Predatory capacity of *Tupiocoris cucurbitaceus* (Hemiptera Miridae) on several pests of tomato

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Abstract

The predator *Tupiocoris cucurbitaceus* (Spinola) is frequently found preying on whiteflies in greenhouse tomato without pesticide use in Argentina. This study evaluated its preying capacity on several pests, determining the 24 hours consumption of females, males, large and small nymphs on nymphs of *Bemisia tabaci* (Gennadius), eggs and larvae of *Tuta absoluta* (Meyrick), nymphs of *Myzus persicae* (Sulzer) and *Tetranychus urticae* Koch females. Whitefly consumption varied between 15.7 and 38.2 nymphs/predator, the largest predation corresponding to the females of *T. cucurbitaceus* that also were most voracious of *T. absoluta* eggs (147.4) and *M. persicae* nymphs (19.8). *T. cucurbitaceus* individuals preyed 3.3 small larvae of *T. absoluta* while predation of large larvae was negligible for all predator stages. Finally, each predator consumed 27.8 *T. urticae* females. This information broadens our knowledge about the prey range and consumption capacity of this predator.

Key words: predation, Miridae, Bemisia tabaci, Tuta absoluta, Myzus persicae, Tetranychus urticae.

Introduction

Tomato Lycopersicon esculentum Mill. is the most widely field and greenhouse vegetable crop in Argentina, with 15500 ha (Ministerio de Agroindustria, 2017). Among the several pests that affect tomato, the whiteflies Trialeurodes vaporariorum (Westwood) and Bemisia tabaci (Gennadius) (Hemiptera Aleyrodidae), and the tomato leafminer Tuta absoluta (Meyrick) (Lepidoptera Gelechiidae) are considered major due to their serious economic losses. Other species, such as the twospotted spider mite Tetranychus urticae Koch (Acarina Tetranychidae), the green peach aphid Myzus persicae (Sulzer) (Hemiptera Aphididae), thrips (Thysanoptera Tripidae) and leaf miners (Diptera Agromyzidae) cause occasional damages (Mitidieri and Polack, 2012). The use of pesticides against these pests is common. However, this practice is not sustainable and may be ineffective. Therefore, the development of more environmentally friendly control strategies, such as biological control, is strongly recommended.

Tupiocoris cucurbitaceus (Spinola) (Hemiptera Miridae) is a predator found in several American countries: Canada, United States, Mexico, Costa Rica, Panama, Colombia, Ecuador, Brazil, Peru, Chile, Argentina and Uruguay (Carvalho, 1947; Carvalho and Ferreira, 1972; Carvalho and Afonso, 1977). In Argentina, it is present extensively (from 23°S to 43°S) and on a variety of host plants including wild and cultivated Geraniaceae, Rosaceae, Compositae, Fabaceae and Cucurbitaceae species; however, it has preference for Solanaceae species (Carpintero and Carvalho, 1993; Carpintero, 1998; 2004). In particular, this predator is found usually preying on the greenhouse whitefly T. vaporariorum on tomato. As T. cucurbitaceus is easy to rear in laboratory, it is being studied as a possible candidate for inoculative biological control (López et al., 2012; Polack et al., 2017). There is also extensive experience in the use of predatory Miridae to control whiteflies in vegetables crops in other countries (Perdikis *et al.*, 2011). For example, *Macrolophus pygmaeus* (Rambur), *Macrolophus melanotoma* (Costa) (= *Macrolophus caliginosus* Wagner), *Nesidiocoris tenuis* Reuter and *Dicyphus tamaninii* Wagner have been used in pest control management programs in field and greenhouse crops in Europe, mainly to control whiteflies, although they contribute also to control thrips, aphids, spider mites and caterpillars (Perdikis *et al.*, 2008; 2011; Urbaneja *et al.*, 2009; Bouagga *et al.*, 2018).

