Chapter 5

Arthropod Fauna Associated to Soybean in Croatia

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Additional information is available at the end of the chapter

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1. Introduction

The importance of soybean (*Glycine max* (L.) Merr.), as today's world leading oil and protein crop, is increasing in Croatia. As a plant species, soybean was registered for the first time in Croatia in 1876. Soybean is relatively new field crop for Croatia. It was grown for the first time in 1910 but, starting with 1970s it became important field crop [1]. In 1981, soybean was cultivated on an area of 3.381 ha. Since that time the area cultivated by soybean has increased considerably, and productivity has also risen steadily. Figure 1 presents the trends in soybean production in Croatia in the period 1993-2010 [2].

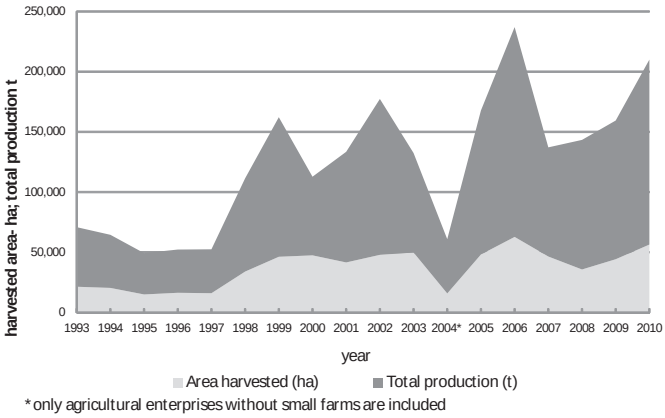

Figure 1. Harvested area and total production of soybean in Croatia, 1993-2010

Today's the area on which soybean is cultivated in Croatia varies, depending on the year, from 30.000 to 50.000 ha. Croatian government statistics [2] show gains in average yielding ability, from 2.160 to 3.000 kg/ha, between 1993 and 2010. Comparing to data from USA [3] on the average yield between 2.197 and 2.896 kg/ha, soybean yields obtained in "regular" years in Croatia are satisfactory. The exceptions in registered yield quantity were observed in extremely dry and warm years 2000, 2003 and 2007 in which yield was between 1.380 and 1.900 kg/ha. Therefore, the main problem of soybean yielding stability is related to vulnerability of soybean production in extreme climatic conditions in which pest outbreaks influence yields negatively. Global climate changes are often discussed by numerous scientists. Besides the increase of global mean temperature [4], the incidence of the years in which extreme conditions are present vs. "regular" years is increasing. This is proved by the fact that in the period from 2000 to 2009, three years with extremely dry and warm conditions were observed. Consequently, to mitigate the negative consequences of pest outbreaks and improve profits soybean growers, in these extreme years, attempt to control the pests which can reduce crop productivity.

Comparing to weeds and diseases, in "regular years" pests are of somewhat less importance for soybean production in Croatia. In different agro-ecosystems, the arthropod fauna of soybean contains a great number of damaging species [5-8]. Soybean pests have not been investigated completely in Croatia. It was reported [8] that in the region where Croatia belongs, soybean crops are attacked by over 180 pests (150 insects and 30 species from other animal classes) among which approximately 25 pest species are the most important.

Some investigations or observations on arthropod fauna of soybean were conducted in the past on the territory of Croatia [9 - 13], and in neighboring countries [14 - 24]. Additionally, some of the species were registered recently as the pests which could cause significant yield damage on soybean [25 - 29].

The most comprehensive overview of the potential arthropod pests' fauna of soybean in Croatia is given by Maceljski [9]. This overview is a result of the literature review and author's long time work experience in entomology. On the other hand, investigations carried out by other scientists in Croatia [10 - 13] and neighboring countries [6-8, 14-24] reported on the presence or harmfulness of some additional species. In the Table 1 arthropod species that are reported as soybean pests both, in Croatia and in neighboring countries are listed.

