RESEARCH ARTICLE

Biological control of thrips pests (Thysanoptera: Thripidae) under greenhouse conditions in Transylvania, Romania

Artúr Botond Csorba^{1,2}, Barna Putnoky-Csicsó², Róbert Koncz², Attila Bandi², Imre-István Nyárádi^{2*}, János Bálint^{2*}

¹Doctoral School in Biology and Sport Biology, University of Pécs, Faculty of Sciences, Ifjúság útja 6., Pécs, Hungary

²Sapientia Hungarian University of Transylvania, Faculty of Technical and Human Sciences, Department of Horticulture, Aleea Sighişoarei 1C, Târgu Mureş/Corunca, Romania.

*Correspondence: János Bálint, balintjanos@ms.sapientia.ro and Imre-István Nyárádi nyaradi@ms.sapientia.ro. Institutional address: Sapientia Hungarian University of Transylvania, Faculty of Technical and Human Sciences, Department of Horticulture, Aleea Sighişoarei 1C, Târgu Mureş/Corunca, Romania.

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ABSTRACT

In present, vegetable cultivation faces significant challenges for two reasons: (i) cultivators have to produce healthy, first-class goods, and (ii) vegetables are allowed to contain only a specified pesticide residue. Development of insecticide resistance as a result of repeated insecticide treatments is causing difficulties to cultivators, who have paid attention to biological control methods over recent years. In Romania, pepper sprouting represents a prominent place in greenhouse vegetable production. Under greenhouse conditions, one of the most dangerous pests of peppers is the western flower thrips (*Frankliniella occidentalis*), which can trigger high economic losses. In recent years, several populations have been found resistant to insecticides and other active compounds, therefore, in our experiments, performed under greenhouse conditions, we used the predatory mite *Amblyseius swirskii* and the pirate bug *Orius laevigatus* against the western flower thrips. In addition to thrips, we monitored the growth and spread of introduced beneficial organisms based on collected flower samples. We also evaluated the relationships between beneficial organisms and greenhouse climate data. Introduction of natural enemies has proven a success of biological plant protection, as thrips did not cause economic losses in our experiment.

Keywords: thrips, biological control, pepper, *Amblyseius swirskii*, *Orius laevigatus*

1. Introduction

Thrips are polyphagous insects from the order *Thysanoptera*. Onion thrips (*Thrips tabaci*) and western flower thrips (*Frankliniella occidentalis*) are the most important pests regarding plant protection under greenhouse conditions. Recently, they have become perhaps the most dangerous pests of vegetables crops, partly because of direct damage from their diet and partly due to their role in spreading viral diseases. (Budai, 2002), (Molnár et al., 2008). In case of mass reproduction, western flower thrips, can cause significant damage by spreading the tomato spotted wilt virus (TSWV). Chemical control of pests does not always produce adequate results, because of chemical resistance (Van Lenteren and Bueno, 2003), or as it may get in conflict with the growing demand of integrated crop production and protection (Budai, 2006).

Integrated pest management consists in combining biological, chemical, biotechnological, and cultivation or crop production processes in a rational manner (Budai, 2006). According to van de Veire et al. (1996) the effects of 31 commonly used pesticides on first larval stage of *Orius laevigatus* were studied. Chitin synthesis inhibitors, such as *flufenoxuron*, *lufenuron*, and *teflubenzuron* exert markedly detrimental effects on the development of larvae of *Orius laevigatus* and the egg-laying ability of adults. Experiments also demonstrated that organophosphates, including *dichlorophos* and *pirimicarb* that belong to the carbamate group, are very toxic to larvae of *Orius laevigatus* in the first stage (van de Veire et al., 1996). Biological plant protection offers many advantages, such as low costs and the lack of adverse effects to humans, animals, and the environment. Rapid proliferation of beneficial organisms and the consequent rapid decline in pest populations can also be of huge benefit (Huffaker, 2012). Integrated pest management for pepper pests is based on thrips control. Venzon and co-workers (2002) performed an experiment, in which the predatory ability of the pirate bug (*Orius laevigatus*) on cucumber leaves was examined. When spider mites and thrips were placed on cucumber leaves separately, *Orius* preferred to consume spider mites. In contrast, when spider mites and thrips were applied to the same cucumber leaf, the pirate bug devoured a higher percentage of thrips.

Predatory mite species and pirate bug species can be used jointly during pest controls. Along with predatory mites, like *Amblyseius cucumeris* and *Amblyseius swirskii*, *Orius sp.* pirate bugs can be used to control the thrips population under greenhouse conditions (Budai, 2006).

Such natural enemies can be introduced starting the flowering phase of peppers as they can also survive, reproduce, and feed on pollen. High humidity helps in the reproduction of predatory mites. In addition to

predatory mites, pirate bugs are able to destroy all forms of thrips and, as they are able to fly well, their predatory ability may exceed that of mites (Budai, 2006).