T. cucurbitaceus is predominantly predatory and although phytophagy can occur in the young nymphal stages, it does not satisfy food requirement for development and reproduction (Orozco Muñoz *et al.*, 2012). López *et al.* (2012) examined some basic biological characteristics and the predatory capacity of *T. cucurbitaceus* on *T. vaporariorum* nymphs and *Sitotroga cerealella* Olivier (Lepidoptera Gelechiidae) eggs as preys, and found that females are more voracious than males and large nymphs, being able to prey about 32 3rd-4th instars of *T. vaporariorum* in 24 hours. So far, there is no published evidence that *T. cucurbitaceus* can prey on other tomato insects. This paper studied the predatory capacity of *T. cucurbitaceus* on four important pests of this crop.

Materials and methods

Sources of insects

Adults and nymphs of *T. cucurbitaceus* used were obtained from colonies reared in the Insectario de Investigaciones para Lucha Biológica (IILB), Instituto de Microbiología y Zoología Agrícola (IMYZA), Instituto Nacional de Tecnología Agropecuaria (INTA), Castelar, Argentina, collected originally in 2009 from greenhouse tomato in La Plata, Buenos Aires province

(34°59'08"S 58°05'15"W). Since then, the colony was refreshed each spring-summer with about 50 insects from local greenhouses. The species was identified by Diego Carpintero from Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina. Following previous experiences on mirid rearing by Agustí and Gabarra (2009), the predator was maintained on tomato and tobacco plants and fed on *S. cerealella* eggs, at 25 ± 5 °C, $65 \pm 15\%$ RH and 14L:10D. Specimens of *B. tabaci*, *T. absoluta*, *M. persicae* and *T. urticae* were also obtained from IILB laboratory rearings maintained on tomato plants. Voucher specimens were preserved in the IILB collection.

The studies were carried out in an environmental controlled chamber at 25 ± 1 °C, $45 \pm 5\%$ RH and 14L:10D photoperiod.

General procedure

Prey consumption was evaluated for four *T. cucurbi-taceus* stages, 2nd-3rd instar (small) nymphs, 4th-5th (large) nymphs, and female (identified by observing the ovipositor and a thicker abdomen compared to males on a stereoscopic microscope) and male adults.

One tomato leaflet ($\sim 3 \times 2$ cm) sustaining the prey was set on a 9 cm diameter and 1.5 cm deep Petri dish lined with filter paper. One predator was set on each arena for 24 hours. Mirid nymphs and adults were only given water for 24 hours before the tests. After exposure, the numbers of live and dead preys (including those with their bodies partly or completely emptied) were recorded under a stereo microscope. There were 8-10 replicates per predator stage and 10 arenas with no mirids as controls. The consumption of the following preys was evaluated in separate experiments:

Bemisia tabaci nymphs

A tomato leaflet with ~100 3rd-4th instars of *B. tabaci* was exposed to each *T. cucurbitaceus* specimen. Tomato seedlings (2-3 leaves, ~20 cm high) were exposed to ~400 *B. tabaci* adults to obtain the whitefly nymphs, into a 21 cm diameter × 40 cm high acetate cage, with the top covered with muslin, for egg-laying during three days. The exposure was made 14 days before the consumption test to ensure the presence of 3rd-4th instar nymphs of *B. tabaci*. The plants were maintained at 25 ± 3 °C, $60 \pm 10\%$ RH and natural light until they were used.

Tuta absoluta eggs

Tomato moth eggs (24-48 hours old) were set on the surface of the tomato leaflet with a fine brush under a stereo microscope. *T. cucurbitaceus* nymphs and males were exposed to 100 eggs, while females to 200 eggs, based on a preliminary test where females had a greater consumption.

Tuta absoluta larvae

Twenty (20) small larvae $(1^{st}-2^{nd} \text{ instar})$ were set on the tomato leaflet and exposed to *T. cucurbitaceus* belonging to one of the four life stages. Predation on large larvae $(3^{rd}-4^{th} \text{ instar})$ of *T. absoluta* was evaluated following the same procedure in a separate test.

Sixty 1st-3rd instar *M. persicae* nymphs were set on the tomato leaflet and exposed to a *T. cucurbitaceus* predator in each replicate.

Tetranychus urticae adults

T. cucurbitaceus small nymphs were exposed to 50 *T. urticae* mites, while large nymphs, males, and females to 60, based on a preliminary test where the early instar nymphs had a lesser consumption.