Besides arthropod species, nematodes are established as potential pests on soybeans in Croatia [11, 12] and in neighboring countries [19]. Jelić [12] established 43 species of phytoparasitic nematodes on 18 localities distributed in east Croatia (region of Slavonia). Identified species belonged to the genera *Ditylenchus* Filipjev, *Meloidogyne* Goeldi, *Paratylenchus* Micoletzky, *Pratylenchus* Filipjev, *Rotylenchus* Filipjev, and *Tylenchorhynchus* Cobb. However significant damages caused by nematodes haven't been recorded jet. Besides mentioned pests, some authors [8, 30] reported that significant damage on soybean crops in Serbia could be caused by other animal species as are *Cricetus cricetus* L., *Microtus arvalis* Pallas and *Lepus europaeus* Pallas as well.

In regular farming practice in Croatia soybean seed is not treated with insecticides. Among the arthorpod pests, mites (*Tetranychus urticae* Koch and *Tetranychus atlanticus* = *T. turkestani*

Ugarov & Nikolskii) could be controlled by the use of acaricides if their populations reach economic threshold (usually in warm and dry years). Other pest species are controlled only occasionally if pest outbreaks occur.

Order	Suborder	Family	Species	Literature source	Croatia	Neighboring countries
Collembola		Smynthuridae	*Sminthurus* sp. Latreille 1802	21		+
Thysanoptera		Thripidae	*Frankliniella intonsa* (Trybom 1985)	21		+
Hemiptera	Heteroptera	Miridae	*Lygus* sp. Hahn 1833	9	+	
			Lygus gemellatus (Heerrich-Schaeffer 1835)	21		+
			Lygus pratensis (Linneaus 1758)	24		+
			Lygus rugulipennis Poppius 1911	8		+
			Halticus apterus (Linnaeus 1758)	9	+	
			Apolygus lucorum (Meyer-Dur 1843)	24		+
		Pentatomidae	*Dolycoris bacarrum* (Linnaeus 1758)	21, 24		+
			Eurydema oleracea (Linnaeus 1758)	24		+
			Nezara viridula (Linnaeus 1758)	9, 26	+	
			Piezodorus sp. Fieber 1861	9	+	
		Anthocoridae	*Anthocoris* sp. Fallen 1814	9	+	
			Orius niger Woolf 1811	6		+
		Nabidae	*Nabis (Nabis) ferus* Linnaeus 1758	6, 9	+	+
			Nabis feroides Wagner 1967	6		+
			Nabis pseudoferus Remane 1949	6		+
	Homoptera	Membracidae	*Sctictocephala bisonia* Koop & Yonke	9, 22, 24	+	+
			Cicadella viridis (Linnaeus 1758)	9	+	
		Aphididae	*Aphis craccivora* Koch 1854	21		+

Order	Suborder	Family	Species	Literature source	Croatia	Neighboring countries
		Diaspididae	*Lepidosaphes* sp. Shimer 1898	21		+
Coleoptera		Elateridae	*Agriotes ustulatus* Schaller 1793	15		
			Agriotes sp. Eschscholtz 1829	15		+
		Scarabaeidae	*Anomala* sp. Schoenherr 1817	21		+
		Anobiidae	*Stegobium paniceum* (Linnaeus 1758)	21		+
		Cocinelidae	*Subcocinella vigintiquatuorpunctata* (Linnaeus 1758)	9, 21	+	+
		Chrysomelidae	*Longitarsus* sp. Berthold 1827	9	+	
			Phylotreta undulata Kutschera 1860	9	+	
			Haltica oleracea Linnaeus 1758	9	+	
		Lathiridae	*Corticaria* sp. Marsham 1802	9	+	
		Curculionidae	*Phyllobius* sp. Germar 1824	9	+	
			Sitophillus sp. Schnherr, 1838	9	+	
Lepidoptera		Gracilariidae	*Phylonorycter insignitella* Zeller 1846	24		+
		Pyralidae	*Etiella zinckenella* (Treitschke 1832)	8, 9, 14, 21	+	+
		Crambidae	*Udea ferrugalis* Hubner 1796	21		+
		Tortricidae	*Olethreutes lacunana* Freeman 1941	21		+
			Grapholita compositella Fabricius 1775	24		+
		Lymanthridae	*Orgya gonostigma* L.	21		+
		Geometridae	*Ascotis selenaria* Dennis & Schiffermuller 1775	21		+
		Nymphalidae	*Vanessa cardui* Linnaeus 1758	9, 20, 23, 24, 25, 27, 28, 29	+	+
		Noctuidae	*Acronicta (Viminia) rumicis (Linnaeus 1758)*	21		+

