Effect of two oviposition feeding substrates on Orius insidiosus and Orius tristicolor (Hemiptera: Anthocoridae)

Maria E. Lorenzo\(^1\)*, Leticia Bao\(^2\), Luciana Mendez\(^3\), Gabriela Grille\(^3\), Olivier Bonato\(^3\), and Cesar Basso\(^2\)

Abstract

Western flower thrips, Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae), is one of the most significant pests of commercial vegetables, fruits, and ornamental crops worldwide, causing both direct and indirect damage. Chemical control is the most common methodology for dealing with F. occidentalis, but this pest lays its eggs inside plant tissues, and adults and larvae feed in concealed locations, which can make chemical control of this pest difficult. As an alternative to chemical control, research attention has been focused on biological control through inoculative augmentation using anthocorid flower bugs of the genus Orius (Hemiptera: Anthocoridae). Although Orius insidiosus (Say) (Hemiptera: Anthocoridae) is an effective predator used worldwide for suppressing populations of western flower thrips, its use on pepper crops in Uruguay (Salto) has not achieved favorable results to date. Taking into account that O. insidiosus can supplement its diet by feeding on pollen and plant tissues, the aim of this study was to assess the effects of pepper fruits compared to bean pods, a vegetable substrate widely used for multiplying this predator, on the duration of the embryonic and nymph developmental stages, survival, fertility, and longevity of this species. Since Orius tristicolor (White) (Hemiptera: Anthocoridae) is present also in the horticultural region of Salto, this species was incorporated into the study in order to evaluate if significant differences exist between these 2 species. When biological parameters were measured, pepper fruits proved to be a more appropriate substrate than bean for the 2 Orius species studied. We reject the hypothesis that an antibiosis effect would explain the difficulties for the establishment of O. insidiosus in the greenhouses of Salto. These results show the need to examine other factors contributing to low establishment of these predatory bugs in greenhouses in Uruguay.

Key Words: biological control; omnivory; plant-feeding predators; pepper; Frankliniella occidentalis; oviposition substrates

Resumen

El trips occidental de las flores, Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae), es una de las plagas más importantes de hortalizas comerciales, frutas y cultivos ornamentales en todo el mundo, causando daño tanto directo como indirecto. El control químico es la metodología más común para el control de F. occidentalis, pero esta plaga pone sus huevos dentro de los tejidos vegetales y los adultos y las larvas se alimentan en lugares ocultos, lo que puede dificultar el control químico de esta plaga. Como una alternativa al control químico, mucha de la atención de la investigación se ha enfocado en el control biológico a través del aumento inoculativo usando insectos antocoridos del género Orius (Hemiptera: Anthocoridae). Aunque Orius insidiosus (Say) (Hemiptera: Anthocoridae) es un depredador eficaz utilizado en todo el mundo para suprimir poblaciones de trips occidentales de las flores, su uso en cultivos de pimiento en Uruguay (Salto) no ha dado buenos resultados hasta la fecha. Teniendo en cuenta que O. insidiosus puede complementar su dieta alimentándose de polen y de tejidos vegetales, el objetivo de este estudio fue evaluar los efectos de los frutos de pimiento, en comparación con las vainas de frijol (un sustrato vegetal ampliamente utilizado para multiplicar este depredador), en la duración de las etapas de desarrollo embrionario y ninfa, supervivencia, fertilidad y longevidad de esta especie. Como Orius tristicolor (White) (Hemiptera: Anthocoridae) está presente también en la región hortícola de Salto, esta especie se incorporó al estudio para evaluar si existían diferencias significativas entre estas dos especies. Cuando se midieron los parámetros biológicos, los frutos de pimiento probaron ser un sustrato más apropiado para las dos especies de Orius estudiadas que las vainas de frijol. Rechazamos la hipótesis de que un efecto de antibiosis explicaría las dificultades para el establecimiento de O. insidiosus en los invernaderos de Salto. Estos resultados muestran la necesidad de examinar otros factores que contribuyen al bajo establecimiento de estos insectos depredadores dentro de los invernaderos en Uruguay.

Palabras Clave: control biológico; omnívoro; depredadores que se alimentan plantas; pimiento; Frankliniella occidentalis; sustrato de oviposición

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ing of significant plant viruses (Kirk & Terry 2003). Direct or cosmetic damage results from feeding and oviposition on plant leaves, flowers, and fruits of more than 200 vegetables and ornamental crops (Lewis 1997; Reitz 2009). Indirect damage is caused by virus transmission, of which the tomato spotted wilt virus is the most important from an economic point of view (De Jager et al. 1995; Maris et al. 2003). In addition to feeding on pollen and plant tissues, adult *F. occidentalis* individuals can supplement their diet with high-protein resources by feeding on other herbivores, such as spider mite eggs (Trichilo & Leigh 1986) and thrips larvae (van Rijn et al. 1995). These omnivorous habits may provide *F. occidentalis* with a competitive advantage over other species, such as *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) in greenhouses, given that a variety of food resources such as pollen and prey are available. Those ecosystem attributes (as well as a warm, protected environment) predispose *F. occidentalis* to permanent establishment and spread, especially in greenhouse crops (Morse & Hoddle 2006).

