

The potential of arthropod assemblages against thrips and aphids in  
greenhouse pepper

PhD thesis

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## 1. INTRODUCTION AND OBJECTIVES

Biological pest management has several advantages over chemical control: resistance builds up less easier; the efficiency against thrips is high; no pre-work or pre-harvest intervals are needed; there are no harmful residues or phytotoxic effects; the impact on the environment is limited; and the demand for manual labor is low (van Lenteren 2000). Despite the advantages of biological pest management, only 5% of the total area of Hungarian greenhouse cultures, approximately 260 hectares, is controlled with biological methods. The ratio of biological control in greenhouse pepper is only 1.5% and it is based on the combined use of the predatory mite *Neoseiulus (Amblyseius) cucumeris* (Oudemans) and the pirate bug *Orius laevigatus* (Fieber) against thrips (Budai et al., 2006). Since none of these natural enemies are endemic to Hungary (Bozai 1997, Kondorosy 1999, Ripka 2006), their application is expensive. To this end the Institute of Plant Protection, Szent István University has launched a research to examine the potential of one of the most frequent (Bogya and Markó 1999, Tóth and Kiss 1999, Samu and Szinetár 2002) spider species of the Hungarian agricultural fields, that is the common crab spider *Xysticus kochi* (Thorell) against thrips. We trusted to find an effective and inexpensive method for the biological control of pests. Isolated single-plant experiments (Nagy et al. 2007, Zrubecz et al. 2007), as well as farm-sized studies in plastic tunnels (Bán et al. 2007) have both concluded that *X. kochi* collected from alfalfa (*Medicago sativa* Linnaeus) and raised in the laboratory did reduce the damage by *F. occidentalis*. However, the considerable time and labor demand of field collection, selection and laboratory rearing of *X. kochi*; and the high rate of spider mortality during these processes did define a certain limit as to the potential range of use of this control method. And although the method using the common crab spider as a sole agent proved inappropriate, the experiences gathered helped us to find a completely inexpensive, simple-to-use and pesticide free approach in pest management.

With regards to increase the role of biological control in greenhouse production, we aimed to find alternative and chemical-free pest management methods that are reasonably priced and at the same time have the same control potential as the expensive, state-of-the-art methods that are commercially available. Introducing arthropod assemblages into polytunnels without any selection reduces the time needed for collection and storage. The lack of selection, however, implies that besides predators, phytophagous arthropods may also be introduced.

We studied long-flowering, abundant wild plants to find arthropod predators that can act as natural enemies of the pests of greenhouse pepper. We wanted to determine the interval

when the number of potential predators is the highest. We examined what controlling effect may the introduction of arthropod assemblages collected from different types of plant communities have in the biological pest management of single plant experiments and in farm-size pepper production. In order to assess the risk of this new approach we studied the phytophagous thrips population of the collected assemblages and arthropod predators alike.

Our secondary aim was to investigate the thrips fauna of greenhouse pepper in the Jászág region and to determine the spread of the western flower thrips since its first appearance in Hungary in 1989. Our tertiary aim was to examine the flower bug assemblages of greenhouse pepper and determine the spectrum of endemic predator species that may play a role in the biological pest management of pepper; and to determine the daily range of the number of predatory flower bug (*Orius* species) adults in greenhouse pepper.

## 2. MATERIALS AND METHODS

### 2.1. Assemblages of alfalfa, nettle, danewort and chervil

Since our aim was to study the ability of arthropod assemblages captured in weeds to supply polytunnels with natural enemies, and the number of individuals and its species composition, we sampled alfalfa (*Medicago sativa*) and nettle (*Urtica dioica*) with sweepnets between 2006 and 2008. During these three years, we sampled alfalfa 24 times with a total of 1025 sweeps, whereas nettle was sampled 26 times, with 1125 sweeps. We sampled danewort (*Sambucus ebulus*) and chervil (*Anthriscus cerefolium*), but found that these two were difficult to sweep and the durations of their flowering periods were short. Due to these experiences, danewort and chervil were sampled only thrice in the year 2007, with a total of 130 and 150 sweeps, respectively.

All collected arthropod specimens were stored in plastic wraps in a refrigerator prior to species determination and in 70% ethanol solution afterwards. Minute pirate bugs (*Orius* spp.), spiders (*Araneae*), ladybugs (Coleoptera: Coccinellidae), phytophagous thrips (Thysanoptera) and predatory thrips (*Aeolothrips intermedius*) were counted and determined to species level. For species determination, we used the following literature: Péricart (1972) for *Orius* species, Heimer and Nentwig (1991) for spiders, Bährmann (2000) for ladybugs and Jenser (1982) for predatory thrips.

## **2.2. The effect of mixed arthropod assemblages in isolated single-plant experiments**

In years 2006 and 2007 we isolated plants to study the effect of arthropod assemblages collected from nettle, alfalfa and danewort. Since we infected one half of the plants artificially with thrips, we had the following set of eight treatments in eight repetitions in both years:

- 1. nullcontrol** (no thrips and no arthropods introduced)
- 2. control** (thrips only)
- 3. nettle** (arthropods swept from nettle)
- 4. nettle + thrips** (arthropods from nettle and thrips)
- 5. alfalfa** (arthropods swept from alfalfa)
- 6. alfalfa + thrips** (arthropods from alfalfa and thrips)
- 7. danewort** (arthropods swept from danewort)
- 8. danewort + thrips** (arthropods from danewort and thrips)

During artificial infection, we introduced thrips adults and larvae to each isolator. The average number of thrips per isolators was 11,70 ( $\pm 3,24$ ) in 2006 and 6,05 ( $\pm 1,78$ ) in 2007. In order to introduce the assemblage of arthropods we sampled both vegetations with 10 sweeps per pepper individuals per occasion.

We sampled the stand in every 10-14 days. When peppers were ripe for harvest, we estimated the size of the area damaged by thrips and other pests; measured the weight of the crop and divided it into quality classes.