Model species:

Orius laevigatus belongs to the family *Anthocoridae* and used widespread in biological control against thrips. (De Puyssseleyr et al., 2011). Females lay their eggs in plant tissues (main steam, veins, flowers). Most *Orius* females lay their eggs at the same location, where they feed, without looking for a separate place. Within a habitat, however, plants with a thin epidermis are selected for laying because young larvae have easier access to food from thin plant tissues (Lundgren et al., 2008), (Lundgren et al., 2009). In the fourth and fifth larval stages, they become of dark brown color (Hamdan and Abu-Awad, 2008). *Orius laevigatus* is an effective predator and has been used since 2005 as a natural enemy against western flower thrips (*Frankliniella occidentalis*) (Arijs and De Clercq, 2004).

Population of *O. laevigatus* is not limited by daylength, rather than by temperature, which may exert the greatest effect on their development. Experiments performed in Spain demonstrate that *O. laevigatus* population remains active all year around, when the winter is mild (Lacasa et al., 1996). Temperature plays a very important role in the hatching of eggs of *Orius* species; from 15 °C to 30 °C, the hatching is 4 times faster and the average hatching time is between 3.3 and 11.7 days. Temperature is also an important factor for the *Orius*' ability to develop and reproduce. *O. laevigatus* develops very slowly at 14 °C and just a few females lay eggs. Between 22 °C and 30 °C females lay many eggs and they develop faster, but the most favorable temperature for their growth is 26 °C (Gergely, 2010).

Amblyseius swirskii is a predatory mite belonging to the family *Phytoseiidae*, which naturally inhabits the eastern Mediterranean (Israel, Cyprus, Turkey, Greece, and Egypt), where it is also common in apples, citrus fruits, and vegetables. It was first used for biological control in California to prevent citrus pests. In Europe it has been utilized for biological control since 2005 (Ragusa and Swirski, 1977), (Dogramaci et al., 2013). As long as ideal conditions exist, the *Amblyseius swirskii* population grows and spreads extremely rapidly in cultures and its efficacy and reproduction rates are greater than *Frankliniella occidentalis* and *Thrips tabaci* (Bolckmans et al., 2005). Other experiments demonstrated that in 12 h, they can consume 4-6 spider mite nymphs (Xu and Enkegaard, 2010). According to Momen and Elsaway (1993) *Amblyseius swirskii* can survive on several pests, but in addition to arthropods, it also feeds on flower pollen. In addition to temperature, humidity plays an important role in the development and reproduction of *Amblyseius swirskii*. In terms of food, various experiments demonstrated that *Amblyseius*

swirskii preferred thrips larvae as food, rather than greenhouse whitefly (Xu and Enkegaard, 2010).

We report here experiments on using predatory mite *Amblyseius swirskii* and the pirate bug *Orius laevigatus* against western flower thrips, under greenhouse conditions. In addition to thrips, we monitored the growth and spread of introduced beneficial organisms. The latter was based on collected flower samples. We also evaluated the relationships between beneficial organisms and greenhouse climate data. Introduction of natural enemies has proven a successful means of biological plant protection, as thrips did not cause economic losses in our experiment.

2. Methods

2.1 Presentation of study area

Experiments were conducted under greenhouse conditions in Glodeni, 15 km away from city of Târgu-Mureș, in the Transylvania region of Romania (Figure 1).

Figure 1. Satellite view of the greenhouse area in Glodeni, 15 km away from city of Târgu-Mureș, Transylvania region, Romania, where the experiments were conducted

The greenhouse consisted of an air-inflated, double layer polyethylene structure, with a 4000 m² area, where the ventilation was provided by the doors located at the end of the greenhouse. The ventilation was controlled by an automatic system (Pola FP line, computerized control of an agricultural environment which changing air is mainly controlled by windows actioning, Italy). Since 2012, soilless cultivation was used (Cocogreen® SaladFusion™ Growbags, United Kingdom). Pepper plants chosen for the experiment were *Bibic F1* (Duna-R, Hungary) from Szentes, Hungary. Plants were grown in twin rows, at a density of 4 plants/m², being trellised to a two-stem pruning system. Altogether 14 800 plants were used in the experiment. In early years, attempts were made to control the thrips species with chemical plant protection, but this method ceased to be successful (because of preharvest interval, PHI), causing 10-15%

yield loss. Supplementary treatments against pathogens and pest used in the greenhouse are listed in Table 1. An attempt was made to introduce a new method that involves biological control, as well as the use of beneficial organisms. Starting 2014, biological control strategy was initiated. The presence of pest species was checked by regular examination of the plants and flowers. At the time of introduction, the predatory species *Amblyseius swirskii* and the pirate bug *Orius laevigatus* were launched. Quantities used in the experiment are shown in Table 2.

