

Bioassay

Companion plants for conservative management of *Tupiocoris cucurbitaceus* (Spinola 1852) (Heteroptera: Miridae: Dicyphini) on greenhouse tomato crops

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Abstract. In recent decades, greenhouse crops relevance increased due to the high demand for products outside their growing season, with tomato standing out as one of the most cultivated crops. In these production systems, insects and mites find optimal conditions for their development, achieving high populations that affect crops. Farmers usually control these populations using chemical insecticides, which affect the health of workers and consumers and have negative effects on the environment. Tomato crops suffer damage by lepidopterans and hemipterans, among which those known as whiteflies (Hemiptera: Aleyrodidae) perform regular attacks, causing yield and quality losses in the final product. Currently, the use of zoophytophagous predators of the Miridae family, Dicyphini tribe, as an alternative for their biological control, has intensified studies on *Tupiocoris cucurbitaceus* (Spinola, 1852), a predator of several species of aphids and whiteflies found in Uruguay. A strategy for the conservative management of those species was designed, using companion plants grown together with the crop. By the time the tomato plants were transplanted, *Calendula officinalis*, *Smallanthus connatus*, *Tithonia rotundifolia*, *Nicotiana tabacum*, *Physalis peruviana* and *Petunia hybrida* plants were established as companion species. These plants were monitored weekly together with the tomato plants, and a greater presence of *T. cucurbitaceus* in the crops with companion plants was found. The results suggest that the incorporated plants were useful for the preservation and retention of predaceous mirids in productive conditions.

Keywords: Whitefly, Aphids, Mirids, Conservative biological control.

In Uruguay, greenhouse production has increased by 41% in the past decade, reaching 500 hectares, being tomato one of the most prominent crops, with 150 hectares in the north and 87 hectares in the south of the country (MGAP DREA 2017). In this production system, attacks by *Trialeurodes vaporariorum* (Westwood, 1856) (Hemiptera: Aleyrodidae) and *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) whiteflies lead to relevant yield losses and to the application of insecticides by producers (Rodríguez & Cardona 2001). Additionally, tomato greenhouse crops provide suitable conditions that favor tomato leafminer *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) as the main crop pest (Desneux et al. 2010).

Globally, the use of biocontrol agents has intensified, wherein the use of zoophytophagous predators of the Miridae family, Dicyphini tribe, stands out (Pérez-Hedo & Urbaneja 2016). Periodic releases of these beneficial insects represent the most widely used strategy in biocontrol programs in tomato greenhouses (Van Lenteren 2012). The use of this type of predators increases the sustainability of biological control programs through greater persistence and ease of establishment, due to a wider range of prey and their feeding on plants in the absence of prey (Symondson et al. 2002).

The use of companion plants seems to provide oviposition and feeding sites useful for predaceous mirids (Parolin et al. 2012). In the Mediterranean region, predatory mirid bugs frequently migrate from spontaneous vegetation surrounding the crops into greenhouses, thus contributing to pest control (Castañé et al. 2004; Ingegno et al. 2009; Perdikis et al. 2011).

In Uruguay and other countries from the neotropical region, the presence of *Tupiocoris cucurbitaceus* (Spinola, 1852) (Hemiptera: Miridae) feeding on various species of insects and mites has been reported. This interaction has been observed on different plants such as

Rubus odoratus (Rosaceae), *Phaseolus vulgaris* (Fabaceae), *Cucurbita* sp. (Cucurbitaceae), *Matricaria chamomilla* (Asteraceae), *Smallanthus connatus* (Asteraceae), *Urolepis hecatantha* (Asteraceae), *Geranium* spp. (Geraniaceae), *Pelargonium hortorum* (Geraniaceae), *Physalis peruviana* (Solanaceae), *Petunia hybrida* (Solanaceae), *Solanum tuberosum* (Solanaceae), *Lycopersicon esculentum* (Solanaceae) and *Nicotiana tabacum* (Solanaceae) (Ferreira et al. 2001; Bado et al. 2005; Logarzo et al. 2005). These plants may be a useful reproduction and/or feeding resource in the absence of prey, although no quantitative information on their use by *T. cucurbitaceus* is available.