## **2.3. The effect of mixed arthropod assemblages in farm-size studies**

We studied commercial pepper plantations (225-400m<sup>2</sup>, 0.055-0.098 acres) in the Jászág and in the Gödöllő regions of Hungary in 2006 and 2007. There were seven locations in both years, with two (an experimental and a control) polytunnels of the same size in each location. Overall, we examined 28 polytunnels during the survey period.

Polytunnels displayed the same number of plants, pepper variety, method of growing and production technology within pairs; whereas pest management methods were different. In order to control pests within polytunnels, we introduced arthropods swept from alfalfa and nettle into treated greenpepper stands. There were 10 chemical treatments in the treated polytunnels in 2006 and 13 chemical treatments in 2007. During the years, control polytunnels needed 29 (and two additions of biological control) and 38 chemical treatments.

We evaluated the potential of mass collected arthropod assemblages against sweet pepper pests by analyzing thrips, aphid and *Orius* content of pepper flowers and also by the crop yield at the end of the growing season.

## **2.4. Introduction methods**

In 2008 we set up five polytunnels in Pusztamonostor to study the effects of two different types of introduction (called A and B) in two dosages (single and triple). We had one polytunnel as a null control. Introduction type A was one introduction per week; type B was three successive introductions per one week; where a single dose refers to introducing 1 sweep of arthropods per 10 pepper individuals; a triple dose (marked as '3x') refers to 3 sweeping per 10 peppers.

Produced by the farmer, all seedlings were checked visually for any signs of pests in every week, prior to having them planted out into polytunnels. Despite these measures, there were 300 seedlings in polytunnel 'A 3x' that were produced elsewhere. There were western flower thrips individuals found in the flowers of these plants soon after they were planted out into the polytunnel.

We introduced arthropod assemblages collected from alfalfa and nettle. In order to quantify the differences between the pest controlling efficiency of the various arthropod introductions, we evaluated and compared the number of thrips, aphids and flower bugs found in the stand and also we measured the size of the damaged surface of the crop.

## **2.5. The daily activity of flower bugs**

In order to study the daily activity of flower bugs we counted the number of *Orius* adults in 2-3 polytunnels per each location in three locations in 2007 and 2008. There were 20 'counting days', consisting of six countings (we examined 200 pepper flowers per each one done in every two hours between 8 am and 6 pm).

## **2.6. Statistical analysis**

For the statistical analysis of our data, we used STATISTICA (StatSoft 6.1). Normality of data was checked by the Kolmogorov-Smirnov adjustment test and a Levene test was used to verify the homogeneity of variance.

We used a one-way ANOVA test in isolated single plant experiments, for comparing different introduction methods and for studying the daily activity of flower bugs. For farm size experiments however, data were compared with a two-way ANOVA. Using ANOVA results, multiple comparisons were done with a Neuman-Keuls test. Our null hypothesis was tested at a significance level of 0,05.

### 3. RESULTS AND DISCUSSION

#### 3.1. Assemblages of alfalfa, nettle, danewort and chervil

##### Species composition

##### *Flower bugs (Orius spp.)*

According to our findings, the most frequent *Orius* species of alfalfa, nettle and danewort were *O. niger* and *O. minutus* (Table 1). We consider this species composition favourable because according to authors (Veire and Degheele 1992, Disselvet et al. 1995, Kohno and Kashio 1998) these particular flower bug species were found suitable in the biological control of thrips. *Orius* species also have the advantage of feeding on other pests including aphids, greenhouse whiteflies, mites and bollworm eggs (Rácz 1989, Alvarado et al. 1997, Sigsgaard and Esbjerg 1997, Blaeser et al. 2004, Rutledge and O'Neil 2005).

##### *Ladybugs*

The three most frequent ladybug species of alfalfa and nettle (Table 1) were *Propylea quatuordecimpunctata*, *Coccinella septempunctata* and *Hippodamia variegata*, all proved highly abundant in alfalfa and nettle stands by a number of earlier observations (Schmid 1992, Zhou and Carter 1992, Lövei 1989, Nicoli et al. 1995, Kalushkov and Hodek 2004, 2005, Burgio et al. 2006). These species, adults or larvae alike, are able to consume a considerable amount of aphids (Lövei 1989, Omkar and Srivastava 2003).

Although ladybugs need to feed on aphids to complete their development, they also feed on thrips, mites, scale insects and on other soft-bodied arthropods (Lövei 1989, Triltsch 1999). According to a study on the seven-spot ladybird, a 1:30 predator:prey ratio provides for an adequate control of onion thrips (Deligeorgidis et al. 2005).

The only disadvantage of ladybugs is that their adults would leave the area as soon as their potential prey items vanish from the polytunnel (Lövei 1989). However, as long as there is prey to feed on, ladybugs are always present on highly infected plants. The rate of migration is directly related to the abundance of aphids (Krivan 2008).

##### *Spiders*

Spiders as generalist predators feed on a broad spectrum of insects (Riechert and Lockley 1984, Nyffeler 1999). According to our samplings and captures, all four weed stands were dominated by juvenile spiders (Table 1) feeding mainly on soft-bodied arthropods including thrips and aphids (Nyffeler et al. 1994).

Although spiders are generalist predators, they do have a preference for certain hunting methods and micro-habitats, which means that most spiders specialize in certain habitats (Nyffeler et al. 1994, Marc és Canard 1997, Marc et al. 1999, Nyffeler 1999). Alfalfa and danewort for example was dominated by the Thomisiid family, while Philodromid spiders preferred nettle and chervil instead. Both families rely on hunting, a method more appropriate for controlling pests than web-building (Young és Edwards 1990). Hunting spiders have a relatively large prey spectrum: they feed on orthopterans, homopterans, heteropterans, lepidopterans, thrips, dipterans, hymenopterans and even on some coleopterans as well (Nyffeler et al. 1994, Marc and Canard 1997). Considering the role of spiders in pest control it is favourable that besides hunting spiders, various types of web-builders were also present in the samples. Assemblages composed of spiders with different hunting strategies can prey more and can be more effective than an assemblage of spiders with similar strategies (Marc et al. 1999, Riechert 1999, Sunderland 1999).