Table 1. Treatments applied in the greenhouse

Date of application (2014)	Pesticide name	Active substance	Applied doses	Method of application
3/27	Biosol	potash soap	2 l/ha	foliar spray
4/9	Teppeki 50 WG	flonicamid	0.14 kg/ha	drench
4/25	Biosol	potash soap	2 l/ha	foliar spray
5/5	Topas 100 EC	penconazole	0.5 l/ha	fog
5/13	Teppeki 50 WG	flonicamid	0.14 kg/ha	drench
5/27	Amistar	azoxystrobin	0.75 l/ha	fog
6/12	Sythane Duplo	myclobutanil	0.5 l/ha	fog
6/25	Amistar	azoxystrobin	0.75 l/ha	fog
7/10	Sythane Duplo	myclobutanil	0.5 l/ha	fog
7/24	Topas 100 EC	penconazole	0.5 l/ha	fog
8/9	Amistar	azoxystrobin	0.75 l/ha	fog
8/22	Topas 100 EC	penconazole	0.5 l/ha	fog
8/30	Sythane Duplo	myclobutanil	0.15 l/ha	fog
9/6	Topas 100 EC	penconazole	0.5 l/ha	fog
9/20	Amistar	azoxystrobin	0.75 l/ha	fog
10/6	Amistar	azoxystrobin	0.75 l/ha	fog
10/17	Topas 100 EC	penconazole	0.5 l/ha	fog

Amblyseius swirskii bags containing adults and larvae were hung on every 4th plant at the height of the first branch. This density enabled the spread and reproduction of predatory mites. The pirate bug, *Orius laevigatus* was applied twice, by the same method. Pruned green plant parts were left in the greenhouse for additional 2-3 days, to allow beneficial organisms to get back to the plants.

2.2 Sampling

Observations and collection (open blooms) were made every second week from March 26 to October 3, 2014. Each time 50 open blooms were randomly selected and collected into a 5 mL tube, filled with 70% ethanol. Samples were analyzed in the laboratories of Sapiientia Hungarian University of Transylvania, in Târgu Mureș (Romania), by pouring their content into Petri dishes. After examination of the open blooms under stereo microscope (Carl Zeiss, Stemi 305 Stereo, Germany), we prepared the thrips and mites on separate slides and identified the species using a compound microscope (Optika B-510BF, Italy).

2.3 Statistical analysis

Data were analyzed with PAST 3 statistical program. Normality of original data was not met, therefore, the nonparametric Kruskal-Wallis test was used, followed by Mann-Whitney U tests to compare varieties (Hammer et al., 2001). Means with different letters in tables represent statistically significant differences.

Table 2. Release time and the number of natural enemies used in the experiment

Release time	Name of the product	Natural enemies	Used amount/ 4000 m ²
2014.03.21	SWIRSKII-MITE+/500	500 A. swirskii/ bag	1000 bags
2014.03.21	THRIPOR laev. / 500	500 O. laevigatus/box	16 boxes
2014.06.27	THRIPOR laev. / 500	500 O. laevigatus/box	16 boxes

3. Results and discussion

During the experiment, a total of 705 mites were collected, and of them 704 were found to be *Amblyseius*

swirskii. One mite could not be identified. In case of thrips, we collected altogether 366 specimens all of them being western flower thrips (*Frankliniella occidentalis*). At first sampling, the number of thrips individuals was very small, but started to increase, and reached a maximum in mid-July (3.3 thrips/flower). Eventually, their number decreased significantly, owing to the introduction of predatory bugs. After releasing the natural enemies, we examined predatory mites in pepper flowers, and we noticed that as the temperature and the population of pirate bugs increased, predatory mites fled to the pepper leaves. So, after May 21, we started examining predatory mites not only in flowers, but also in 5 randomly selected pepper leaves. We experienced the largest number of predatory mite individuals on July 16. At that time, an average of 11,375 predatory mites were found on one single pepper plant. Subsequently, we investigated the development of mites in correlation with humidity. When the number of hours and days with unfavorable relative humidity increased, the number of predatory mites decreased suddenly. Figure 2 shows the number of hours below 15 °C that adversely affected the development of *Orius laevigatus*. After the introduction of pirate bugs, the number of hours below 15 °C began to increase, causing a decrease in the number of predatory bugs. In July, another pirate bug installation was done; at that time, the number of hours with unfavorable temperatures was already zero, which helped the predatory bug to reproduce and develop. From the end of August, the number of hours below 15 °C increased again, with the simultaneous decrease in the number of *O. laevigatus*. Figure 3 exhibits the relationship between pirate bugs *Orius laevigatus* and blooming flowers. After the introduction of *Orius laevigatus*, the number of flowers started to increase, which was favorable for *O. laevigatus*, as well. After April 17, 2014, because of cold temperatures, the number of blooming flowers began to decrease significantly, which affected adversely the pirate bugs, so, the number of their individuals also decreased.

