Preference of Orius insidiosus and Orius tristicolor (Hemiptera: Anthocoridae) for Host Plants in Olfactometry and Free-Choice Experiments

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Preference of *Orius insidiosus* and *Orius tristicolor* (Hemiptera: Anthocoridae) for host plants in olfactometry and free-choice experiments

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Abstract

The western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is considered one of the most important pests of greenhouse crops (Gillespie & Vernon 1990; Salguero Navas et al. 1991; Bitterlich & McDonald 1993), limiting the productivity of these crops. *Frankliniella occidentalis* and other thrips species feeding damage, excretes phytotoxic substances, and transmits important viruses. Control with chemical insecticides often is ineffective because endophytic oviposition and the cryptic habits of the pest provide protection. In Uruguay, the biological control program of this pest in sweet pepper crops is at risk due to the low settlement rate and high dispersal of releases of predator *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). Previous studies have ruled out an antibiosis effect as the cause of dispersal; therefore, we hypothesized antixenosis (non-preference) for the sweet pepper plants as the cause of poor biological control by *O. insidiosus*. The effect of olfactory stimuli from different structures of strawberry, corn, and sweet pepper plants (sumuco and blocky type) on the behavior of *O. insidiosus* was evaluated in olfactometry and free-choice cage experiments. Since *Orius tristicolor* (White) (Hemiptera: Anthocoridae) occurs naturally in the area, it was included also in the study with the aim of assessing whether there are differences in behavior between the species. *Orius tristicolor* may act as a complementary biocontrol agent or competitor on sweet pepper. Y-tube experiments showed no preference for plant volatiles in any combination, and response to volatile stimuli generally was poor. However, in the free-choice cage experiment, females of both species of *Orius* preferred the flowering strawberry plants over the flowering sweet pepper plants, which could explain the low establishment of *O. insidiosus* when released on pepper with neighboring strawberry fields. Given that horticultural greenhouses in Uruguay and in many other countries are open, this information can be very useful in designing the spatial and temporal management of different crops on a production field, which enhances the effectiveness of these predatory species.

Key Words: thrips; antixenosis; plant volatile; sweet pepper; strawberry

Resumen

El trips occidental de las flores, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), es una plaga clave para el cultivo de pimiento dulce, donde causa daños al alimentarse, excreta sustancias fitotóxicas y transmite importantes virus. El control con insecticidas químicos no resulta efectivo, debido a la oviposición endófita y los hábitos cripticos le brindan protección. En Uruguay, el programa de control biológico de esta plaga en el cultivo de pimiento dulce está en riesgo debido a la baja tasa de establecimiento y la alta dispersión de las liberaciones del depredador *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). Estudios previos permiten descartar un efecto de antibiosis como causa de la dispersión, por lo cual se planteó como hipótesis la existencia de antixenosis o no preferencia por la planta de pimiento dulce como causa de un control biológico deficiente por parte de *O. insidiosus*. Se evaluó el efecto de estímulos olfativos provenientes de diferentes estructuras de plantas de frutilla, maíz y pimiento dulce (tipos sumuco y california) sobre el comportamiento de *O. insidiosus* en experimentos de olfactometría, y de libre elección en microparcela. Dado que *Orius tristicolor* (White) (Hemiptera: Anthocoridae) también está presente en el área, se incluyó en el estudio con el objetivo de evaluar si existen diferencias en el comportamiento entre ambas especies. *Orius tristicolor* podría actuar como un agente controlador complementario o competidor en pimiento dulce. Los experimentos con tubo en Y no mostraron preferencia por los compuestos volátiles de las plantas en ninguna combinación, y la respuesta a los estímulos volátiles en general fue pobre. Sin embargo, en el experimento de libre elección en microparcela, las hembras de ambas especies de *Orius* prefirieron las plantas con flores de frutilla sobre las plantas con flores de pimiento dulce, lo que podría explicar el bajo establecimiento de *O. insidiosus* cuando se libera en pimiento dulce con los campos de frutilla cercanos. Dado que los invernaderos hortícolas en Uruguay y en muchos otros países están abiertos, esta información puede ser muy útil para diseñar el manejo espacial y temporal de diferentes cultivos en un campo de producción, lo que mejora la efectividad de estas especies depredadoras.