Based on the potential of *T. cucurbitaceus* as predator of pests affecting tomato crops (López et al. 2012; Polack et al. 2017), in this paper it is hypothesized that plants used by *T. cucurbitaceus* may be useful as companion plants for the preservation of this predator. Their apparent attraction, alternative feed source and oviposition sites could promote the natural regulation of insects attacking the crop. The aim of this work was to assess the effect of incorporating companion plants on the population of *T. cucurbitaceus* in tomato greenhouses. Specifically, we evaluated 1) the effect of the presence of *T. cucurbitaceus* on tomato insect pests, 2) whether *T. cucurbitaceus* uses companion plant species differentially and 3) whether there is any effect on *T. cucurbitaceus* when companion plants are preserved over two seasons. Additionally, species of spontaneous vegetation growing around greenhouses and used by *T. cucurbitaceus* were identified.

Nine wooden and Poly Film tomato greenhouses, of 500 m² on average, located in grassland matrix areas of Rocha, Castillos, and La Paloma in the department of Rocha (Uruguay), were selected. Early (September to March) and late (January to May) tomato crops, all grown in mollic soil, were chosen for the experiment. The horticultural farms where the experiment was carried out have

the following locations: Rocha: 34°28'44.99"S 54°21'11.21"O, 34°28'49.70"S 54°21'15.82"O, Castillos: 34°11'28.09"S-53°51'6.79"O, 34°11'42.04"S-53°50'45.92"O, 34°10'16.81"S 53°52'52.52"O, and La Paloma: 34°36'21.05"S-54°10'1.52"O.

The companion plants were seeded in a Medityr SMP 250 growth chamber, kept at 24.6 ± 0.2 °C, $45 \pm 3\%$ HR and 12:12 h (L:D), then transplanted into 300 cm³ pots with Jiffy® substrate and placed in cages with tulle mesh in a greenhouse located at Centro Universitario Regional del Este (CURE). At the time of tomato crop transplantation, three species of Asteraceae: *Calendula officinalis*, *S. connatus*, *T. rotundifolia* and three species of Solanaceae: *N. tabacum*, *P. peruviana* and *P. hybrida* were established as companion species, directly in the soil, in the same manner as the crop, in nine greenhouses. Companion plants were distributed in bushes, every four meters, on an alternate basis, following the central row of columns and at the corners within the greenhouses. The usual management of the producer without companion plants was carried out in two greenhouses as a control treatment. After transplantation, the application of conventional fungicides and insecticides was restricted in the greenhouses with companion plants, in order not to interfere with the spontaneous colonization of predatory mirid bugs.

Population levels of different arthropods at each greenhouse were assessed weekly during two growing seasons, through visual observation and counting. As the upper strata of the plants are dominated by whitefly adults, and being the easiest stage to count (Basso et al. 2001), the abundance of these insects in the two most recent fully developed leaves in 20 random tomato plants from each greenhouse was registered. The presence or absence of *T. cucurbitaceus* in each plant was recorded. In the same plants, the presence of other phytophagous insects and beneficial insects (predators and parasitoids) was also registered by searching the whole tomato plant (Mitidieri & Polack 2012). The presence of *Misumenops pallidus* (Keyserling, 1880) (Araneae: Thomisidae) both in the crop and companion plants was also assessed, because this spider has been reported to have a certain feeding preference for Miridae (Cheli et al. 2006).

During the first three months following the transplantation of the crop, samplings directed to mirids were carried out with manual aspirator on specimens of spontaneous vegetation surrounding the greenhouses. For the purpose of detecting new refuges for predatory mirid bugs, Cucurbitaceae, Solanaceae and Asteraceae plants were monitored, as these families were identified as preferred by *T. cucurbitaceus* (Ferreira et al. 2001; Bado et al. 2005; Logarzo et al. 2005). Samples were labeled and preserved in alcohol 70°, for subsequent identification in the laboratory of the specimens found.

We adjusted three different statistical models to assess 1) the effect of *T. cucurbitaceus* on whitefly abundances in tomato plants, 2) which companion plant species was most used by *T. cucurbitaceus* and aphids, and 3) whether there were any differences in *T. cucurbitaceus* relative abundance between greenhouses that kept companion plants for two seasons vs. greenhouses that kept companion plants for only one season.