Order	Suborder	Family	Species	Literature source	Croatia	Neighboring countries
			Chloridea dipsacea L. (*Heliothis viriplaca* (Hufnagel, 1766))	21		+
			Phragmatobia fuliginosa (Linnaeus 1758)	21		+
			Autographa gamma (Linnaeus 1758)	7, 8		+
			Helicoverpa armigera (Hubner 1808)	7, 8		+
			Mamestra sp. Ochensheimer 1816	7, 8		+
Diptera		Cecidomyidae	*Clinodiplosis trotteri* = *Anabremia trotteri* (Kieffer 1909)	21		+
			Acarolestes tetranychorum (Kiefer 1909)	21		+
		Anthomyiidae	*Delia platura* (Meigen 1826)	8		+
		Agromyzidae	*Lyriomyza congesta* (Becker 1903)	21		+
Prostigmata		Tetranychidae	*Tetranychus urticae* Koch 1836	7, 8, 9, 10, 12, 13, 16, 17, 19	+	+
			Tetranicuhus atlanticus = *T. turkestani* Ugarov & Nikolskii 1937	7, 8, 9, 10, 16, 17, 19, 24	+	+
			Tetranychus tumidus Banks 1900	24		+

Table 1. Arthropod species established to damage soybean in Croatia and neighburing countries

In only one investigation which was carried out in Serbia [21] beneficial fauna on soybean was recorded. Only three predatory species were established, *Coccinella septempunctata* L. (Coleoptera: Coccinelidae), *Chrysopa carnea* Stephens (Neuroptera: Chrysopidae) and *Acarolestes tetranychorum* Kief. (Diptera: Cecidomyidae). There are no similar investigations conducted in Croatia but, out of all species listed [9] as potential members of entomofauna of soybean, two species (*Anthocoris sp.* Fallen and *Nabis (Nabis) ferus* L.) are listed as potential beneficial insects.

The subject of pest control is rarely discussed without the reference to the concept of integrated pest management (IPM). IPM is essentially a holistic approach to pest control that

seeks to optimize the use of a combination of methods to manage whole spectrum of pests within particular cropping system. IPM relies heavily on biological controls with a perspective chemical input only as a last resort. For effective control, there needs to be an understanding of a pest's interaction with its environment. This is so called concept of "life system" which was initially conceived by Clark et al. [31] to reinforce the idea that population cannot be considered apart from the ecosystem with which it interacts. The life system consists of the pest population plus its "effective environment". Most ecological pest management concentrates on the agro-ecosystem, defined as "effective environment" at the crop level [32]. Monitoring in insect pest management can be used to determine the geographical distribution of pests, to assess the effectiveness of control measures, but in its widest sense monitoring is the process of measuring the variables required for the development and use of forecast to predict pest outbreaks [33]. Such forecasts are an important component of pest management strategies because a warning of the timing and extent of pest attack can improve the efficiency of control measures. For successful pest control according to the principles of IPM it is of great importance to have deep knowledge in harmful and beneficial arthoropods in particular agro-ecological conditions.

The study was conducted to determine the harmful and beneficial arthropod fauna during the soybean growing season, and based on their dynamic of occurrence and abundance to identify the harmful and beneficial species of greater importance for soybean production in Croatia.