### *Thrips*

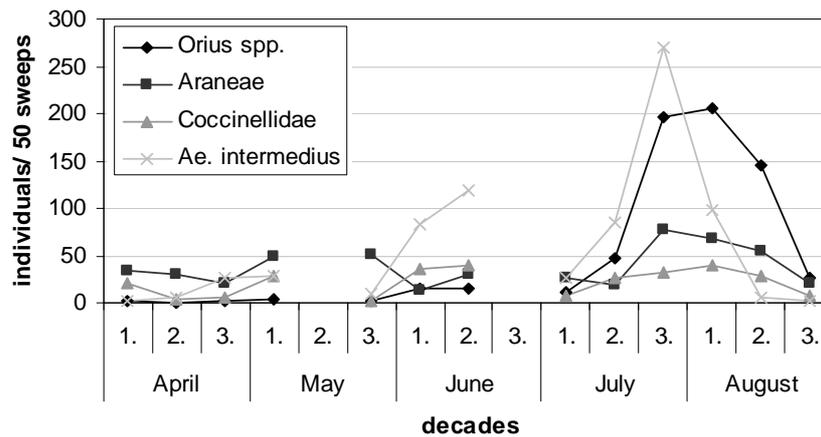
Besides predatory thrips, most of the thrips fauna of alfalfa is composed of phytophagous species that feed on a wide range of plants, so when introduced to the polytunnel, they do present a threat to pepper (Table 1.) Also captured there are phytophagous thrips in low abundancy that do not damage the pepper such as *Odontothrips confusus* and *Sericothrips bicorinis* that dwell on leguminous plants; or *Aptinothrips* and *Limothrips* species that inhabit grasses (Jenser 1982). Arthropod assemblages captured from nettle proved less harmful than those collected from alfalfa. The two most important phytophagous thrips of green pepper, *T. tabaci* and *F. occidentalis* were also present in nettle, but to a significantly lesser extent: 7% and 1%, respectively. Similarly to assemblages captured in alfalfa, the most frequent thrips species of danewort and chervil was the onion thrips.

**Table 1** The most frequent *Orius* spp., ladybug and thrips species and spider families captured by sweepnet (Jászszág and Gödöllő, 2006-2008)

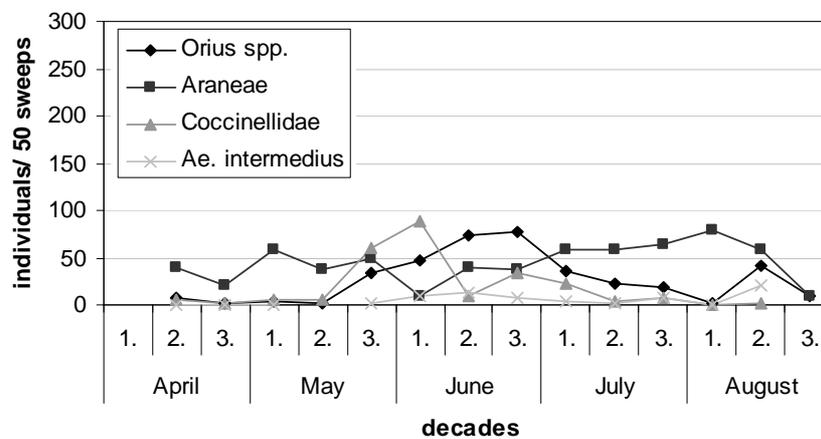
		<b>Alfalfa</b>	<b>Nettle</b>	<b>Danewort</b>	<b>Chervil</b>
<b><i>Orius</i> spp.</b>	larvae	24%	52%	45%	33%
	adult	76%	48%	55%	67%
	number of species	3	3	3	1
	most important species	<i>O. niger</i> 76% <i>O. minutus</i> 20% <i>O. majusculus</i> 3%	<i>O. niger</i> 64% <i>O. minutus</i> 35% <i>O. majusculus</i> 1%	<i>O. niger</i> 51% <i>O. minutus</i> 46% <i>O. majusculus</i> 3%	<i>O. majusculus</i> 100%
<b>Araneae</b>	juvenile	71%	64%	79%	43%
	subadult	10%	12%	11%	33%
	adult	17%	20%	6%	17%
	number of families	15	14	11	8
most important families	Thomisidae 42% Therididae 14% Araneidae 12% Linyphiidae 9% Philodromidae 8%	Philodromidae 41% Thomisidae 22% Araneidae 10% Therididae 9%	Thomisidae 69% Araneidae 10%	Philodromidae 47% Araneidae 24% Thomisidae 20%	
<b>Coccinellidae</b>	larvae	19%	13%	0%	0%
	adult	81%	87%	100%	100%
	number of species	10	9	5	3
	most important species	<i>Propylea quatuordecimpunctata</i> 26% <i>Coccinella spetempunctata</i> 25% <i>Hippodamia variegata</i> 23%	<i>Propylea quatuordecimpunctata</i> 44% <i>Coccinella spetempunctata</i> 21% <i>Hippodamia variegata</i> 11% <i>Adalia bipunctata</i> 10%	<i>Propylea quatuordecimpunctata</i> 33% <i>Coccinula quatuordecipustulata</i> 25%	<i>Propylea quatuordecimpunctata</i> 66%
<b>Thysanoptera</b>	larvae	19%	36%	40%	8%
	adult	81%	64%	60%	92%
	number of species	26	23	7	7
	most important species	<i>Thrips tabaci</i> 35% <i>Aeolothrips intermedius</i> 28% <i>Frankliniella intonsa</i> 17% <i>Frankliniella occidentalis</i> 5%	<i>Thrips utricae</i> 77% <i>Thrips tabaci</i> 7% <i>Frankliniella occidentalis</i> 1%	<i>Thrips tabaci</i> 49% <i>Thrips utricae</i> 46%	<i>Thrips minutissimus</i> 40% <i>Thrips tabaci</i> 35% <i>Frankliniella intonsa</i> 15%

### Number of individuals collected

According to the average capture results of the three year study, alfalfa stands were able to supply natural enemies of pepper pests in great abundance between late July and early August (Figure 1), whereas from nettle, the chance to collect predatory arthropods in significant numbers was the highest between the third decade of May and the beginning of July (Figure 2).



