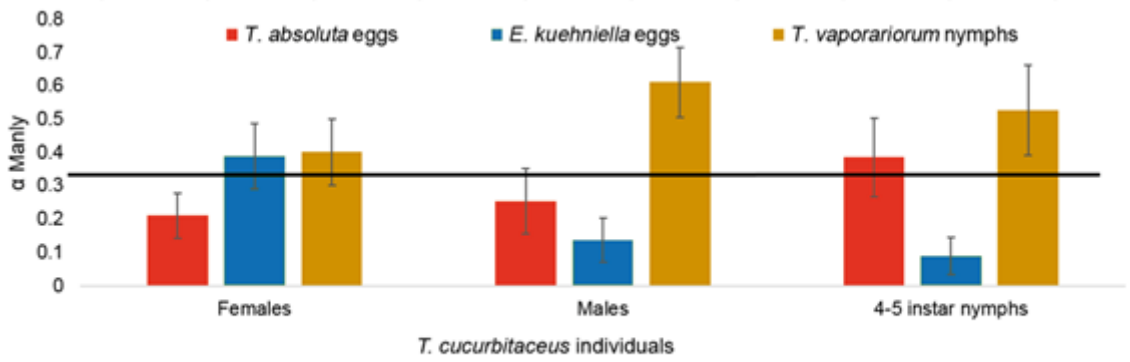

Figure 5

Preference Index (Manly's α) of 4-5th instars nymphs, females, and males of *T. cucurbitaceus* for *T. absoluta* and *E. kuehniella* eggs, and *T. vaporariorum* nymphs, after the first 30 m of the trial. The black line indicates indifference (α= 0.33). The bars indicate ±SE.

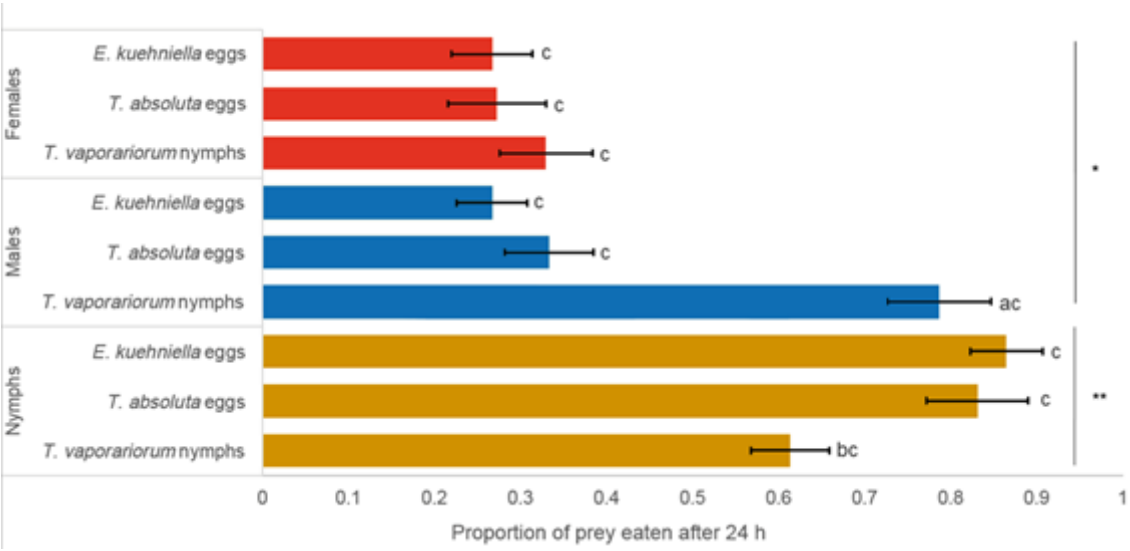

Figure 6

Proportion (mean) of each prey item eaten by *T. cucurbitaceus* individuals (4-5th instars nymphs, females, and males) in 24 h. The bars indicate ±SE. Asterisks denote significant differences between *T. cucurbitaceus* individuals (4-5th instar nymphs, females, and males), and different letters shown significant differences in the interaction (p-value<0.05).