To assess whether the presence of *T. cucurbitaceus* affected the abundance of whitefly in tomato plants (model 1) we fitted a generalized linear model using the abundance of whitefly in the total sampled plants for each date and greenhouse as the response variable and the presence of *T. cucurbitaceus* in each date and greenhouse as the explanatory variable (i.e., if at least one of the 20 plants was used by *T. cucurbitaceus* we recorded it as "presence"). This model was fitted using a negative binomial probability distribution.

To assess which companion plant species was most used by *T. cucurbitaceus* (model 2) we used the proportion of plants occupied by the insect as a response variable, in each date and greenhouse. As it is a proportion, we fitted a generalized linear model with beta distribution. Then, to detect differences between plant species, we compared paired means through Tukey's test. We followed the same procedure to assess if aphids used companion plant species differentially. Moreover, to assess the effect of the proportion of tomato plants with *T. cucurbitaceus* on the proportion of plants with *M. pallidus* and *Jalysus* sp., we fitted a beta regression as well.

To assess if there were differences in *T. cucurbitaceus* relative abundance between greenhouses that kept companion plants for two seasons vs. greenhouses that kept companion plants for only one season (model 3), we used data from two greenhouses that kept companion plants for two seasons. In this model we compared the proportion of plants with *T. cucurbitaceus* in year 2 of the aforementioned greenhouses with the proportion of plants with *T. cucurbitaceus* in the rest of the greenhouses (i.e., those that kept companion plants for only one year). For that purpose, we fitted a generalized linear model with beta distribution (Douma et al. 2019).

Analyses were carried out using the software R (R Core Team 2021). The regression models with beta probability distribution were fitted with the glmm TMB library (Brooks et al. 2013). Due to the presence of zeros, in order to use beta distribution, the scale of the response variable was changed using the correction proposed by Smithson & Verkuilen (2006).

The most abundant phytophagous insects observed were *T. vaporariorum*, *T. absoluta*, *Edessa meditabunda* (Fabricius, 1794) (Hemiptera: Pentatomidae) and the aphids complex of the genus *Myzus* sp. (Hemiptera: Aphididae). Among the beneficial insects, the following parasitoids were found: *Encarsia formosa* (Gahan, 1924) (Hymenoptera: Aphelinidae) on whitefly on *S. connatus* and *P. peruviana* plants; *Trissolcus* sp. (Hymenoptera: Scelionidae) on egg masses of *E. meditabunda* on *N. tabacum* plants; and *Aphidius colemani* (Dalman, 1820) (Hymenoptera: Braconidae) parasitizing aphids on *C. officinalis*. Specimens of the genus *Jalysus* sp. (Heteroptera: Berytidae) were found feeding on mirids, and *Tupiocoris chlorogaster* (Berg, 1878), *Campyloneuropsis cincticornis* (Stål, 1860), *Engytatus varians* (Distant, 1884) and *T. cucurbitaceus* were also registered.

The presence of *T. cucurbitaceus* was registered in all the greenhouses with companion plants. Additionally, whitefly counts were significantly lower in plants with presence of *T. cucurbitaceus* ($p=0.000342$) (Fig. 1A). The rate of tomato plants with presence of *T. cucurbitaceus* was significantly higher in greenhouses after the second year of management ($p=0.00819$), (Fig. 1B). No significant reduction of damage by *T. absoluta* on tomato plants was observed in the situations with the higher rate of plants with *T. cucurbitaceus* ($p=0.174$).

Aphid colonies found on plants of *C. officinalis* (Calendula), *S. connatus* (Smallanthus) and *N. tabacum* (Tobacco) were significantly greater than on the crop ($p=0.001$) (Fig. 2A). The plant type had a significant effect on the proportion of plants occupied by *T. cucurbitaceus* ($z=22.38$, $p<0.001$). The highest probability of occurrence of *T. cucurbitaceus* was verified on companion plants of the species *S. connatus* (Smallanthus) and *N. tabacum* (Tobacco), compared to tomato plants (Tobacco vs. Tomato: $z=-4.784$, $p<0.01$; Smallanthus vs. Tomato: $z=-2.544$, $p<0.05$). The other companion species did not show differences with the tomato crop (Fig. 2B).