The most common methodology for dealing with *F. occidentalis* is chemical control, especially in virus-sensitive crops, where a large number of specific treatments are carried out to control thrips. However, it is difficult to control *F. occidentalis* with chemicals because this species lays its eggs inside plant tissues, and the adults and larvae feed in concealed locations, such as flower buds, which protect the pest from insecticides (Jensen 2000; Brødsgaard 2004). Furthermore, in recent yr, failures in the chemical control of this species have been reported because of the emergence of different levels of resistance to various insecticides, including organochlorines, organophosphates, carbamates, pyrethroids, and spinosad (Jensen 2000; Bielza 2008). These resistance phenomena are facilitated by the short generation time, high fertility, and the presence of haploid males in which the resistance genes can be rapidly selected (next generation) when the parents are exposed to pesticide treatments (Espinosa et al. 2002).

As an alternative to chemical control, research attention has been focused on biological control through inoculative augmentation (i.e., deliberate introductions of mass-reared natural enemies into cropping systems with most of the control being provided later by the offspring of the released organism) (van Driesche & Bellows 1996). In this control method, the quality of the organisms released can determine, among other factors, the success of biological control (van Lenteren 1991). The artificial breeding conditions of natural enemies, compared with those of the habitat where they will be released, could influence their later effectiveness. Quality control of the released natural enemies must be carried out to avoid failures in the control of the pests because of the process of their mass production (Bigler 1989, 1994).

Phytoseid mites of the genus *Amblyseius* (Mesostigmata: Phytoseiidae) or antlovorid flower bugs of the genus *Orius* have been used for the biological control of *F. occidentalis* (Ramakers 1995). In particular, *O. insidiosus* was shown to be an effective predator that suppressed populations of western flower thrips in field peppers grown during a period when thrips were rapidly colonizing and developing in the flowers (van de Veire & Degheele 1992; Funderburk et al. 2000; Viglianichino 2013). This antlovorid preys on both larval and adult thrips (Ramachandran et al. 2001; Chow et al. 2010), as well as on aphids, psyllids, mite larvae, flies, and other arthropods (Funderburk et al. 2000; Junget al. 2011).

Antlovoridae also may feed on the sap of vascular plants (van den Meiracker & Ramakers 1991; Coll & Ridgeway 1995; Naranjo & Gibson 1996; Corey et al. 1998). They also may consume pollen; however, they are considered omnivorous. The chemical properties of plants and prey differ greatly, and their relative nutritional values for the omnivore are likely to be a major determinant on the proportion of plant and prey consumption by *O. insidiosus* in the continuum between herbivorous to predaceous habits (Coll & Izralevich 1997). The interactions between plant-prey-omnivore influence the behavioral (plant selected for oviposition) and physiological (plants differ in their suitability for the insect’s growth, survival, and reproduction) aspects (Coll 1996).

As a consequence, plant hosts may have a dramatic effect on several components of *O. insidiosus* fitness, mostly related to the preference for oviposition in adults and the performance of the offspring (Coll 1998). Physical and chemical factors, including plant volatiles, the texture and thickness of the plant tissue, or even the combination of both, influence selection of oviposition substrates (Richards & Schmidt 1996). *Orius* spp. females lay their eggs inside plant tissues; therefore, the preference of females for a given substrate depends on the ease of oviposition insertion, or even the appropriateness of different plants or specific plant tissues for development of the eggs (Coll 1996). It is known that plants have different defense mechanisms for protection from insects which can affect their fertility, longevity, growth, or oviposition (Painter 1951). The preference-performance hypothesis predicts that insects will use plants that provide higher offspring fitness (Balagawi et al. 2013; Clark et al. 2011).

Since 2011 in Uruguay, *O. insidiosus* has been used for pest control in *Capsicum annuum* L. (Solanaceae) pepper crops in greenhouses in the horticultural region of Salto. This predator is released as part of a strategy of biological control through inoculative augmentation, which also includes the predator mite *Amblyseius swirskii* Anthias-Henriot (Mesostigmata: Phytoseiidae). However, the populations of *O. insidiosus* showed a low rate of increase in those crops into which it was released in Salto, even though its prey, *F. occidentalis*, is easily available. This leads to significant damage to the crops caused by thrips, and interest in the reasons why population growth of this predator has failed under these crop conditions. After studies carried out in the Entomology Laboratory of the Faculty of Agronomy of Uruguay, poor quality of the released predators was disregarded as a cause of their low establishment in the crops. The lots of *O. insidiosus* supplied by Brometan Company SA (Buenos Aires, Argentina) were tested, and the criteria defined by the International Organization for Biological Control (IOBC) were fulfilled, i.e., a sex-ratio ≥ 45% of females (n = 100) and a fertility ≥ 30 eggs per female in 14 d (van Lenteren 2003).