**Figure 1** Number of predatory arthropods collected from alfalfa per decade (based on the average sweepnet capture of years 2006-2008)



**Figure 2** Number of predatory arthropods collected from nettle per decade (based on the average sweepnet capture of years 2006-2008)

### **3.2. The effect of mixed arthropod assemblages in isolated single-plant experiments**

Arthropod assemblages collected from various weeds were unable to reduce thrips damage, that exceeded economical threshold by varying between 2-5%, treatment pending. We also have to note however, that in order to easily recognize and monitor any damage done by thrips in our isolated single-plant experiments, we chose a variety that was claimed highly

sensitive to thrips damage (Emese). We assume that the level of thrips damage would have been significantly lower, had we used a resistant pepper variety. The relatively high level of damaged surface in null control peppers especially in 2006 may also be attributed to the fact that we used a sensitive variety. Contrary to our expectations, the nature and range of thrips damage on peppers not infected with thrips and on artificially infected peppers was found similar. In other words, introducing phytophagous thrips did not increase the level of damage. One possible reason behind this observation is that at a certain, high level of density, thrips began to compete with each other.

Although the level of thrips damage remained uncontrolled, other, non-thrips damages increased when the arthropod assemblages were introduced into the plant isolators. The most frequent pest damages were done by cotton bollworms and snails. We have also noticed viral damages on many of the plants. Although we did not test the pepper plants for the actual viral infection, the sightings of secondary damages allowed us to suspect the presence of aphids as vectors. Our overall impression was that introducing arthropods reduced both the quantitative and the qualitative yield of pepper.

According to our isolated single-plant experiments the origin of the arthropod assemblages had no significant influence on the yield of isolated green pepper. Whether collected from alfalfa, nettle or danewort, none of the arthropod assemblages were effective in controlling the pests of polytunnel pepper.

That fact that the amount of arthropods per plant was a hundred times more in the isolated single-plant experiments than in the farm-size studies may account for the former having been significantly less successful than the latter. If we had introduced the same (large) rate of collected arthropods to the polytunnels used in farm-size studies, than we would have probably experienced its negative effects there as well. Since there is a definite need to study the effect of the origin of the collected arthropod assemblages in isolated single-plant experiments, we suggest using the same or similar amount of arthropods as applied in farm-size studies, that is 1-3 sweeps per plant.

### **3.3. The effect of mixed arthropod assemblages in farm-size studies**

#### *Crop yield*

We found no significant difference between the economic value of pepper harvested in experimental and control polytunnels. During the two-year experiment, in all surveyed locations, pepper yield of treated polytunnels was 1,58% higher than that of control polytunnels. The quality and quantity of the pepper yield of treated and control tunnels were

similar in both years. The amount of first class quality pepper collected in control polytunnels exceeded that of treated polytunnels with 2,51 %; while in the case of second class pepper with 1,61 %.

*Species composition of the thrips and flower bug fauna of pepper flowers*

Three native *Orius* species were found in both years in treated and control polytunnels (Table 2). In year 2006, in Boldog 1, *O. laevigatus* has been released by farmers but the recapture was scarce, as no individuals were found in 2007. *O. niger* was the most abundant species in treated and control polytunnels. The second most frequent *Orius* species of pepper flowers was *O. minutus* in 2006, whereas in 2007, it was *O. majusculus*.

**Table 2** Percentage distribution (mean  $\pm$  SD) and the amount of totally collected individuals of *Orius* spp. and phytophagous thrips populations of experimental and control polytunnels (Hungary, Jászság and Gödöllő regions, 2006-2007)

	treated polytunnels			untreated polytunnels		
	mean	SD	individual	mean	SD	individual
<b>2006</b>						
<i>Orius</i> larvae	<b>56,86</b>	$\pm 6,29$	<b>240</b>	<b>56,68</b>	$\pm 8,27$	<b>172</b>
<i>Orius</i> adults	<b>43,13</b>	$\pm 6,29$	<b>151</b>	<b>41,32</b>	$\pm 8,27$	<b>101</b>
<i>O. laevigatus</i>	0,00		0	1,39	$\pm 1,39$	1
<i>O. majusculus</i>	7,25	$\pm 2,28$	11	7,73	$\pm 3,05$	7
<i>O. minutus</i>	15,82	$\pm 3,72$	23	10,71	$\pm 2,32$	10
<i>O. niger</i>	76,30	$\pm 4,56$	117	80,19	$\pm 3,13$	83
<b>thrips larvae</b>	<b>44,96</b>	$\pm 4,77$	<b>1582</b>	<b>41,31</b>	$\pm 4,29$	<b>659</b>
<b>thrips adults</b>	<b>55,04</b>	$\pm 4,77$	<b>1940</b>	<b>58,69</b>	$\pm 4,29$	<b>1015</b>
<i>Aeolothrips intermedius</i>	0,52	$\pm 0,16$	11	1,91	$\pm 0,63$	21
<i>Frankliniella intonsa</i>	19,45	$\pm 3,35$	402	28,60	$\pm 6,32$	390
<i>Frankliniella occidentalis</i>	49,56	$\pm 5,44$	949	33,05	$\pm 4,16$	301
<i>Thrips</i> spp.	21,76	$\pm 2,10$	452	31,44	$\pm 4,10$	264
Other species	8,70	$\pm 2,87$	126	5,00	$\pm 1,25$	39
<b>2007</b>						
<i>Orius</i> larvae	<b>50,16</b>	$\pm 5,00$	<b>401</b>	<b>47,01</b>	$\pm 4,30$	<b>337</b>
<i>Orius</i> adults	<b>49,84</b>	$\pm 5,00$	<b>352</b>	<b>52,99</b>	$\pm 4,30$	<b>383</b>
<i>O. laevigatus</i>	0,00		0	0,00		0
<i>O. majusculus</i>	7,68	$\pm 2,01$	24	19,45	$\pm 4,77$	59
<i>O. minutus</i>	6,08	$\pm 1,73$	26	8,91	$\pm 1,68$	36
<i>O. niger</i>	86,29	$\pm 2,23$	302	71,64	$\pm 4,53$	288
<b>thrips larvae</b>	<b>46,65</b>	$\pm 4,35$	<b>5251</b>	<b>44,39</b>	$\pm 5,04$	<b>3179</b>
<b>thrips adults</b>	<b>53,35</b>	$\pm 4,35$	<b>5786</b>	<b>55,61</b>	$\pm 5,04$	<b>4197</b>
<i>Aeolothrips intermedius</i>	2,86	$\pm 0,87$	105	5,35	$\pm 1,41$	170
<i>Frankliniella intonsa</i>	14,86	$\pm 2,64$	926	22,00	$\pm 3,17$	1102
<i>Frankliniella occidentalis</i>	39,57	$\pm 7,77$	2751	25,84	$\pm 4,84$	845
<i>Thrips</i> spp.	39,96	$\pm 6,69$	1796	44,73	$\pm 5,80$	1987
Other species	2,76	$\pm 1,02$	208	2,07	$\pm 0,52$	93