Feeding of the spider *M. pallidus* on *T. cucurbitaceus* was confirmed. This species could contribute to the regulation of the population of these zoophytophagous mirids, which may cause damage to the crop when present in high densities and scarcity of prey. In fact, the rate of *M. pallidus* increased significantly as the rate of *T. cucurbitaceus* increased ($p<0.001$). A similar behavior and response were registered for *Jalysus* sp, which significantly increased its presence when the rate of plants with *T. cucurbitaceus* increased ($p=0.00115$).

Finally, in the spontaneous vegetation surrounding the crops, *T. cucurbitaceus* was recorded in *Senecio selloi* (Asteraceae), *Cleoeme* sp., (Capparaceae) and *Solanum sarrachoides* (Solanaceae), these being new records of host plants for the species, which may be a useful resource for its conservation.

The lesser number of whiteflies associated to higher abundance of *T. cucurbitaceus* (i.e., the predator) in tomato with companion plants could be explained by the direct predatory action as well as by indirect effects. Firstly, some Dicyphini mirids can trigger the synthesis of defensive substances in tomato plants, which enable them to repel the whitefly and the tomato leafminer. Additionally, these substances may attract parasitoids such as *E. formosa* (Pérez-Hedo et al. 2015). These herbivores induced plant volatiles (HIPVs) have been identified after the action of species such as *Nesidiocoris tenuis* (Reuter, 1885) or

recently *Macrolophus basicorni* (Stål, 1860) (Miridae: Dicyphini). After feeding activation, various metabolic pathways such as abscisic acid, salicylic acid and jasmonic acid make plants unattractive for whiteflies and attractive for conspecific predators and hymenopteran parasitoids (Pérez-Hedo et al. 2015; Silva et al. 2022).

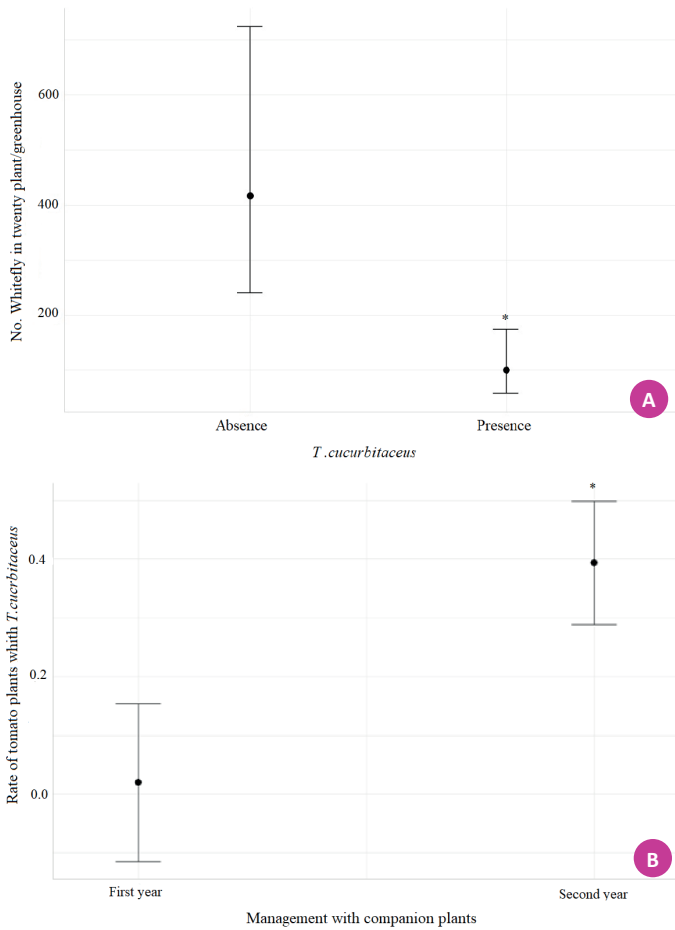


Figure 1. (A) Total number of whiteflies in 20 plants per greenhouse in the absence and presence of *T. cucurbitaceus*, (B) Proportion of tomato plants with *T. cucurbitaceus* in greenhouses on first and second years of management with companion plants (Where confidence intervals do not overlap treatments are different * $p < 0.001$).