Palabras Claves: trips; antixenosis; volátiles de planta; pimiento dulce; frutilla
crops worldwide. In several countries in South America, it is designated as one of the most limiting pest insects of sweet pepper crops (Capsicum annuum L.; Solanaceae) in greenhouses (Carrizo 1998). Adults and immature stages of this species cause damage to plants by puncturing and then sucking the cellular content of plant tissues (Hunter & Ullman 1989), producing white surface lesions in the epidermis. In addition, they secrete phytotoxic substances when they feed, causing deformation of the foliage in all plant structures and organs (Bosco et al. 2008; Castresana et al. 2008). They also can cause indirect damage by transmitting viruses such as tomato spotted wilt virus (tomato black plaque virus) and impatiens necrotic spot virus (Whitfield et al. 2005). Tomato spotted wilt virus is one of the viruses that causes major damage to a wide range of crops (Fanigilulo et al. 2014) and F. occidentalis is one of the most efficient species in its transmission (Whitfield et al. 2005). Tomato spotted wilt virus has a wide host range and worldwide distribution (Sherwood et al. 2000). This virus may lead to major economic losses on tomato, lettuce, pepper, eggplant, french beans, broad beans, celery, and different ornamental plants (Rosselli et al. 1996). Insecticide applications are not effective in controlling thrips in these systems because females place their eggs under the epidermis of plant leaves, and both immature and adult stages have cryptic habits that give them some protection against toxic substances (Hansen et al. 2003; Helyer & Brobyn 2008). Frequent applications of chemical controls harm the complex of their natural enemies, promote the rapid development of resistance (Castresana et al. 2008), and generate risks to human health from acute and chronic exposure (Damalas & Eleftherohorinos 2011).

As an alternative to chemical control, the use of generalist natural enemies in sweet pepper crops is used widely and has been proven successful (van Lenteren et al. 2018). The release of the predator Amblyseius swirsikki (Athias-Henriot) (Acar: Phytoseiidae) together with Orius insidiosus (Say) (Hemiptera: Anthocoridae) may manage the pepper pests Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae) and Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae) successfully (Funderburk et al. 2000; Chow et al. 2010; Calvo et al. 2014).

In Uruguay, the incidence of F. occidentalis affects more than 80% of the sweet pepper production area, with significant losses in production (Maeso et al. 2013). Additional damage is caused by the tobacco whitefly, B. tabaci, which in combination have traditionally forced growers to apply chemical insecticides repeatedly on the sweet pepper crop with little effect. In the current decade, biological control in protected horticultural crops has made great progress in Uruguay, where the release of A. swirsikki has reduced the incidence of B. tabaci on sweet peppers (Buenahora & Basso 2015). At the same time, the release of O. insidiosus in numerous greenhouses reached a low settlement rate and did not result in a reduction in the populations of F. occidentalis and their crop damage. These results contrast with the statements of Funderburk et al. (2000) indicating that O. insidiosus is effective in suppressing populations of thrips in field sweet pepper crops. It is even indicated that O. insidiosus remains in that crop for up to 6 mo after having eliminated F. occidentalis (van den Meiracker & Ramakers 1991).

Uruguay is part of the distribution area of O. insidiosus (Bentancourt & Scatoni 2001; Carpentero 2002), but not of Orius laevigatus (Fieber) (Hemiptera: Anthocoridae), which is the most widely used species in Europe for biological control of thrips with successful results in sweet pepper (Jacas et al. 2008; van Lenteren 2012). In Europe, the varieties of sweet pepper planted belong preferably to the blocky type, unlike Uruguay, where all varieties are of the lamuyo type (Orius works well in both types of sweet pepper).

Studies conducted by Lorenzo et al. (2019) made it possible to rule out that settlement and effectiveness difficulties of O. insidiosus in the pepper greenhouses in Uruguay were due to an antibiotic effect. Discounting that the low settlement of O. insidiosus is associated with the use of chemical insecticides for the control of other pests because there was a strict control of the products used for these purposes, it was hypothesized that an effect of antixenosis or non-preference was operating in relation to the sweet pepper plant. It should be noted also that the greenhouses used in Uruguay are open and are surrounded by 2 other common hosts of O. insidiosus (strawberry and corn) which are cultivated simultaneously with sweet pepper crops and coincide in their period of flowering in the field.