The notion of the natural settling (immigration) of native *Orius* bugs is supported by the fact that in 2007, the second most frequent species of treated and control polytunnels besides

*O. niger* was not *O. minutus* that occurred both in alfalfa and nettle, rather, it was *O. majusculus*. Bosco et al. (2008), conducting a survey in Northern-Italy, among continental climatic conditions similar to those found in Hungary also proved that the two most frequent species of greenhouse pepper were *O. niger* and *O. majusculus* that settled around late June or early July into pesticide-free polytunnels.

Phytophagous thrips represented 95-99% of the total thrips population of polytunnel samples (Table 2). In treated polytunnels in 2006, the dominant thrips species was *F. occidentalis*, while in 2007, *F. occidentalis* and *T. tabaci* had the same occurrence rate. In control tunnels in 2006 phytophagous thrips populations were composed of *F. occidentalis*, *T. tabaci* and *Frankliniella intonsa* (Trybom) with nearly similar abundance, while in 2007 the dominant species was *T. tabaci*.

#### *The number of Orius individuals per flower*

The number of *Orius* bugs was significantly higher (ANOVA, F (1, 112)= 8,68, p= 0,004) in treated polytunnels than in control ones.

In year 2007, *Orius* populations of experimental and control polytunnels showed no significant difference (ANOVA, F (1, 222)= 0,07, p= 0,789).

In the year 2006 there were 10-15 chemical treatments in the untreated polytunnels against thrips. At the same time, the lack of pesticides and the introduction of mixed arthropod assemblages definitely had a positive effect on the number of flower bugs.

Since Biobest and Koppert advise the introduction of 50-1,000 *Orius* individuals per 100m<sup>2</sup> (Ocskó 2010) (considering 5 peppers/m<sup>2</sup> and 4 flowers/pepper plants) the calculated amount for preventive purposes is 0.025 individuals/flower, 0.05 individuals/flower for controlling moderate cases and 0.5 individuals/flower for severe ones.

In 2006 the average density of flower bugs in both experimental polytunnels was found 3-4 times larger than the suggested amount needed to control moderate cases (0.05 individuals/flower). The relatively high number of individuals may be explained by the lack of chemical control. The only measure taken was a treatment against cotton bollworms, that is, the introduction of *Bacillus thuringiensis*, a measure that has no effect on flower bugs. We also have to note that these two polytunnels were located on the outskirts of the settlement, where the surroundings may have also contributed to the natural immigration of beneficial arthropods into the polytunnels.

Considering that no *Orius* bugs were artificially introduced to and no pesticides were applied in the two null control polytunnels at locations Gödöllő and Jászfényszaru 3 in 2007,

their exceptionally high *Orius* content (0.35 individuals/flower; that is 700 individuals/100m<sup>2</sup>) suggests that these arthropods must have naturally immigrated from outside as well. From late June to early July the number of *Orius* sp. approached 1 per flower in these locations (2000 individuals/100m<sup>2</sup>), which was twice the amount deemed suitable to control a severe thrips infestation.

The amount of *Orius* bugs needed to control an average level of thrips infestation was exceeded in five experimental polytunnels in both years; whereas there were only four control polytunnels in 2006 and three in 2007 where the average number of *Orius* bugs reached or exceeded said level. Except for two, extremely infected polytunnels, the average thrips infection of the polytunnels never exceeded the economic threshold of 1 thrips per flower.

According to our findings, a continuous presence of 0.05-0.1 *Orius* bugs per flower (that is: 1-2 individuals/m<sup>2</sup>) can successfully keep the number of thrips below the level of economic importance. In the case of a suddenly raised thrips invasion however, the above mentioned amount of *Orius* bugs (0.025 individuals/flower or 0.5 individuals/m<sup>2</sup>) is not enough, not especially in the case of the western flower thrips. Similarly to our findings, Schelt in the Netherlands (1999) and Tommasini and Maini in Italy (2001) have also found 1-2 *O. laevigatus* per square meter a sufficient measure to control thrips in greenhouse sweet pepper. Chamber et al. in Great-Britain however (1993) used five times the amount (that was 5-10 individuals/m<sup>2</sup>) to effectively control thrips for a prolonged period of several months.

#### *The number of thrips per flower*

In year 2006, the number of phytophagous thrips was significantly higher (ANOVA, F (1, 112)= 8,60, p= 0,004) in the experimental tunnels than in the control ones.

In year 2007, the number of phytophagous thrips of experimental and control polytunnels showed no significant difference (ANOVA, F (1, 222)= 3,34, p= 0,068). In contrast with 2006, thrips level exceeded the average of 1 thrips per flower in four experimental and four control tunnels.