As a first step to answer this question, the effect of the host plant on the biological parameters of *O. insidiosus* (duration of embryonic and nymphal stages, survival, fertility, and longevity) was determined. Because *O. tristicolor* also is abundant in the horticultural region of Salto (Carpintero 2002; Ribeiro & Castiglioni 2008), this species was incorporated into the study in order to evaluate if differences between these 2 *Orius* species existed.

In the current study, fruits of the variety of pepper most widely planted in Salto (‘Bilano’ variety, ‘Lamuyo’ type) were used, and their effects were compared with those caused by bean pods *Phaseolus vulgaris* L. (Fabaceae), a feeding-oviposition substrate widely used for breeding *Orius* spp. in laboratory (Castañé & Zalom 1994; Richards & Schmidt 1996; Mendes et al. 2005).

**Materials and Methods**

**INSECT COLONIES AND EXPERIMENTAL CONDITIONS**

Specimens of *O. insidiosus*, *O. tristicolor*, and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) used in the experiments were mass-reared in the Faculty of Agronomy (Salto, Uruguay) Entomology Lab-
oratory. Colonies of *O. insidiosus* were established from individuals coming from Argentina, purchased from the Brometan Company SA (Buenos Aires, Argentina) that distributes *O. insidiosus* to the horticultural growers in Salto. Individuals of *O. tristicolor* were collected from corn *Zea mays* L., sorghum *Sorghum* spp. (both Poaceae), and strawberry *Fragaria* sp. (Rosaceae) in fields located in the vicinity of the city of Salto, Uruguay (31.350°S, 57.883°W).

Colonies were reared in the laboratory for at least 1 yr before their use in the experiments. The anthocoridae were fed with *E. kuehniella* eggs, an excellent protein source for these predators (Mendes et al. 2002; Zambrano 2009; Sobhy et al. 2010). The colonies were kept under controlled conditions, i.e., 25 ± 1 °C, 65 ± 10% RH, and a 16:8 h (L:D) photoperiod, according to the protocol described by Mendes and Bueno (2001). Rearing was carried out in glass boxes (35 cm L × 20 cm W × 14 cm H) containing corrugated paper or vermiculite, a source of water (wetted cotton) and food (*E. kuehniella* eggs) ad libitum. Females and males from each of the 2 *Orius* species were put in each box; the substrate for oviposition was either green bean (*P. vulgaris*) pods, about 15 cm long (Castañé & Zalom 1994; Richards & Schmidt 1996; Mendes et al. 2005) or recently set pepper fruits (*Capsicum annuum*) 14 cm long (Castañé & Zalom 1994) under a cardboard controlled conditions, i.e., 25 ± 1 °C, 65 ± 10% RH, and a 16:8 h (L:D) photoperiod, according to the protocol described by Mendes and Bueno (2001). Rearing was carried out in glass boxes (35 cm L × 20 cm W × 14 cm H) containing corrugated paper or vermiculite, a source of water (wetted cotton) and food (*E. kuehniella* eggs) ad libitum.

### DURATION OF THE EMBRYONIC AND POST-EMBRYONIC DEVELOPMENT

In order to obtain cohorts of *O. insidiosus* and *O. tristicolor*, 117 females and 39 males were extracted from the offspring of each species. They were then distributed in arenas constituting of plastic boxes (Briden, Montevideo, Uruguay) (20 cm in diam) covered with polyethylene extendible film (Naka SA, Montevideo, Uruguay). As oviposition substrate, 4 pods of *P. vulgaris* (about 15 cm long) or 7 peppers (about 1 cm long) were placed in each box. Thirteen boxes were used for each species, and 9 males and 3 females were introduced into each of them. After 24 h, adults were removed, and the eggs laid in the pods and pepper fruits were counted under a stereoscopic microscope (Nikon SMZ 1B-X35, Nikon Corporation, Montevideo, Uruguay) in each box. Pods and peppers were gathered into 7 groups of 30 eggs each. Each group was considered a replication, and the pods and peppers from each replication were put in plastic boxes (Briden, Montevideo, Uruguay) (20 cm in diam) covered with polyethylene extendible film (Naka SA, Montevideo, Uruguay) and kept until the nymphs hatched, following the procedure reported by Avellaneda et al. (2016).