It has also been found that plants activated by the feeding of the Miridae may trigger responses of the metabolic pathway of jasmonate in neighboring plants (Pérez-Hedo et al. 2021). In this sense, companion plants such as tobacco could play an important role in inducing resistance in neighboring plants and recruiting predators.

Furthermore, the pruning of tobacco allows this species to behave as biannual, standing out as the main refuge during periods without tomato crops in the greenhouses. In contrast, the other companion species have strictly annual cycles, thus representing a lesser potential as oviposition and conservation sites for *T. cucurbitaceus*.

The higher proportion of colonies of aphids on Calendula, Tobacco and Smallanthus compared to tomato crops would enable their consideration as trap plants. Additionally, in these plants *T. cucurbitaceus* was found more frequently and can be therefore considered as insectary plants (Parolin et al. 2012). Since these plants can be alternative food sources and oviposition sites for *T. cucurbitaceus*, they could be considered in future conservation biological control programs.

The results highlight the role of Tobacco, Calendula and Smallanthus as companion plants in the generation of niches to preserve and retain beneficial insects, since their incorporation into greenhouses was useful for the preservation and refuge of the predaceous mirid *T. cucurbitaceus*.

The use of these companion plants decreased the incidence of aphids and whiteflies on the crop, and could therefore contribute to the regulation of these insects in ecological production systems.

The maintenance of companion plants during the winter period enabled the preservation of *T. cucurbitaceus* and a significantly higher colonization of this predator on the crop in the subsequent production cycle.

The presence of *T. cucurbitaceus* observed in species of the spontaneous vegetation surrounding greenhouses makes it possible to consider these plants as additional potential resources for their inclusion in conservative management systems.

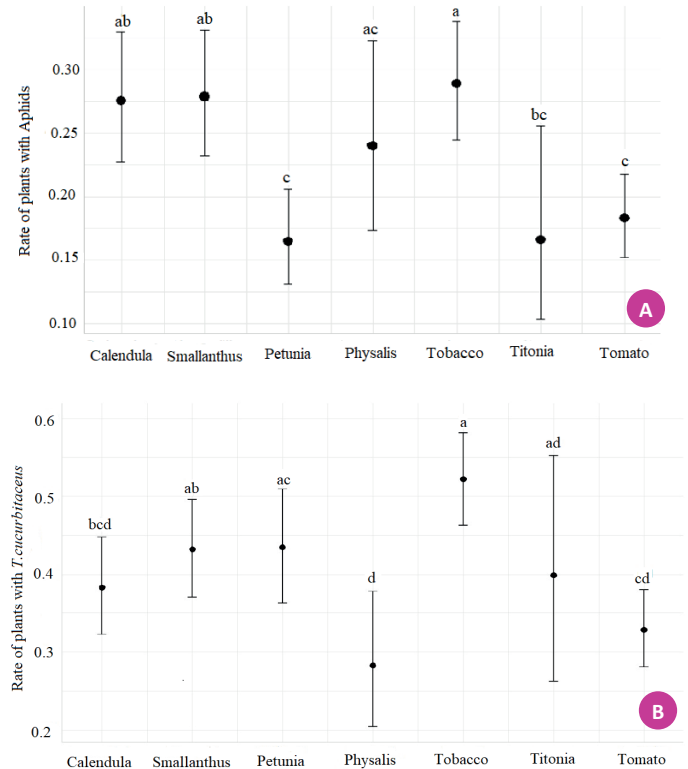


Figure 2. (A) Rate of companion and tomato plants with presence of aphids, (B) Occurrence of *Tupiocoris cucurbitaceus*, expressed as a rate, in the different plant species. Plants species sharing the same letters denote a lack of statistical difference between them.

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Authors' Contributions

JPB: Design of the proposal, production of companion plants, field trips, processing of samples in the laboratory, data processing and drafting of the paper. NA: Field trips, review and proofreading of the paper. JA: Statistical analysis and graphic illustrations. CF: Identification of plants, and EC: Design of the proposal, review and proofreading of the paper.

Conflict of Interest Statement

The authors declare no potential conflict of interest.

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