It is known that the olfactory system allows insects to distinguish the specific volatile compounds emitted by plants (Visser 1986; Bernays & Chapman 1994). These chemical compounds are useful cues for the insect to locate and recognize the plant as a suitable host, which are used to act as inhibitors or stimulants for feeding or oviposition (Campbell et al. 1986; Smith & Boyko 2006). The preferred behavior of females for oviposition derives from particular chemical, physical, and nutritional characteristics of plants (Lundgren & Feren 2006; Lundgren et al. 2008). In this way, antixenosis or non-preference allows certain genotypes of plants to be incompatible with a specific insect, preventing insect establishment, feeding, or oviposition (Painter 1951). Anthocorids display this behavior, which allows them to select the oviposition substrates by physical and chemical factors (Richards & Schmidt 1996; Griffin & Yeargan 2002). It should be considered that Orius, although being highly appreciated as entomophagous, lay their eggs inside plant tissues (Lundgren & Feren 2006; Lundgren et al. 2008) and may even complete their development on certain foods of plant origin (Cocuzza et al. 1997). In recent studies, the phytophagous feeding behavior of O. laevigatus on sweet pepper was shown to trigger defensive responses in the plant. Plant induced defenses may contribute to the repelling or attraction of pests or natural enemies. Specifically, O. laevigatus-punctured sweet pepper plants induced repellency for the whitefly B. tabaci and the thrips F. occidentalis (Bouagga et al. 2018).

Based on the above, the study of preference or non-preference (antixenosis) for possible hosts may help explain the possible causes of low settlement and reproduction of O. insidiosus on sweet pepper. At the same time, it was of interest to compare the behavior of this species with O. tristicolor, which is normally found in corn (Zea mays L.; Poaceae) and strawberry (Fragaria sp.; Rosaceae) crops (Carpintero 2002; Ribeiro & Castiglioni 2008) near the sweet pepper greenhouses. As with O. insidiosus, it is common to find O. tristicolor in corn panicles due to the abundance of pollen, and in the styles of female flowers near stigmas (Corey et al. 1998), and in strawberry associated with flowers (Saini et al. 2003). Therefore, knowing the behavior of O. tristicolor in the cultivation of sweet pepper will let us know if it can be released together with O. insidiosus and contribute to the control of thrips in this crop.

In view of these questions, the purpose of this study was to characterize the preference of O. insidiosus and O. tristicolor for 3 of their host plants. Preference for cultivated varieties of sweet pepper, strawberry, and corn were evaluated under Y-tube olfactometer conditions and in free-choice experiments.

Materials and Methods

PLANT MATERIAL AND INSECTS

Studies were carried out in the laboratory and in a greenhouse of Plant Protection-Entomology at the Experimental Station of the School of Agronomy, Universidad de la República in Salto, Uruguay. Orius insidiosus and O. tristicolor were obtained from the field and maintained in our laboratory at 25 ± 1 °C, 65 ± 10% RH, fed with eggs of Ephesia...
The initial choice of the insect was recorded, and a response was considered positive when, within 10 min, the insect remained inside the Y-tube. If the insect did not enter the decision zone within the established period, the test was recorded as null.

All treatments were evaluated in a darkened space to focus the experiment on the volatile substances generated by the plants introduced into the Y-tube 5 min before the start of each test. Between tests, the olfactometer was cleaned with 70% acetone to remove possible traces of contaminants or pheromones. The acetone was left to evaporate for 1 min at ambient conditions. In addition, to avoid any bias, the sources of odors were alternately switched to the other arm of the olfactometer after every 5 females tested, and the plant material was replaced after every 10 females.