To determine the duration of embryonic development, the number of eggs and first-stage nymphs present in each replication were counted every 24 h until no new nymphs were found. In order to calculate the duration of the nymphal developmental stage, the nymphs found daily were collected from the arenas, and were separated in order to prevent cannibalism or mutual interference, as reported for *Orius* species in the absence of food or under a high density of individuals (van den Meiracker 1999). They were placed in Petri dishes (about 5 cm in diam) containing cotton wetted with distilled water, white filter paper (0.5 cm²), and *E. kuehniella* eggs ad libitum as prey. Each box was covered with polyethylene extensive film with high transparency in order to avoid escapes.

The duration of each nymphal stage was registered daily until adult emergence; the exuviae from the ecdisis was extracted using a fine paint brush. The sex-ratio was then determined by observing the abdominal region of adults (Ferragut & González 1994) under a stereoscopic microscope (Nikon SMZ 1B-X35, Nikon Corporation, Montevideo, Uruguay).

### SURVIVAL OF EGG AND NYMPHS

The eggs (laid on bean pods and pepper fruits) were monitored and observed daily in order to determine the percentage of eggs hatched and the survival rate of embryos. Hatched eggs can be differentiated because of the opening of the operculum that remains suspended from the egg. After hatching of the eggs, nymphs were individualized and observed daily in order to record the change of stage and determine nymphal survival; the exuviae from the ecdisis was extracted. This process was carried out until the last exuviae from the imaginal molting (nymph V) was obtained. A stereoscopic microscope (Nikon SMZ 1B-X35, Nikon Corporation, Montevideo, Uruguay) was used for observing the morphological changes of the nymphs.

### LONGEVITY AND FERTILITY OF FEMALES

Petri dishes (5 cm in diam) were used as experimental units. They contained white sulphite paper (0.5 cm²), *E. kuehniella* eggs as food ad libitum, and cotton that was wetted daily with distilled water in order to keep the humidity elevated. In each box, a section of pod from *P. vulgaris* (about 3 cm long) or a pepper fruit (about 1 cm long) was placed as an oviposition substrate. Seventeen couples of anthocoridae from each species, ≤ 24 h old, were selected and introduced into an experimental unit. Bean pod sections and pepper fruits were removed daily and replaced by fresh ones until the death of the female. In case the male died before the female, he was quickly replaced (Avellaneda et al. 2016).

For each ovipositing female, the number of eggs laid was registered daily, and the periods of pre-oviposition, oviposition, and post-oviposition were determined, as well as the total fertility (total number of eggs per female), and the daily fertility (daily number of eggs per female). Longevity was calculated including all the females, even if no longer ovipositing.

### STATISTICAL ANALYSIS

A 2-way ANOVA with *Orius* species and host plant as 2 different factors was used to check the effect of oviposition-feeding substrate on the duration of the pre-imaginal development stages, the total duration of the development (egg to adult), pre-oviposition, oviposition, and post-oviposition periods, fertility, and longevity of *O. insidiosus* and *O. tristicolor* females. Before running the ANOVA programs (*P* < 0.05), the normality of experimental errors as well as the homoscedasticity were checked. When the ANOVA reflected a significant difference, means were separated using the Tukey test. For data of embryonic and post-embryonic stages survival, the interaction between species and host plant was analyzed using a GLM (binomial distribution, and species and host plant as factors). All the statistical analyses were performed using the R Core Development Team statistical program (2014).

### Results

#### DURATION OF THE EMBRYONIC AND POST-EMBRYONIC DEVELOPMENT

Both *O. insidiosus* and *O. tristicolor* showed a shorter duration of the embryonic stage on bean pods than on pepper fruits (*F* = 257; *df* = 3; *P* < 0.001). Comparison between the 2 species indicated that *O. insidiosus* needed less time than *O. tristicolor* to complete the egg stage on both oviposition substrates (*P* < 0.05) (Table 1). An effect of the species (*F* = 104; *df* = 1; *P* < 0.001), the host plant (*F* = 663; *df* = 1; *P* <
When the 2 species were compared, nymphal survival rate on bean pods was lower than on pepper fruits. Simultaneously, *O. insidiosus* needed less time to develop from egg to adult on both substrates than *O. tristicolor* (Figs. 2 & 4).

Despite the fact that the 2 species reached the adult stage on both substrates, the duration of the pre-imaginal development in both species was lower on bean pods than on pepper fruits. Simultaneously, *O. insidiosus* needed less time to develop from egg to adult on both substrates than *O. tristicolor* (Figs. 2 & 4; Table 2). An effect of the host plant on nymphal survival (*P* = 0.001; $\chi^2 = 6.99$) was observed, but there was no effect of the species ($P = 0.001; \chi^2 = 0.52$).