2. Materials and methods

Research was conducted on experimental field located in Zagreb. The soybean variety Zlata (BC Institute Zagreb, Croatia) was planted on April 27th 2010 on an experimental area of 162 m^2. The average plant density was 630.000 plants/ha. Soybean variety Zlata belongs to the maturity group "0" and according to the information given by producers [34] it has a "good" tolerance to pests and diseases. In order to control weeds gyphosate (pre-sowing), metribuzin, metholachlor and clomazone (in the phase of the first trifoliate - V1, according to [35]) and bentazon (in the phase of the third trifoliate - V3) were applied.

Sweep net sampling consisted of making a set of 50 sweeps across three rows of soybeans while walking down the row [36]. A 30 cm diameter sweep net was used. Sampling began when soybeans were in the beginning of flowering (R1) on June 24th 2010 and continued through September 9th 2010 when plants reached physiological maturity (R7). Weekly sampling was done on the same day each week in late morning. It was performed for 12 weeks. At each sampling date four samples were collected.

Whole plant counts were conducted on 10 plants per each of four replicates. As it was proposed by Kogan and Pitre [36] randomly selected plants were initially scanned for large, often fast moving species. After the initial scan, both sides of each leaf on the plant were searched, as were petioles, axils and stems. Additionally, one leaf per plant was collected at each whole plant count date to establish mite population by leaf inspection. Therefore, four

samples each containing 10 leaves were transported to laboratory to be examined under the stereomicroscope and all life stages of mites were counted [37]. Whole plant counts and leaf collection began one week later than sweep net sampling i.e. on July 1st 2010 and continued through September 9th 2010. It was performed for 11 weeks.

All collected insects were identified to the family or genus and species (if possible). For identifying insects identification keys were used [38-42].

Based on the number of all individuals, cenological characteristics (dominance and frequency) of the insect orders and families (where appropriate) were determined [43].

The dominance was calculated by Balogh formula:

$$D1=\frac{a_1}{\sum a_i}x100$$

Where: a_1 = number of identified specimens of one species;

Σ_{a1} = total number of all collected specimens.

The frequency was calculated by Balogh formula:

$$C_{a1}=\frac{U_{a1}}{\sum U_i} \text{ x } 100$$

Where: U_{a1} = number of samples with identified species;

$\sum U_i$ = total number of samples.

3. Results and discussion

The total catch was 1357 specimens which belong to six orders: Thysanoptera, Hemiptera, Coleoptera, Lepidoptera, Diptera and Prostigmata (Table 2).

Out of 1357 specimens, only 73 individuals (5.37%) belong to beneficial fauna (mostly predators), while all other collected specimens are herbivorous and therefore potential pests on soybean. All found beneficials belonged to predators and majority of them (70 individuals) belong to Hemiptera what confirms the statement of Ketzschmar [44] that predaceous Hemiptera are usually more abundant in soybean fields than all other insect predators combined. In earlier investigations [21] conducted in Serbia no predaceous Hemiptera have been found while more recent investigations in Serbia [6] and in Croatia [9] stated that they are present in soybean crops. All predaceous Hemiptera feed on a wide range of hosts and may extend this polyphagy to plant feeding to some extent [45]. Such plant feeding causes no damage to row crops but almost certainly has survival value for the predators by maintaining populations where prey are scarce or absent. Some of the species which belong to family Pentatomidae are also recognized as predators [45]. Since some of the individuals collected in our

investigation were classified as family Pentatomidae but, were not identified to the species, it is possible that some of them are predaceous as well.

ORDER	FAMILY	GENUS	SPECIES	TOTAL NUMBER OF INDIVIDUALS CAPTURED BY		
				SWEEP NET	WHOLE PLANT COUNTS	LEAF INSPECTION
Thysanoptera		*		52	12	
Hemiptera	Miridae	*Halticus*	*apterus*	44	3	
		Lygus Hahn 1833	sp.	4		
	Nabidae	*Nabis*	*ferus* Linnaeus 1758	55		
	Anthocoridae	*		3	8	
	Pentatomidae	*		12		
		Nezara	*viridula* Linnaeus 1758	472	181	
		Piezodorus	sp. Fieber 1861	28		
	Membracidae	*Stictocephala*	*bisonia* Kopp & Yonke 1977	2		
	Cicadellidae	*Cicadella*	*viridis* Linnaeus 1758	3		
Coleoptera	Coccinelidae	*		3		
	Chrysomelidae	*Phyllotreta*	*undulata* Kutschera 1860	4		
		Haltica	*oleracea* Linnaeus 1758	7		
		Longitarsus Berthold 1827	sp.	2	1	
	Latridiidae	*Corticaria* Marsham 1802	sp.	21		