The reader is advised to take time to consider the temporal distribution of the thrips population of the four null control polytunnels. The number of thrips was relatively high in all the four polytunnels until mid-June, when it dropped near to nil, the lack of chemical control measures and/or the lack of introducing arthropod assemblages notwithstanding. We assume that the lack of pesticides resulted in predatory arthropods feeding on thrips having been able to immigrate into polytunnels from the surrounding plant stands and natural assemblages.

### The number of aphids per flower

We found no significant difference between the average number of aphids per pepper flowers in treated and in control polytunnels neither in 2006 (ANOVA,  $F(1, 112) = 0,52, p = 0,473$ ) nor in 2007 (ANOVA,  $F(1, 222) = 0,78, p = 0,379$ ).

Aphids were not considered a significant pest in any of the polytunnels in any of the years; although in some cases during the May and June invasion of aphids peppers were treated with paraffin oil.

### 3.4. Different methods of introducing mixed arthropod assemblages

Apart from the effect on phytophagous thrips, the different frequency of introduction and the amount of collected arthropods per introduction had no significant effect on any of the studied arthropod taxa. According to our assumption it was not the method of introduction that counted. In the polytunnel where there were three introductions per week for example, some of the seedlings were initially infected with western flower thrips at planting time. This probably contributed to the significantly higher number of thrips in that particular polytunnel. Our experience points out the importance of pest-free basic material (plants) in biological pest management. We have also found that the western flower thrips did not spread between polytunnels, as its ratio was 35% in the polytunnel with the initial infection and only 1-4% in polytunnels where the initial pepper stock was free of thrips (Table 3).

**Table 3** The percentage distribution of *Orius* and thrips species while examining the effect of the various methods of introduction of arthropod assemblages. (mean  $\pm$  SD) (Pusztamonostor, 2008)

	Null Control		A 1 $\times$		A 3 $\times$		B 1 $\times$		B 3 $\times$	
	mean	SE								
<b><i>Orius</i> larvae</b>	<b>69,77</b>	<b><math>\pm 9,24</math></b>	<b>50,25</b>	<b><math>\pm 8,31</math></b>	<b>54,25</b>	<b><math>\pm 8,39</math></b>	<b>61,01</b>	<b><math>\pm 9,59</math></b>	<b>54,28</b>	<b><math>\pm 8,40</math></b>
<b><i>Orius</i> adults</b>	<b>30,23</b>	<b><math>\pm 9,24</math></b>	<b>49,75</b>	<b><math>\pm 8,31</math></b>	<b>45,75</b>	<b><math>\pm 8,39</math></b>	<b>38,99</b>	<b><math>\pm 9,59</math></b>	<b>45,72</b>	<b><math>\pm 8,40</math></b>
<i>O. majusculus</i>	11,11	$\pm 11,11$	0,00	$\pm 0,00$	0,00	$\pm 0,00$	0,00	$\pm 0,00$	2,78	$\pm 2,78$
<i>O. minutus</i>	11,11	$\pm 11,11$	3,57	$\pm 3,57$	12,50	$\pm 10,03$	5,00	$\pm 5,00$	15,23	$\pm 9,05$
<i>O. niger</i>	77,77	$\pm 14,70$	96,43	$\pm 3,57$	87,50	$\pm 10,03$	95,00	$\pm 5,00$	81,94	$\pm 9,94$
<b>thrips larvae</b>	<b>57,06</b>	<b><math>\pm 6,14</math></b>	<b>46,15</b>	<b><math>\pm 5,07</math></b>	<b>47,95</b>	<b><math>\pm 4,13</math></b>	<b>58,21</b>	<b><math>\pm 4,33</math></b>	<b>53,84</b>	<b><math>\pm 3,84</math></b>
<b>trips adults</b>	<b>42,97</b>	<b><math>\pm 6,14</math></b>	<b>53,85</b>	<b><math>\pm 5,07</math></b>	<b>52,05</b>	<b><math>\pm 4,13</math></b>	<b>41,79</b>	<b><math>\pm 4,33</math></b>	<b>46,16</b>	<b><math>\pm 3,84</math></b>
<i>Ae. intermedius</i>	18,74	$\pm 8,16$	27,04	$\pm 5,58$	16,43	$\pm 4,67$	35,86	$\pm 7,40$	24,53	$\pm 6,67$
<i>F. intonsa</i>	3,20	$\pm 1,62$	10,03	$\pm 2,48$	7,10	$\pm 2,23$	3,43	$\pm 1,54$	5,78	$\pm 1,63$
<i>F. occidentalis</i>	2,71	$\pm 1,33$	0,52	$\pm 0,36$	35,62	$\pm 4,31$	1,43	$\pm 0,70$	2,12	$\pm 1,04$
<i>Thrips</i> spp.	70,85	$\pm 7,88$	61,54	$\pm 6,46$	38,41	$\pm 5,52$	55,32	$\pm 7,28$	64,15	$\pm 6,10$
other species	4,50	$\pm 1,56$	0,88	$\pm 0,43$	2,43	$\pm 0,87$	3,95	$\pm 1,50$	3,41	$\pm 1,76$

We found it interesting that despite of the large number of western flower thrips in the polytunnel where there were three introductions per week, the number of *Orius* species was

the lowest among all polytunnels. Our conclusion was that flower bugs preferred polytunnels where endemic phytophagous thrips were abundant. The range of the ratio of striped thrips in pepper flowers coincides with the findings of Lévyayné and Tóth (2008) who experienced that striped thrips prefer polytunnels infected with onion thrips to polytunnels infected with the western flower thrips, thus concluding that the frequency of predatory thrips is the lowest in polytunnels infected with the western flower thrips. We also have found that given certain conditions, that is when their ratio exceeds 16-36%, predatory thrips species can significantly control the population of phytophagous thrips. Except for the Pusztamonostor location, our experiences however, were the opposite in 2006 and 2007: the ratio of predatory thrips remained as low as between 2-5%, and this low ratio had quite an insignificant potential in pest control.