**LONGEVITY AND FERTILITY OF FEMALES**

The pre-oviposition period was not altered by the substrate in any of the species studied: *O. insidiosus* on pepper fruits compared to bean pods (*P* = 0.175) and *O. tristicolor* on pepper fruits compared to bean pods (*P* = 0.864) (Table 3). The oviposition period was longer on pepper fruits than on bean pods for both species. When the 2 species were compared, the oviposition period on bean pods was longer for *O. tristicolor* than for *O. insidiosus* (*F* = 14.09; *df* = 3; *P* = 0.0001). Similarly, on pepper fruits, the oviposition period was longer for *O. tristicolor* than for *O. insidiosus* (*F* = 14.09; *df* = 3; *P* = 0.005). The post-oviposition period was not affected by substrate in any of the species considered (*F* = 0.38; *df* = 3; *P* = 0.766) (Table 3).

Longevity was significantly affected by the oviposition-feeding substrate (*F* = 9.24; *df* = 3; *P* < 0.001), but only for *O. tristicolor* where this period was longer on pepper fruits than on bean pods (*P* = 0.008). *Orius tristicolor* showed greater longevity than *O. insidiosus* on both substrates (*P* = 0.049) (Table 3).

Total fertility was affected only by the oviposition-feeding substrate for *O. tristicolor* (*F* = 11.67; *df* = 3; *P* = 0.001), with a higher number of eggs per female on pepper fruits than on bean pods (*P* = 0.024). *Orius tristicolor* laid a higher number of eggs per female than *O. insidiosus* on both substrates (Table 3).

Daily fertility of *O. insidiosus* females on bean pods and pepper fruits was stabilized after 19 and 15 d, respectively, without significant differences between substrates (Figs. 1 & 3). On the other hand, cumulated daily fertility of *O. tristicolor* females on these 2 substrates was stabilized after 12 and 16 d, respectively, being higher on bean pods (Figs. 2 & 4).

**SURVIVAL OF EGGS AND NYMPHS**

The oviposition substrate affected the egg survival of both species, and survival was greater on pepper fruits than on bean pods (*P* = 0.001; $\chi^2$ calculated = 17.52; $\chi^2$ theoretical = 7.81; *df* = 3; *α* = 0.05). The survival rate of embryonic *O. insidiosus* on pepper fruits was 61.9% and 47.6% on bean pods (calculated value = 0.219 > critical value = 0.120), whereas the survival rate of embryonic *O. tristicolor* on pepper fruits was 63.0% and 49.0% on beans (calculated value = 0.114 > critical value = 0.105). No significant differences between species were detected when compared for embryonic survival on either bean pods (calculated value = 0.014 < critical value = 0.136) or pepper fruits (calculated value = 0.014 < critical value = 0.132) (Table 2). An effect of the plant (*P* = 0.001; $\chi^2$ = 11.21) on embryonic survival was found, but there is no effect of the species ($P = 0.001; \chi^2 = 2.75$).

The substrate also influenced nymphal survival rate of both species ($\chi^2$ calculated = 48.04; $\chi^2$ theoretical = 7.81; *df* = 3; *α* = 0.05; *P* < 0.001). In both cases, survival was higher on pepper fruits than on bean pods. When the 2 species were compared, nymphal survival rate on bean pods was greater for *O. tristicolor* (75.7%) than for *O. insidiosus* (60.0%) (calculated value = 0.157 > critical value = 0.126), whereas on pepper fruits, no differences between species were detected (calculated value = 0.052 < critical value = 0.098) (Table 2). An effect of the host plant on nymphal survival (*P* = 0.001; $\chi^2 = 6.99$) was observed, but there was no effect of the species ($P = 0.001; \chi^2 = 0.52$).

**Table 1.** Duration (d) (mean ± confidence interval) of the egg stage, the stages of nymphal development, and the total for the pre-imaginal period of *Orius insidiosus* and *Orius tristicolor* on bean pods and pepper fruits under controlled conditions: 25 ± 1 °C, 65 ± 10% RH, and a 16:8 h (L:D) photoperiod.