ORDER	FAMILY	GENUS	SPECIES	TOTAL NUMBER OF INDIVIDUALS CAPTURED BY		
				SWEEP NET	WHOLE PLANT COUNTS	LEAF INSPECTION
	Curculionidae	*			1	
		Phyllobius Germar 1824	sp.	5		
		Sitophillus Schnherr, 1838	sp.	1		
Lepidoptera		*			2	
	Nymphalidae	*Vanessa*	*cardui* Linnaeus 1758	7		
	Noctuidae			15		
Diptera	Nematocera	*			1	
		*		16		
Prostigmata	Tetranychidae	*Tetranychus*	*urticae* Koch 1836			387
TOTAL				759	211	387

* Identification was not possible; Beneficial species are marked in grey;

Table 2. Arthropod species established during the soybean vegetation in 2010 by three different methods

Using the entomological net, 759 individuals were collected, whereas 211 individuals were gathered by whole plant counts and 387 individuals by leaf inspection.

3.1. Sweep net sampling

Number of arthropod individuals collected by sweep net sampling was the highest among the three methods applied. Using these methods, species belonging to 20 different systematic categories were collected. The collected individuals belonged to five insect orders, Thysanoptera, Hemiptera, Coleoptera, Lepidoptera and Diptera. The abundance of insect orders established by sweep net sampling is shown in Figure 2.

Order Hemiptera was present in the sweep net sampling in the highest abundance (82.48%). The same order was the most frequent. It was present in 87.5% of all samples obtained by sweep net sampling. Order Coleoptera was present in 57.5% of all samples and was designated as constant. Other orders (Thysanoptera, Lepidoptera and Diptera) were less frequent; they were present in 30-37.5% of all samples. Investigations conducted in different agro-ecosystems showed that the sweep net sampling is the most effective method to collect different

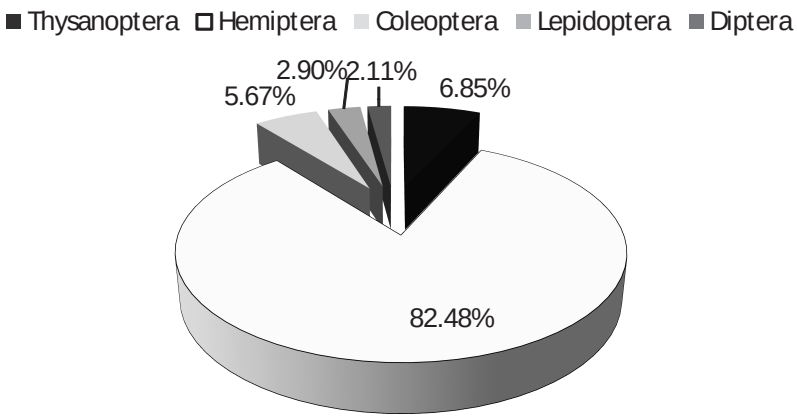

Figure 2. Abundance of insect orders collected by sweep net sampling of soybean crop, Zagreb, 2010

leaf feeding pests as are leafhoppers [46], lepidopterous larvae [46, 47], leaf feeding Coleoptera [48] and phytophagous Pentatomidae [49] as well as predaceous Hemiptera [45].