Statistical analysis

To estimate the preys consumed, mortality recorded for *T. absoluta* small larvae, *M. persicae* nymphs and *T. urticae* adults was corrected substracting the mean mortality in the controls. In *B. tabaci* nymphs and *T. absoluta* eggs, the mortality in the controls were negligible (< 0.5 individuals/replicate) and consequently these data were not corrected.

Differences in predation items among *T. cucurbitaceus* stages were analysed using a one-factor ANOVA for *T. absoluta* eggs and larvae, and for *M. persicae* nymphs. Means were compared by the Tukey test at the 0.05 level. Since their data did not meet the assumptions of normality and homoscedasticity, *B. tabaci* and *T. urticae* consumptions were analysed using a Kruskal-Wallis test. A post-hoc comparison of the means was performed using Dunn's method at $P \le 0.05$. Statistica for Windows (StatSoft, 2000) was used for the analysis. The results are presented as means \pm standard error.

Results

Bemisia tabaci nymphs

A general trend with a greater consumption for females was observed, with intermediate values for males/large nymphs, and lesser predation levels for small nymphs. The greatest number of nymphs consumed corresponded to female *T. cucurbitaceus*, preying 38.20 ± 4.65 nymphs of *B. tabaci*. This value differed significantly from the consumption of small predator nymphs (15.7 ± 4.34 whitefly nymphs). The level of consumption of male and large nymphs was intermediate and not significantly different from those of the other predator stages (Kruskal-Wallis test = 8.76; p = 0.033) (table 1).

Tuta absoluta eggs

Significant differences in predation of tomato moth eggs occurred between stages/sexes of *T. cucurbitaceus*. Predator females were the most voracious $(147.45 \pm 9.48 \text{ eggs})$, while male and large nymphs preved more (on average 68.88 ± 3.95 eggs) than small nymphs (26.80 ± 10.78 eggs) (F = 39.02, DF = 3.36; P < 0.01) (table 1).

Tuta absoluta larvae

No significant differences in predation of small larvae of *T. absoluta* occurred between stages/sexes of *T. cucurbitaceus* (F = 1.25; DF = 3.36; p = 0.30). On average, *T. cucurbitaceus* individuals preyed 3.36 small larvae in 24 hours (table 1).

Prey	Stage of <i>T. cucurbitaceus</i>			
	Female adult	Male adult	Large nymph	Small nymph
<i>B. tabaci</i> nymphs	38.2 ± 4.6 a	28.8 ± 3.9 ab	30.9 ± 9.0 ab	15.7 ± 4.3 b
T. absoluta eggs	147.4 ± 9.5 a	$64.4 \pm 3.6 \text{ b}$	72.6 ± 5.9 b	$26.8 \pm 10.8 \text{ c}$
T. absoluta small larvae	3.6 ± 1.1 a	1.9 ± 0.9 a	4.6 ± 1.1 a	3.3 ± 0.7 a
<i>M. persicae</i> nymphs	19.8 ± 2.7 a	12.8 ± 2.3 ab	13.0 ± 2.0 ab	$7.2 \pm 1.8 \text{ b}$
T. urticae females	25.9 ± 2.1 a	28 ± 3.3 a	27.0 ± 4.6 a	30.1 ± 1.3 a

Table 1. Prey consumption by different stages of *T. cucurbitaceus* (mean \pm standard error). Means followed by different letters in each row are significantly different (Kruskal-Wallis test and ANOVA, P < 0.05).

Very few predators (6 out of 36) were able to prey on large *T. absoluta* larvae, and when they did, it was in very low numbers. The average consumption was less than 1 larva/bug/24 hours (from 0.08 for small nymphs to 0.36 for female *T. cucurbitaceus*), producing data not analysed statistically.

Myzus persicae nymphs

Females of *T. cucurbitaceus* were significantly more voracious on *M. persicae* (19.75 \pm 2.70) than small nymphs (7.25 \pm 1.83). Consumptions of males and large nymphs were intermediate and did not differ from that of the other stages/sexes (12.49 \pm 1.47 aphids/predator) (F = 5.33; DF = 3.28; p < 0.0049) (table 1).