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Oviposition substrate (mean ± confidence interval)</th>
<th>Oviposition substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bean pod</td>
<td>Pepper fruit</td>
</tr>
<tr>
<td>Egg</td>
<td>4.37 ± 0.09 a</td>
<td>6.07 ± 0.13 b</td>
</tr>
<tr>
<td>Nymph I</td>
<td>2.25 ± 0.09 a</td>
<td>2.73 ± 0.08 b</td>
</tr>
<tr>
<td>Nymph II</td>
<td>2.22 ± 0.08 b</td>
<td>2.95 ± 0.10 c</td>
</tr>
<tr>
<td>Nymph III</td>
<td>2.33 ± 0.10 a</td>
<td>2.34 ± 0.09 a</td>
</tr>
<tr>
<td>Nymph IV</td>
<td>2.14 ± 0.06 a</td>
<td>2.34 ± 0.09 b</td>
</tr>
<tr>
<td>Nymph V</td>
<td>1.22 ± 0.08 a</td>
<td>2.10 ± 0.10 b</td>
</tr>
<tr>
<td>Egg + nymph</td>
<td>14.52 ± 1.77 a</td>
<td>18.53 ± 0.19 b</td>
</tr>
</tbody>
</table>

Different letters in a row indicate significant differences (ANOVA + SNK, *P* = 0.05).

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**Table 2.** Survival rate (%) in egg and nymphal stages of *Orius insidiosus* and *Orius tristicolor* on 2 oviposition substrates (bean pods and pepper fruits) under controlled conditions: 25 ± 1 °C, 65 ± 10% RH, and a 16:8 h (L:D) photoperiod.

<table>
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<tbody>
<tr>
<td></td>
<td>Bean pod</td>
<td>Pepper fruit</td>
</tr>
<tr>
<td>Egg</td>
<td>47.6 ± 0.09 a</td>
<td>61.9 ± 1.6 b</td>
</tr>
<tr>
<td>Nymph</td>
<td>60.0 ± 0.09 a</td>
<td>81.9 ± 1.6 b</td>
</tr>
</tbody>
</table>

Different letters in a row indicate significant differences ($\chi^2$, *P* = 0.05).
An effect of the species ($F = 107.7; \text{df} = 1; P < 0.001$), and of the plant-species interaction ($F = 103; \text{df} = 1; P < 0.001$) on the longevity and fecundity of the females was found, but an effect of the host plant ($P = 0.82$) on these variables was not observed.

### Discussion

The duration of the egg development of *O. insidiosus* on bean pods observed in the current study was similar to that reported by van den Meiracker (1999) (4.6 d) and Tommasini et al. (2004) (4.2 d), who studied this species on the same vegetable substrate. Our findings were similar to data reported for *O. tristicolor* by Askarri and Stern (1972) (5 d) at 25.5 °C on petioles from bean leaves; however, the findings were different than that reported by Avellaneda et al. (2016) when they evaluated *O. insidiosus* on bean pods. Care must be taken when comparing results from different authors because of the influence exerted by the different experimental methods used, the variability in the populations of predators used in each study, or the previous history of the offspring from which adults used for the oviposition were extracted (Iranipour et al. 2009; Avellaneda et al. 2016).

The physical effect of the oviposition substrate may have been influenced by the fact that both *Orius* species completed their embryonic development more rapidly on bean pods than on pepper fruits. Eggs are inserted into vegetable tissues, so the time for egg development may be affected by the ease of insertion of the ovipositor, or perhaps the appropriateness of the different plants or sections of plants for the development of the eggs (Coll 1996).

The egg survival values observed were close to those reported for *O. insidiosus* by Richards and Schmidt (1996) and Tommasini et al. (2004), who obtained 56% and 63% of eggs hatched in bean pods, respectively. Little information is available about the biology of *O. tristicolor* on the same 2 substrates. It must be taken into account that the principal role of the substrate is to provide water and the appropriate conditions to avoid egg desiccation, and to allow complete egg development (van den Meiracker 1999). According to Nolasco (2008), after 3 d at 20 to 25 °C, bean pods lose 60% of their humidity. Therefore, the lower viability of eggs laid on bean pod compared to pepper fruits may be related to the rapid dehydration of bean after being cut. Perhaps the viability of the eggs obtained in our study would be different if whole pods were used on bank plants as refuge for *Orius* species. The humidity variations in the oviposition substrate also may be the principal factor affecting the survival of nymphs, because the *E. kuehniella* eggs cannot provide the moisture needed (Schmidt et al. 1995). The lack of humidity is the principal cause of mortality in the immature stages of *O. insidiosus* (Schmidt et al. 1998).

Tommasini et al. (2004) and Avellaneda et al. (2016) obtained a total time of immature development of *O. insidiosus* on bean pods similar to that we observed in the current study, when this species was fed with *F. occidentalis* adults (14.1 d) or with *E. kuehniella* eggs (15 d). In contrast, for *O. tristicolor*, Pinto et al. (2004) and Hokkanen et al. (2002) reported higher values on bean pods than those found in the current study, with a duration from egg to adult of 33.3 and 32 d at 26 °C, respectively, when this species was fed with *F. occidentalis* and *Tetranychus urticae* Koch (Acarina: Tetranychidae).