3.2. Whole plant count

The lowest number of individuals was established by whole plant count method. The majority of established individuals belonged to order Hemiptera. Only few Thysanoptera and Lepidoptera were established by this method. Some authors stated that this method is suitable for larvae of Lepidoptera [46, 47] and for phytophagous thryps [50]. There are no data on any damage caused by any phytophagous thrips in Croatia while *Vanessa cardui* L. was recorded in previous investigations as important pest.

3.3. Leaf sampling and inspection

The only species established by leaf inspection was *T. urticae*. It was proved that this method is good for establishing population of phytophagous thrips [48], whiteflies [49] and mites [35]. It is obviously that out of these three groups, only phytophagus mites were present in experimental field.

3.4. Collected species: abundance and importance

Sampling arthropod populations is a cornerstone of basic research on agricultural ecosystems and the principal tool for building and implementing pest management programs. The purpose of sampling is dual, it is a research method for defining the nature and dynamics of communities in agricultural ecosystems and it is also a mean for providing pest management decision. The purpose of sampling in our investigation was to get deep knowledge on pest and beneficial species present in soybean crop. Conducted investigation encompassed three most common sampling methods for investigations of soybean arthropod fauna. The

need to encompass all three methods is confirmed later by the fact that species identified by each particular method differ. By employed methods we were able to get all relevant data on above ground arthropod fauna that could be found on soybean canopy. We did not aim to collect information on underground soybean arthropods and ground predators in soybean fields. To collect this information we should use common methods for sampling soil arthropods, soil samples and extraction [51] or pitfall trapping [48]. Some of earlier researches on soybean arthropod fauna in the region collected information on underground soybean arthropods but, no research did pay attention on ground predators in soybean field. No researches among all conducted [6-29] did pay attention to abundance and frequency of particular orders, genus or species as well, so it is not possible to compare if there are some discrepancies with the results of previous researches.

Individuals that belonged to the order Thysanoptera have been found by sweep net sampling in highest abundance than by whole plan counts, and they haven't been found by leaf inspection at all. In Serbia one phytophagous thrips species (*Frankliniella intonsa* Trybom) on soybean has been identified [21]. Since in our investigation thrips were not established by leaf sampling and inspection, it could be concluded that they did not feed and develop on soybean. It might be that those species are predaceous because it is reported [52] that thrips are natural enemies of different pests. Important predaceous genera of thrips are *Aelothrips* Haliday, *Franklinothrips* Back, *Scolothrips* Hinds, *Leptothrips* Hood, *Karnyothrips* Watson and *Podothrips* Hood. Within the genus *Aeolothrips*, the species *Aeolothrips intermedius* Bagnall is distributed throughout western and eastern Europe [53], the middle East and India but now it can be considered cosmopolitan [54]. Comparative tests by many authors [53, 55, 56] using different types of prey (including various species of Thysanoptera), suggested that both the larvae and the adult females are generic predators, even though they present marked dietary preferences. In Italy [57] *A. intermedius* was detected in association with various different phytophagous Thysanoptera, which included *T. tabaci* but also frequently *F. occidentalis* on many different plant species including legume species *Medicago sativa* L. Predaceous thyrips belonging to the genus *Aelothips* are reported as important predators of *T. urticae* in soyben crops in north-eastern Italy [58]. *Franklinothrips* sp. adults and larvae are generalist predators and attack a wide variety of arthropod pests including two spotted spider mite (*T. urticae*) [59]. Genus *Scolothrips* is counting six species in Europe [60], and one of them, *Scolothrips longicornis* Priesner is a predator of *T. urticae* [61] and *T. turkestani* [62]. Both pest species are registered as soybean pests in Croatia. Genus *Leptothrips* is not present in Europe [60]. Genus *Karnyothrips* is counting three species in Europe [60]. Some species are reported as predators of scale insects [63]. Genus *Podothrips* is known as grass-living genus. It counts only two species in Europe present only in Italy and Cyprus [60]. Since identification to the species was not possible, we cannot state which species of Thysanoptera were present.