Tetranychus urticae adults

All life stages of *T. cucurbitaceus* preyed a similar level of *T. urticae* female adults (27.75 ± 1.51 spider mites/predator) (Kruskal-Wallis test = 2.84; p = 0.42) (table 1).

Discussion

Our results indicate that *T. cucurbitaceus* can prey on different species that affect tomato. The pattern of predation of *T. cucurbitaceus* on *B. tabaci* was similar to that registered on *T. vaporariorum* nymphs and *S. cerealella* eggs (López *et al.*, 2012). Likewise, Bonato *et al.* (2006) observed that the stage of development of *M. caliginosus* influenced the consumption rate of whitefly nymphs being the female adults more voracious due to their reproductive requirements.

The consumption of *B. tabaci* nymphs by *T. cucurbitaceus* females was slightly greater to that when fed on *T. vaporariorum* nymphs, and much greater for males and large nymphs (32 nymphs/female) (López *et al.*, 2012). The physical and chemical characteristics of both whitefly species (*e.g.* nutritional content, cuticular lipids, size, shape, setae) could explain these differences.

No other records in the literature exist of *B. tabaci* predation by other predacious Miridae with a Neoptropical origin. When compared to Palearctic mirids, *T. cucurbitaceus* had herein a greater daily consumption of *B. tabaci* nymphs than that registered by Barnadas *et al.* (1998) for the nymphs and adults of *D. tamaninii* Wagner (4.1 and 6.7 nymphs, respectively) and *M. caliginosus* (2.7 and 4.5 nymphs, respectively). In addition, it was similar to the predation rate recorded by Bonato *et al.* (2006) for *M. caliginosus* nymphs (about 24)

nymphs) and Ziaei Madbouni *et al.* (2017) for *N. tenuis* females (34.40 nymphs).

Herein, the capacity of T. cucurbitaceus to prey on T. absoluta is reported for the first time to the best of our knowledge. We found that this mirid preyed successfully on eggs of the tomato moth, being the females the most voracious stage/sex. Consumption recorded in our study exceed levels registered by van Lenteren et al. (2016; 2017) for the females of the Neotropical mirids Campyloneuropsis infumatus Carvalho (51 eggs), Engytatus varians (Distant) (91.1 eggs) and Macrolophus basicornis (Stal) (100.8 eggs), and by Arnó et al. (2009) for M. pygmaeus and N. tenuis (about 110 eggs). They were also considerably greater than the predation rates recorded by Urbaneja et al. (2009) for M. pygmaeus and N. tenuis adults (about 55 eggs), and by Ingegno et al. (2013) for Dyciphus errans (Wolff) (about 10 eggs), although these authors offered a limited number of eggs to each adult predator. Large nymphs of T. cu*curbitaceus* preyed more eggs of *T. absoluta* than the 5th instar nymph of M. pygmaeus (35.70 eggs) and N. tenuis (50.60 eggs) (Michaelides et al., 2018) although, according to Arnó et al. (2009), small nymphs of these European mirids had a greater consumption (> 50 eggs) compared to T. cucurbitaceus.

The existing records on consumption of lepidopteran eggs by *T. cucurbitaceus* (López *et al.*, 2012) report 29 preyed eggs of *S. cerealella*/female and 16.3 eggs/male or nymph on tomato in 24 hours period. Burla *et al.* (2014) found that *T. cucurbitaceus* preyed 137 eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera Pyralidae) over its entire lifespan, with a daily consumption never greater than 15 eggs/individual predator. In a first instance we may explain this difference in predation rates by the smaller size of the *T. absoluta* egg (0.36×0.22 mm) (EPPO, 2005) as compared to that of *S. cerealella* (0.51×0.29 mm) and *E. kuehniella* (0.60×0.27 mm) (Arbogast *et al.*, 1980). However, factors associated with the nutritional value of each of these lepidopteran eggs might also play a role.