Mendes et al. (2005) pointed out that females exposed to more tender tissues (rather than stiffer tissues) showed a lower pre-oviposition time. In our case, the beginning of the oviposition of females of the 2 species was similar for both substrates, which would indicate that no effect of substrate could be detected.

#### Table 3. Pre-, post-, and oviposition periods (d), longevity of females (d), and total fertility (eggs per female). (Mean ± confidence interval) of *Orius insidiosus* and *Orius tristicolor* on 2 oviposition-feeding substrates (bean pods and pepper fruits) under controlled conditions: 25 ± 1 °C, 65 ± 10% RH, and a 16:8 h (L:D) photoperiod.

<table>
<thead>
<tr>
<th></th>
<th><em>Orius insidiosus</em></th>
<th></th>
<th><em>Orius tristicolor</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-oviposition</td>
<td>Oviposition</td>
<td>Post-oviposition</td>
<td>Longevity</td>
</tr>
<tr>
<td>Bean pods</td>
<td>3.2 ± 0.8 a</td>
<td>8.4 ± 2.1 a</td>
<td>4.3 ± 1.4 a</td>
<td>15.6 ± 2.1 c</td>
</tr>
<tr>
<td>Pepper fruits</td>
<td>2.8 ± 0.2 ab</td>
<td>11.6 ± 1.0 b</td>
<td>6.0 ± 0.6 a</td>
<td>17.4 ± 0.9 bc</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td><em>Orius tristicolor</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bean pods</td>
<td>2.4 ± 0.2 b</td>
<td>12.5 ± 0.9 b</td>
<td>4.9 ± 0.6 a</td>
<td>18.1 ± 0.9 b</td>
</tr>
<tr>
<td>Pepper fruits</td>
<td>2.3 ± 0.2 b</td>
<td>15.0 ± 1.2 c</td>
<td>6.9 ± 0.5 a</td>
<td>20.9 ± 1.2 a</td>
</tr>
</tbody>
</table>

Different letters in a column indicate significant differences (ANOVA + SNK, $P = 0.05$).

![Fig. 1. Cumulative daily fertility for *Orius insidiosus* females on 2 oviposition substrates: bean pods and pepper fruits.](image1)

![Fig. 2. Cumulative daily fertility for *Orius tristicolor* females on 2 oviposition substrates: bean pods and pepper fruits.](image2)
A significant influence of the substrate on the oviposition period was observed for both species. In the case of *O. insidiosus* on bean pods, this period was similar to that reported by Avellaneda et al. (2016) (9.21 d), whereas Mendes et al. (2005) reported more extended periods when this species was bred on the same substrate and fed with *E. kuehniella* eggs (27.8 d). Our results suggest that both structures and plant sections (bean pods or pepper fruits) are accepted by *O. insidiosus* and *O. tristicolor* females for oviposition despite the detected differences.

Differences in fertility and longevity between both substrates and between the 2 species could be related to the physico-chemical composition of the plant, as perceived by the predator during the oviposition activity (Richards & Schmidt 1996), and to the physical structure and internal anatomy of the plants. These plant conditions not only have direct implications on the females, but also on the ability of their offspring to use the same plant as a food resource (Lundgren et al. 2008). The complex tri-trophic relationship between the predators of the genus *Orius* and the plant has been evidenced, because the plant, apart from being an oviposition substrate, also is used as a source of water and nutrients by these predators, which are phytophagous to a certain extent during their immature stages (Coll 1996; Cocuzza et al. 1997; Corey et al. 1998). Although other factors may affect the biological characteristics of *Orius* species, such as temperature, photoperiod, and the type of prey (Argolo et al. 2002; Mendes et al. 2002), the nutritional quality and the temperature are the most influential factors (Iranipour et al. 2009). In our study, temperature and photoperiod conditions were common for all individuals; therefore, the only variable that would explain the observed differences is the vegetable substrate.

The fact that pepper fruits proved to be an appropriate substrate for the expression of the biological characteristics of the *Orius* species studied in a controlled environment allows for discarding an antibiosis effect that would explain the difficulties for establishing *O. insidiosus* in the greenhouses in Salto, Uruguay. Furthermore, pepper fruits allowed for maximizing most of the biological characteristics studies when they were compared with the values observed on bean pods.

These results present the need to evaluate other factors that may be affecting the establishment of *Orius* species in greenhouses. According to Urbaneja et al. (2003), the speed of installation of these predators can be determined by several factors that may affect the moment of release: the genetic species or variety, the food or prey available, environmental conditions, chemical residues, and interspecific relationships that may arise with other arthropods. Taking these factors into account, it is important to emphasize that the pepper hybrids used in Uruguay are the Lamuyo type, which are different from those planted in most of the regions of the world where *Orius* species are successfully used for the control of thrips on pepper (the Blocky type).