Figure 2. Dependence of the number of *O. laevigatus* individuals on critical temperature, from March to September 2014. (Mann-Whitney U test, $p < 0.05$). Different letters indicate statistically significant differences.

Figure 3. Interaction between the number of *O. laevis* individuals and blooming flowers. Measurements were performed in year 2014. (Mann-Whitney U test, $p < 0.05$). Different letters indicate statistically significant differences.

Figure 4. Dependence of the number of *A. swirskii* individuals on critical temperature, from March to September 2014. (Mann-Whitney U test, $p < 0.05$). Different letters indicate statistically significant differences. (Mann-Whitney U test, $p < 0.05$). Different letters indicate statistically significant differences.

Figure 5. Interactions between the number of *A. swirskii* individuals and Thrips. Measurements were performed in year 2014. (Mann-Whitney U test, $p < 0.05$). Different letters indicate statistically significant differences.

Figure 6. Relationship between the number of *A. swirskii* individuals and thrips. Dates refer to year 2014. (Mann-Whitney U test, $p < 0.05$). Different letters indicate statistically significant differences.

To overcome this situation, another installation was carried out in July 2014, as mentioned above. During the examination period, the temperature was almost adequate for predatory mites, until the end of summer. Figure 4 reveals that there were a very few hours in March, when the temperature dropped below 15 °C. Nevertheless, from late April to mid-May, the number of hours with temperatures below 15 °C began to increase. From the end of May to the end of August, temperatures became more favorable for predatory mites, hence, their number started to increase until the end of September. In Figure 5 we show the interaction between the number of *Orius laevigatus* and thrips. After the first introduction of *Orius laevigatus*, the number of thrips increased slightly, given that pirate bugs regulated the reproduction. As a result of cold temperatures in April-May, the number of *O. laevigatus* decreased, causing a sudden increase in the number of thrips. In July, after the second introduction, pirate bugs diminished the number

of thrips successfully, again. Figure 6 exhibits the relationship between the number of *Amblyseius swirskii* predatory mites and thrips. After the introduction of the predatory mite, both the number of thrips and the number of predatory mites started taking off. In mid-July, both mites and thrips reached their maximum number. Owing to the high number of individuals and predation, starting August the number of thrips decreased significantly. Figure 7 provides insight in the interaction between the *Orius laevigatus* and the *Amblyseius swirskii*. As a result of low temperatures at the end of April and beginning of May, the number of pepper flowers decreased, consequently, the number of pirate bugs also decreased. At the same time, it was noticed that predatory mites migrated back from the leaves to the flowers. As it has proven unable to control the thrips on their own, *Orius laevigatus* population was reinstalled. Following reinstalling, predatory mites migrated back from flowers to the leaves.

Figure 7. Interaction between the number of *A. swirskii* individuals and *O. laevigatus* (Mann-Whitney U test, $p < 0.05$). Dates refer to year 2014. Different letters indicate statistically significant differences.

4. Conclusions

After the installation of *Orius laevigatus*, natural enemies of thrips, the number of pirate bugs started growing rapidly. Possibly as a result of intra-guild predation by *O. laevigatus*, a lower population of *A. swirskii* was observed as compared to the experiments reported by Calvo et al. (2012), and the population of mites did migrate from flowers to leaves. In addition, Farkas et al. (2016) demonstrated that under laboratory conditions *Orius laevigatus* nymphs can prey on *Amblyseius swirskii*. Other authors reported the risks caused by combined release of mites and pirate bugs, triggered by intraguild predation (Skirvin et al., 2007), (Shakya et al., 2009). As a result of low temperatures in April 2014, pepper flowers became sparse, which resulted in a sudden decline in the number of *O. laevigatus* individuals. According to Sanchez and Lacasa (2002) *O. laevigatus* population could remain at low levels or at least decline, because of the lack of sufficient thrips preys. As the number of pirate bugs decreased, thrips began to multiply in the flowers, getting rid of the pressure caused by predators. First it was observed that the number of thrips in flowers increased, which caused some damage in the crops. Then, mites returned to the flowers from leaves because of the decrease in number of individuals of pirate bugs. As the number of thrips steadily increased, it was necessary to introduce another predatory bug population for preventing subsequent damage. After reinstallation, climatic conditions became favorable, so the *O. laevigatus* population started to grow and the number of thrips suddenly decreased. Overall, we can conclude that the applied biological plant protection has proven satisfactory in the protection against thrips. The population density remained below the estimated economic threshold range of 0.7-2.1 thrips individuals per flower, reported by Park et al. (2007).

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