T. cucurbitaceus preyed also on the smaller larvae $(1^{st} \text{ and } 2^{nd} \text{ instars})$ of *T. absoluta*, without differences in larval predation between sexes or life stages. Consumption of large larvae was negligible. The same pattern was found by Urbaneja *et al.* (2009) and Ingegno *et al.* (2013) for *M. pygmaeus*, *N. tenuis* and *D. errans*, with a preference for the smallest larvae of *T. absoluta* because they are less mobile and smaller in size than the large ones. *T. cucurbitaceus* consumed 3.3 larvae for every 20 larvae offered. This rate was similar to that recorded by

Ingegno *et al.* (2013) for *D. errans*, and greater than the consumption rates of *M. pygmaeus* and *N. tenuis* (Arnó *et al.*, 2009; Urbaneja *et al.*, 2009). In these last two cases, the adult specimens were exposed to a limited number of larvae (5) established inside their galleries, a fact that could have diminished the consumption rate of the predators. On the contrary, in our work larvae were offered to predators set on the leaves, not inside galleries, increasing their exposure to predation and consequently their mortality.

Several studies have revealed the predation capacity that some mirids have on aphids. Species such as M. pygmaeus, M. caliginosus, N. tenuis and D. tamaninii have been reported preying on M. persicae, Aphis gossypii Glover and Macrosiphum euphorbiae (Thomas) (Alvarado et al., 1997; Perdikis et al., 1999; Castañé et al., 2002; Moayeri et al., 2006; Valderrama et al., 2007; Maselou et al., 2015; 2018). Herein, T. cucurbitaceus also preyed on nymphs of M. persicae. Females fed on almost 20 aphids, a value similar to those registered by Messelink et al. (2015) and Maselou et al. (2015) for M. pygmaeus and D. tamaninii, and greater than that by Perdikis et al. (1999) for M. pygmaeus, with *M. persicae* as prey in all the cases. On the other hand, T. cucurbitaceus consumption was lesser than that recorded for M. caliginosus and D. tamaninii on A. gossypii and M. euphorbiae, and for N. tenuis preying M. persicae (Alvarado et al., 1997; Valderrama et al., 2007). In the case of large nymphs of T. cucurbitaceus, their consumption was equivalent to that of M. pygmaeus, M. caliginosus and D. tamaninii (Alvarado et al., 1997; Perdikis et al., 1999; Maselou et al., 2018). Finally, small nymphs of T. cucurbitaceus preyed more *M. persicae* nymphs than $2^{nd}-3^{rd}$ instar nymphs of *M. pygmaeus* (Perdikis *et al.*, 1999).

The average number of T. urticae adults killed by T. cucurbitaceus was 27.75, similar to that recorded by McGregor et al. (1999) for Dicyphus hesperus Knight when exposed to 30 mite adults. Contrary to whiteflies, aphids and moth eggs, there was no difference in mortality cause by different life stage/sexes of the predator. However, when T. urticae females were exposed to small nymphs of T. cucurbitaceus, they were not all fully or partially consumed. From 46 to 73% of them were killed but not consumed, i.e., leaving the full internal content of their bodies. The appearance of these dead mites was clearly differentiable from those naturally dead, which were in some cases desiccated or collapsed. This result would indicate that the small nymphs of T. cucurbitaceus could have had some difficulty to handle this mite prey, hurting some to the point of killing them but without feeding on them. Fantinou et al. (2008) recorded this kind of predatory behaviour of killing without consumption for M. pygmaeus, and attributed it to the predator's difficulty in seizing large preys.

Mirid predators that show both phytophagy and zoophagy can alternate preys with plant food, and thus survive if one of these is scarce. Since *T. cucurbitaceus* has a limited phytophagy (Orozco Muñoz *et al.*, 2012), the presence of some kind of prey seems to be determinant for the suitable performance of this predator as a biological control agent. Its proper establishment in the crop will require synchronizing its introduction with the presence of the prey, or alternatively adding some nutritional supplement in the field (such as lepidopteran eggs) if the pest is scarce. Thus, the ability of *T cucurbitaceus* to eat different types of prey can be considered positive, since it will be able to choose for other sources of food apart from whiteflies, thus contributing to maintaining its own population and to control other pests.

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