In Uruguay, greenhouses are not closed with a physical barrier or an insect-proof net that could prevent the exit of predators before they can be established. *Orius* species have great mobility, to which we must add the flight capacity of adults, so that they can easily move from one place to another and thus locate new prey. In addition, it is a polyphagous control agent that can be used in a wide range of crops. The horticultural system in Uruguay presents a great diversity of crops (corn, strawberry, sorghum, etc.) that may attract predators to them.

On the other hand, greenhouses do not have supplementary lighting to avoid reproductive diapause that could be affecting populations and interfering with their effectiveness as biological control agents. *Orius* females, in conditions of short d and low temperatures, enter reproductive diapause; this behavior was verified in *O. insidiosus*, *O. laevigatus* (Tommasini & Nicoli 1995), and *O. tristicolor* (Gillespie & Quiring 1993; van den Meiracker 1994). *Orius insidiosus* in temperate regions, and with photoperiods less than 13 h and mean temperature of 25 °C, enters into reproductive diapause (van den Meiracker 1994). This factor could limit its use as a natural enemy in winter conditions. Determining a suitable flowering banker plant system for *O. insidiosus* could improve opportunities for using the predator to control thrips by providing a source of supplementary food in the form of pollen (Frank 2010; Huang et al. 2011). Setting such a system in the greenhouse would allow *O. insidiosus* to establish and increase its population, offering growers the option of preventative introductions. An effective banker plant should provide a location for feeding and reproduction, as well as allowing nymphs to reach the adult stage quickly, ensuring a high survival rate, and supporting population growth. The Black Pearl ornamental pepper is currently used as a banker plant by some commercial growers, because it has been shown to support *O. insidiosus* in the absence of prey (Wong & Frank 2012, 2013). Studies by Wong and Frank (2012, 2013) indicate that pollen from Black Pearl peppers may result in larger populations of *O. insidiosus* by increasing the longevity of the predator when prey are absent, reduce development time, and increase likelihood of survival to adult.

The failure of *O. insidiosus* to establish itself in some greenhouses also could be attributed to the use of several insecticides. Currently, growers still use pesticides to reduce pest numbers before natural enemies are released, or when pest numbers reach economically damaging levels. Despite the potential effectiveness of biological control, many crop protection practices are primarily based on broad spectrum chemical pesticides that are noxious to beneficial arthropods (Desneux et al. 2007). Pesticides could induce multiple lethal and sublethal effects in individuals (Desneux et al. 2007), and these effects could have

![Fig. 3. Survival curves (in %) of *Orius insidiosus* females on 2 oviposition substrates under controlled conditions (25 ± 1 °C, 65 ± 10% RH, and a 16:8 h [L:D] photoperiod): bean pods and pepper fruits.](image1)

![Fig. 4. Survival curves (in %) of *Orius tristicolor* females on 2 oviposition substrates under controlled conditions (25 ± 1 °C, 65 ± 10% RH, and a 16:8 h [L:D] photoperiod): bean pods and pepper fruits.](image2)
an important impact on the population dynamics of natural enemies (Stark & Banks 2003). Sublethal effects could impair the physiology (e.g., neurophysiology, development, longevity, fecundity, and sex-ratio) and the behavior (e.g., mobility, orientation, feeding, host searching, oviposition, and mating) of natural enemies (Arnó & Gabarra 2011; Stara et al. 2011).

Furthermore, it would be interesting to study the compatibility of O. insidiosus and A. swirskii, which also is released in the same pepper greenhouses, as was studied on rose by Chow et al. (2010). In fact, intraguild predation, with Orius acting as an intraguild predator and Amblyseius as an intraguild prey, may be affected by different factors (Lucas 2005). However, A. swirskii is used in greenhouses for pepper without problems, controlling the populations of F. occidentalis, but Orius spp. are not established and do not colonize well in greenhouse crops; therefore, it is expected that intraguild predation should not be regarded as totally or partially responsible for this lack. Finally, it is necessary to check for the existence of antixenosis or no-preference, a phenomenon that results in the insect avoiding the plant for oviposition, feeding, or shelter (Painter 1951). This mechanism, which adversely affects the behavior of a given insect, impeding it to interact with certain genotypes of its hosts, also could be responsible for the fact that O. insidiosus individuals mass-released on pepper crops in Salto could only scarcely be recovered over the growing cycle of those crops.

Acknowledgments

We are grateful to the authorities of the Estación Experimental de la Facultad de Agronomía (EEFAS), and to the colleagues from the Department of Vegetal Protection in EEFAS and the Entomology unit in Montevideo for their collaboration during the entire study period. This study was supported by a grant from the Agencia Nacional de Investigación e Innovación (ANII/INNOVAGRO) that funded a project titled: “Contribution to the biological control of pests of horticulture in the region of Salto.”

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