We found that the number of flower bug individuals in both null control and treated polytunnels (where we introduced arthropod assemblages with different methods) was high enough to provide suitable protection against phytophagous thrips. While the amount of flower bugs deemed necessary for a successful control is 0,05 individuals per flower, in all treated polytunnels the number of flower bugs reached or exceeded 0,1 individuals per flower. We assume that this accounts for the low number of thrips; as the number of thrips per flower remained below the economic threshold of 1 individual per flower even in the polytunnel infected with western flower thrips, and in the case of other treatments, the number of thrips was even lower. We found the importance of aphids similar to that of thrips – they presented no threat to pepper in any of the polytunnels. This we contribute to the fact that during the peak of invasion in May and early June, the number of ladybug adults and larvae was exceptionally high on infected plants. We found as many as four to five pupae which we considered a positive sign of a successful reproduction.

As the figures for the number of individuals of studied pest taxa found within the null control polytunnel were similar in polytunnels controlled by arthropod assemblages, it seems reasonable that given pesticide-free conditions, predatory arthropods are able to migrate into polytunnels at an even frequency to have a sufficient control of pests within.

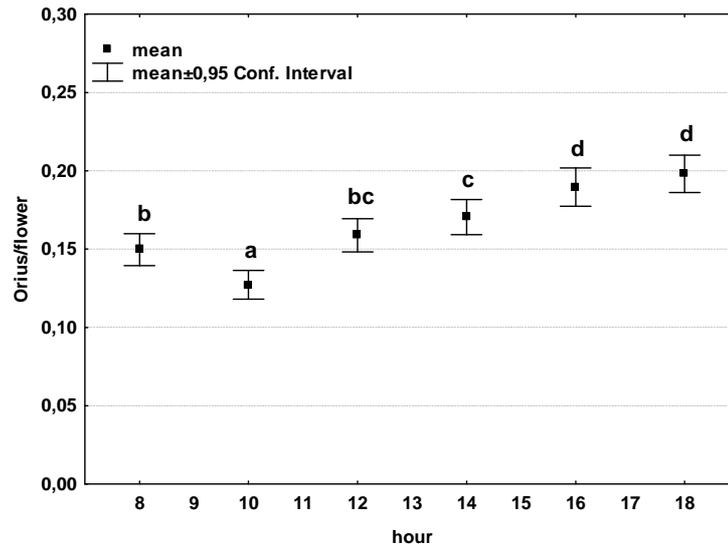
Although the various treatments (different methods of introduction) resulted in no significant difference in the number of predators and phytophagous arthropods, the frequency of introduction and the amount of arthropods introduced per introduction did have an effect on the damaged area. According to our studies, the proportion of the damaged area measured between 0,6-2,6%, which is economically acceptable. We found no important economic

damage in the null control polytunnels either. A series of introductions was able to decrease the size of the damaged area more efficiently than the one introduction per week method. When we increased the per introduction amount of arthropods in a series of introductions, it had no effect on the damaged area. On the other hand, when we increased the amount of arthropods introduced in a weekly introduction, the controlling effect was found increased as well.

We assume that such differences in the size of the damaged area can be attributed to predatory arthropods that dwell outside the flowers. Supposedly spiders played an important role in decreasing thrips damage, because the more frequent the introduction and the higher number of arthropods per introduction the more effective the arthropod assemblage proved to be. Since some of our earlier studies have found that the common crab spider was likely to leave the polytunnel shortly after introduction (Bán 2006, Bán et al. 2007b, Bán et al. 2007c), we assume that other spider taxa follow a similar behavioral pattern. This might account for the single dose per week not being effective enough. Multiple introductions however, kept replacing departing spiders with fresh ones, thus keeping the number of spiders high and their species composition rich enough to provide for an effective pest control. Large doses of spiders were not only able to directly control thrips populations and thrips damage by preying on them; but also had an indirect effect: their presence indirectly urged thrips individuals feeding on the pepper crop to leave the plants (Riechert and Lockley 1984, Marc et al. 1999, Sunderland 1999). Similar observations have been made in studies involving ladybugs (Minoretti and Weisser 2000), so we assume the presence of predators preying on aphids had a similar indirect effect on the size of the damaged area.

### **3.5. The daily activity of flower bugs**

Our two year study found that the number of *Orius* adults per pepper flower significantly varies according to the time of the day (one-way ANOVA,  $F(5, 6114) = 22,11$ ,  $p = 0,000$ ). Their number was decreasing between 8 to 10 AM, and it kept increasing between 10 AM to 6 PM (Figure 9). The number of *Orius* adults within pepper flowers was the lowest at 10 AM (the difference was significant) and was the highest at 4 PM and 6 PM.



**Figure 3** The daily activity of *Orius* adults according to the summarized results (we found no significant difference between two incidents (time-points) bearing the same letter; one-way ANOVA, Newman-Keuls test,  $p > 0,05$ ,  $df = 6108$ )

#### 4. CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

This three-year study has shown that alfalfa and nettle stands were able to supply organic pest control with the natural enemies of pests of greenhouse crops. These generalist predator taxa can be collected in high abundance from mid-May to late June from nettle and due to the infavourable effect of mowing, from June to mid-August from alfalfa. A direct introduction of a non-selected assemblage of arthropods collected from alfalfa and nettle is a cost effective method to protect the infected crop. According to our experience, using a mixed assemblage of arthropods as a supplement to conventional treatment may reduce the amount of pesticides needed against thrips and aphids to one-third of the original amount. Zero selection however, allows the introduction of not only predators, but of phytophagous arthropods (thrips and aphids) as well. These may cause damages to the crop inside the polytunnels. On areas where some of the wild flora is infected with tomato spotted wilt virus (TSWV) the economic importance of this potential threat should be examined prior to incorporating our method into pest control measures as mixed arthropod assemblages may contain the vectors of the viral disease.