**Results**

**Y-TUBE EXPERIMENTS**

Females of *O. insidiosus* preferred stimuli from strawberry flowers compared to those from sweet pepper flowers ($\chi^2 = 5.565$; *P* = 0.018), while females of *O. tristicolor* preferred the stimuli originated in the sweet pepper leaves over the control (pure air) ($\chi^2 = 9.520$; *P* = 0.002). In the other combinations tested, no differences were found in the response to odor stimuli (Tables 1, 2).
Females of *O. insidiosus* showed no preference between blocky or lamuyo type sweet pepper foliage (*χ² = 0.320; *P* = 0.572), and the same result was obtained when evaluating blocky sweet pepper flower compared to lamuyo sweet pepper flower (*χ² = 3.769; *P* = 0.052; α = 0.05), although the value obtained is at the limit, being almost significant (Table 3). The females of *O. tristicolor* also did not show a preference for blocky or lamuyo type sweet peppers, either in foliage (*χ² = 0.077; *P* = 0.782) or flower (*χ² = 1.796; *P* = 0.181) (Table 3).

###-choice-tests-experiments

After 24 h of being released, females of both species preferred strawberry plants over sweet pepper plants (*O. insidiosus*, *χ² = 4.721, *P* = 0.030; *O. tristicolor*, *χ² = 4.321; *P* = 0.038) (Table 4).

The females of the two species of *Orius* did not show a different preference between lamuyo or blocky type sweet pepper plants (*O. insidiosus* − *χ² = 0.450; *P* = 0.371; *O. tristicolor* − *χ² = 1.389, *P* = 0.239) (Table 4).

###Discussion

In the process of colonization of a host, the behavior of females, including those belonging to Anthocoridae, usually includes a sequence of patterns consisting of orientation, landing, and recognition under different selection pressures. Currently, the mechanisms *Orius* spp. employ to locate their host in complex mixed-vegetation habitats remain poorly understood. All steps involve different senses and occur at varying distances from the plant surface; because olfactory and visual cues are perceptible at distance, they play a determinant role in plant location by foraging insects (Schoonhoven et al. 1998).

It is worth emphasizing the importance of visual search cues in *Hemiptera*, such as aphid and whitefly (Pickett et al. 1992; Isaacs et al. 1999). Ohno & Takekoto (1997) and Furihata et al. (2019) established that *Orius* spp. responded to different colors of traps, when they demonstrated that blue sticky traps were more effective for monitoring studies than white or yellow ones. Studies by Patt et al. (2011) showed that visual stimuli improve olfactory responses to the volatile compounds of the host plant. In fact, to initiate the process of colonization of a host, an olfactory stimulus may not be sufficient to generate a search for resources and oviposition (Visser 1986; Bernays & Chapman 1994; Finch & Collier 2000; Awmack & Leather 2002). The low response rate obtained in the Y-tube bioassays was due possibly to insufficient olfactory stimulation. In general, when a high percentage of test insects do not show a response in the Y tubes, the species does not respond to odor stimuli (this is not the case with *Orius* spp.) or the olfactory stimulus is not sufficient to induce search behaviors (Drukker et al. 2000; Bernardo 2015; Ardanuy et al. 2016).

It also should be noted that the size of the leaf or flower offered (amount of plant material = 1 g) as a source of odor may not have been sufficient. This could explain why the females of *O. insidiosus* showed no preference for the foliage and flowers of sweet pepper, strawberry, or corn in relation to fresh air (control). Another aspect to consider is that cutting leaves or using structures and not whole plants could

###table-1-offactory-response-of-females-of-orius-insidiosus-and-orius-tristicolor-to-stimuli-from-foliage-and-flower-of-strawberry-corn-and-pepper-plants-in-the-arm-+-when-offered-against-clean-air-in-the-arm-0-

| Source smell on the arm (+) | n (+) | n (−) | n (0) | % (+) | χ² | *P* |
|-----------------------------|-------|-------|-------|-------|----|-----|
| **Orius insidiosus**        |       |       |       |       |    |     |
| Strawberry foliage         | 6     | 12    | 22    | 15    | 2.778 | 0.096 |
| Corn foliage               | 9     | 15    | 16    | 22.5  | 2.083 | 0.148 |
| Pepper foliage             | 7     | 13    | 20    | 17.5  | 2.500 | 0.114 |
| Strawberry flower          | 18    | 12    | 10    | 45    | 1.667 | 0.197 |
| Corn flower                | 10    | 17    | 13    | 25    | 2.667 | 0.102 |
| Pepper flower              | 12    | 16    | 12    | 30    | 0.276 | 0.599 |
| **Orius tristicolor**      |       |       |       |       |    |     |
| Strawberry foliage         | 7     | 13    | 20    | 17.5  | 2.500 | 0.114 |
| Corn foliage               | 10    | 14    | 16    | 25    | 0.750 | 0.386 |
| Pepper foliage             | 16    | 5     | 8     | 40    | 9.520 | 0.002 |
| Strawberry flower          | 14    | 14    | 12    | 35    | 0.071 | 0.789 |
| Corn flower                | 19    | 12    | 9     | 47.5  | 2.323 | 0.128 |
| Pepper flower              | 14    | 15    | 11    | 35    | 0.069 | 0.790 |

*n* (+, −) = number of predators that reached the end of the (+) or (−) arm of the Y-tube; *n* (0) = number of predators that did not respond within a 10 min test; % (+) = percentage of individuals that reached the end of the arm (+); *P* = critical level.
Table 3. Olfactory response of females of *Orius insidiosus* and *Orius tristicolor* to stimuli from foliage pepper type blocky (on the arm [+] or flower pepper type blocky [on the arm [+] when offered against foliage pepper type lamuyo (on the arm [−]) or flower pepper type blocky (on the arm [−]) when offered against flower pepper type lamuyo (on the arm [−]) of an olfactometer of Y-tube.

| Source smell on the arm (+)                  | n (+) | n (−) | n (0) | % (+) | χ²     | P     |
|----------------------------------------------|-------|-------|-------|-------|--------|-------|
| Foliage pepper type blocky                   | 13    | 11    | 13    | 32.5  | 0.320  | 0.572 |
| Flower pepper type blocky                    | 17    | 9     | 11    | 42.5  | 3.769  | 0.052 |
| Foliage pepper type lamuyo                   | 14    | 12    | 14    | 35    | 0.077  | 0.782 |
| Flower pepper type lamuyo                    | 15    | 11    | 15    | 37.5  | 1.796  | 0.181 |

n (+, −) = number of predators that reached the end of the (+) or (−) arm of the Y tube; n (0) = number of predators that did not respond within a 10 min test; % (+) = percentage of individuals that reach the end of the arm (+); P = critical level.

Table 4. Response of females *Orius insidiosus* and *Orius tristicolor* to sweet pepper plant type blocky and type lamuyo, and strawberry plant in the free-choice test.

|                      | Sweet pepper plant type lamuyo | Strawberry plant | χ²     | P     |
|----------------------|-------------------------------|------------------|--------|-------|
| Female *O. insidiosus* choice rate (n = 72) | 33                            | 51               | 4.721  | 0.030 |
| Female *O. tristicolor* choice rate (n = 72) | 30                            | 47               | 4.321  | 0.038 |

|                      | Sweet pepper plant type lamuyo | Sweet pepper plant type blocky | χ²     | P     |
|----------------------|-------------------------------|-------------------------------|--------|-------|
| Female *O. insidiosus* choice rate (n = 72) | 25                            | 30                            | 0.450  | 0.371 |
| Female *O. tristicolor* choice rate (n = 72) | 20                            | 29                            | 1.389  | 0.239 |


dichotomous data observed (Madadi et al. 2008). Cut plants may have different odor profiles; the volatile flowers, undamaged leaves, and leaves attacked by herbivores often exhibit different emission patterns from each other (Dudareva & Negre 2005), i.e., each plant structure has a specific odor profile (Karlsson et al. 2009).

The release of volatile defense compounds by plant substrates should not be ruled out either, because it has been reported that in other species of Solanaceae, such as tomatoes, substances derived from sesquiterpenes can repel insects (Frelichowski & Juvik 2005; Álvarez Gil 2015). Recent results by Bouagga et al. (2018) indicate that O. laevigatus punctures in the sweet pepper plants induced the release of an altered blend of volatiles and activation of the jasmonate acid and salicylic acid signaling pathways. These results highlight a potential tritrophic interaction in which the predator is able to induce defensive responses in the plant that reduces feeding by the pest.