If pest control dares to take one step further from using a mixed assemblage of arthropods, a method described and examined in this paper, it may strive to a ‘conserving strategy’ that includes preserving and maintaining the conditions where natural enemies thrive in natural agro-ecosystems (Budai 2006). Experimental locations where no chemicals have

been applied for years can demonstrate well that a suitable environment, and the phenomenon of natural enemies migrating into polytunnels, the cheapest and best method of prevention a producer can ever have do control pests effectively. The simplest and cheapest method is to produce alfalfa and also, to leave nettle grow unmowed near the greenhouses. This leads to creating a natural balance between predators and pests inside the crop and in the surroundings of the greenhouses. It also helps to reduce the chance of an outbreak.

Upon summarizing the various results of our experiments we came to the conclusion that the potential of mixed arthropod assemblages deserves further studying, preferably in a wider range of use of the assemblage. As for crops, we advise plantations in unheated polytunnels with a similar spectrum of pests to that of green pepper (such as peppers, aubergines or tomatoes). In the case of polytunnel pepper growing, on the basis of our three year experience we suggest to make the following adjustments to the method:

1. The tested pepper variety should be less sensitive to thrips damage.
2. Seedlings should be free of pests (especially of western flower thrips).
3. The ideal method to collect and introduce mixed arthropod assemblages includes the following elements:
  - after 25-30 sweeps, arthropods may be stored in linen bags for a maximum of 1-2 hours before having them evenly distributed inside polytunnels
  - the best time for sweeping alfalfa is one week prior to mowing and between late May to early July in nettle
  - collection and introduction should start a week or two after the seedlings have been planted out
  - the amount of arthropods per introduction should be at least 1 sweeps per 10 pepper individuals
  - a series of introduction is preferred (one series comprises of three introductions per week)
  - a series should be repeated in two or three week intervals
  - three series per growing season might prove sufficient, although the actual amount of arthropods needed depends on the size and composition of the pest population
4. The number and presence of pests and their predators should be monitored every week. To avoid incorrect data and conclusions, due to the daily fluctuation of the activity of the arthropods, this check-up should be done at the same time of the day.

5. In the case of outbreaks we suggest using environmentally friendly pesticides at hotspots. Chemical treatment however, is not compatible with the introduction of arthropod assemblages.
6. We suggest arranging and maintaining the habitat diverse both inside and outside the polytunnels to provide flowering plant stands for as long as possible, preferably throughout the complete vegetation period. The less this requirement is met (in the case of polytunnels located within a town or village) the more the success of the method is at risk.
7. Finally, growers and analyzers of production should understand that one method alone – such as introducing arthropod assemblages – can not solve all the problems of pest management; and as no two years are the same, there are yearly fluctuations in the success of organic pest management.

## 5. NEW RESULTS

Our research provides the following new results:

1. We determined the *Orius*, spider, ladybug and thrips assemblages of alfalfa, nettle, danewort and chervil. On gathering these data we set the best suitable time for their collection and the potential number of collected individuals per each collection date.
2. We determined the effect of arthropods collected from alfalfa, nettle and danewort on the size of the damaged area of pepper and on the overall quality of green peppers in single plant isolated plants.
3. We determined the effect of arthropods collected from alfalfa and nettle on the size of the damaged area, on crop yield and on thrips and flower bug populations of farm-size polytunnel green pepper.
4. We determined the thrips assemblage of polytunnel green pepper stands in the Jászág and in the Gödöllő regions, which lead us to determine the status of western flower thrips infection within these stands.
5. We determined the *Orius* assemblages of polytunnel green pepper stands in the Jászág and in the Gödöllő regions. We also have established a list of endemic species that have a significant potential in the biological control of phytophagous thrips.
6. We determined the daily fluctuation of the *Orius* adults dwelling inside pepper flowers.
7. We elaborated a cheap, environmentally friendly and easy method to control thrips and aphids in green pepper in unheated polytunnels.

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## 7. LIST OF RELATED PUBLICATIONS

### 7.1 Approved articles in Hungarian

**Bán G.**, Nagy A., Zrubecz P. és Tóth F. (2007): Első tapasztalatok a közönséges karolópók (*Xycticus kochi* Thorell) nyugati virágtripsz (*Frankliniella occidentalis* Pergande) elleni felhasználásáról üzemi méretű hajtattott paprikában. *Növényvédelem* 43 (5): 169-174.

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**Bán G.** és Tóth F. (2009): Tripszek és levéltetvek elleni védekezés vegyes ízeltlábú-együttesrel hajtattott paprikában. *Növényvédelem* 45 (1): 5-14.

**Bán G.**, Pintér A., Fetykó K., Orosz Sz., Veres A. és Tóth F. (2010): A betelepített vegyes ízeltlábú-együttes felhasználási lehetősége hajtattott paprika biológiai védelmében. *Állattani Közlemények* 95 (1): 11-23.

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Tóth F., Veres A., Orosz Sz., Fetykó K., Brajda J., Nagy A., **Bán G.**, Zrubecz P., Szénási Á.: (2006) Landscape resources vs. commercial biocontrol agents in protection of greenhouse sweet pepper – a new exploratory project in Hungary. *IOBC wprs Bulletin* 2006 Vol. 29 (6):129-132.

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**Bán G.**, Fetykó K. and Tóth F. (2010): Predatory arthropod assemblages of alfalfa and stinging nettle as potential biological control agents of greenhouse pests. *Acta Phytopatologica et Entomologica Hungarica* Vol. 45 (1): 125-134.

**Bán G.**, Fetykó K. and Tóth F. (2010): Application of mass-collected, non-selected arthropod assemblages to control pests of greenhouse sweet pepper in Hungary. *North Western Journal of Zoology* (elfogadott kézirat)

### 7.3 Abstracts (Hungarian)

**Bán G.**, Nagy A., Tóth F. (2006): A közönséges karolópók (*Xysticus kochi*) nyugati virágtripsz (*Frankliniella occidentalis*) elleni alkalmazása során felmerülő technológiai kérdések (dózis, felülkezelés) vizsgálata hajtatott paprikán. 52. Növényvédelmi Tudományos Napok, Budapest, 2006. február 23-24., 10. p.

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