In free-choice experiments, the difference found in the preference of females of *O. insidiosus* and *O. tristicolor* for the volatiles emitted by flowering strawberry plants compared to those of flowering sweet pepper plants would confirm that the females use chemical and olfactory cues to recognize their plant host, and that these cues favored strawberry. In the case of strawberry, volatile compounds have been studied widely in fruits; however, there are no studies that evaluate the aromatic differences between the organs of the plant or the whole plant at different phenological stages (Pérez-Rubio 1994). It should be considered that in polyphagous insects, the developmental stage of the host plant can determine the preference, where the main hosts emit a set of very similar volatiles, but both host and non-host plants are more attractive to many species during the flowering stage (Rajapakse 2006). The positive response behavior to the volatile emitted by plants in the flowering stage may be dependent on the response of the anthocorids to this stage of the plant where they feed on pollen and oviposit. It is known that females, during the search for hosts for oviposition, seek to ensure that the plant guarantees the best developmental stage of immature stages (Schoonhoven et al. 2005; Carroll et al. 2006), a principle known as “mother knows best” (Singer et al. 1988; Schoonhoven et al. 2005). As various authors indicated, sweet pepper produces numerous volatile compounds and at least 14 of them are released during flowering and fructification (Addesso et al. 2011), phenological stages when sweet pepper plants are highly attractive to *Orius* spp. adults. The principal compound emitted during these stages is (E)-beta-ocimene (Addesso et al. 2011). This monoterpene has been reported to affect the behavior of adults of some species Coleoptera (Magalhães et al. 2012). Other volatile compounds from *Capsicum* spp. also induce behavioral or electrophysiological activity in different species (Muñiz et al. 2014).

Although the results of this study arise from experiments under controlled conditions in a laboratory or greenhouse, they can be considered useful for informing the management of these predators in the field. These results suggest that females of *Orius* would be able to discriminate between different host plants, and that in particular both *O. insidiosus* and *O. tristicolor* show greater preference for strawberry crops than sweet pepper crops, which could explain the low rate of establishment of the *Orius* females in the sweet pepper crop when they are released on it. It should be noted that in Uruguay, unlike in other horticultural areas, greenhouses are not closed and sweet pepper often is surrounded by other crops, including strawberry. The evaluated host plants coincide with each other in their period of cultivation and flowering in the field. Sweet pepper under greenhouse (113 ha in the country) and strawberry under micro or macro tunnel (55 ha in the country) are produced from Mar to Dec and coincide in their flowering periods (MGAP 2016). The first pepper and strawberry blossoms coincide with the end of corn cultivation, and it is very common to find corn in the surroundings of the greenhouses with the presence of *Orius*. Although these results contrast with those stated by van den Meiracker & Ramakers (1991) and Funderburk et al. (2000), where these authors described the establishment and effectiveness of *O. insidiosus* to control *F. occidentalis* on sweet pepper, they do not indicate whether the greenhouses in these systems were closed or open, or whether there were crops in the vicinity of the sweet peppers that could have interfered with predator establishment.

It should be reiterated that, as evidenced by Lorenzo et al. (2019), there is no effect of sweet pepper antibiosis on *O. insidiosus*; therefore,
if there were no other repellent stimuli present it would remain and reproduce on the sweet pepper crop. The type of sweet pepper grown in Uruguay (lamuco) did not have an influence on this non-preference either, because no differences were observed in preference for lamuco or blocky sweet peppers. Although females of the species *O. tristicolor* were attracted to stimuli from sweet pepper foliage as opposed to pure air, they also were attracted to strawberry plants in the micro-plot test, which could explain their absence on sweet pepper crops while still being found in other hosts surrounding pepper greenhouses.

As a complement, a tool to improve the establishment and colonization by *Orius* could be banker plants located inside the greenhouse. Native species with good flowering and good acceptance by *Orius* should be investigated. Determining a suitable flowering banker plant could improve opportunities for using the predator to control thrips by providing a source of supplementary food in the form of pollen (Huang et al. 2011).

Given that the horticultural greenhouses in Uruguay and in many other countries are open, these results regarding *Orius* preference for different crops may be very useful to aid in the design of spatial and temporal management of different horticultural crops on a production field in order to enhance the effectiveness of these predatory species.

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