Individuals belonging to six families of the **order Hemiptera** were identified in our research. Four families belong to the **suborder Heteroptera** (so called typical bugs) and two families belong to the **suborder Homoptera.**

Family Miridae was presented by genus *Lygus* sp. Four individuals were captured. Identification to species was not possible. Species belonged to the genus *Lygus* were reported by

different authors [6, 8, 21, 24] to feed on soybean crops in Bosnia and Herzegovina and Serbia as well as in Croatia [9] without causing serious damages. More numerous were individuals of *Halticus apterus.* This species is distributed through Mediterranean region [64]. It was reported to feed on soybean only in Croatia [9]. Other research showed that it feeds on some legume plants such as *Medicago sativa* L., *Lotus corniculatus* L. and *Trifolium repens* L. in Italy, and also to be able to cause damages on onions and Gallium [64]. Since it was not reported as serious pest of soybean anywhere, it should be monitored in the future but the probability for this species to become important pest of soybean is low.

Family Nabidae was represented with one species, *N. ferus.* The same species was previously reported in Croatia [9] and in Serbia [6]. Additionally two other species of this genus, *N. feroides* and *N. pseudoferus,* were reported in Serbia [6]. The density of *N. ferus* was moderate, total of 55 individuals were captured. This species was reported as common predator species in Ukraine [65]. Aphids are the principal prey insects for this species, but numerous other families are acceptable, including other bugs [65]. Because of its possible importance in soybean agro-ecosystems, the dynamic of the appearance of this species will be further analyzed.

Eleven individuals belonging to **family Anthocoridae** were captured in our investigation. Family Anthocoridae was previously reported in soybean in Croatia [9] and in Serbia [6]. This family is mentioned as one of the most important predaceous family of Heteroptera in soybean crops [45]. Within the family Anthocoridae, members of the genus *Orius* occur as predators in soybean fields all around the world [45]. The species *Orius niger* Woolf has been found in soybean fields in Serbia [6]. In some areas, species of the genus *Anthocoris* Fallen are probably also important predators in soybean [45]. Captured individuals were not identified to the species so it is not possible to discuss which genus was the most abundant in our investigations.

Among the established Hemiptera, **family Pentatomidae** was the most abundant. Altogether 512 individuals were found in sweep-net samples and 181 individuals by whole plant counts. The most abundant species was the southern green stink bug, *Nezara viridula* L. This species was reported as present in Croatia [9]. Recently the serious damages caused by this species were reported in Croatia [26]. It is not mentioned as serious pest in neighboring countries, while it was mentioned as serious threat to soybeans in Italy [58, 66]. It was reported [49] as one of the most abundant phytophagous stink bugs on soybean worldwide among of almost 40 species of stink bugs that have been found on soybean. Due to high number of captured individuals and registered damages caused by this species, it might be identified as one of the potential pests on soybean in Croatia. Therefore the dynamic of the appearance will be further analyzed. Species belonging to phytophagous genus *Piezodorus* were captured in lower number. Genus *Piezodorus* was reported as possible pest genus in Croatia [9] and in Italy [58]. The importance of the species *P. guildinii* Westwood is increasing in USA as well as in Brazil. This species was observed for the first time in southern Louisiana in 2000 and since 2002, it has been a significant pest of soybean [67]. At present, *Eustichus heros* (F.) and *P. guildinii* are more widespread and occur in greater numbers than *N. viridula,* and *P. guildinii* is principally responsible for the green bean syndrome observed

in Brazilian soybean [68]. Genus *Piezodorus* is counting three species in Europe [60]. Twelve individuals, members of family Pentatomidae remained unidentified. It is possible that some of them are phytophagous. Also it is possible that some of them are predators because species which belong to family Pentatomidae are also recognized as predators [45].

Two families each represented with one species from the **suborder Homoptera** have been collected in low numbers. *Stictocephala bisonia* was reported to feed on soybean in Croatia [9], Serbia [21] and Bosnia and Herzegovina [24]. The second identified species was *Cicadella viridis*. This species was registered to feed on soybean in Croatia [9] but without significant damage. Within the USA, potato leafhopper (*Empoasca fabae* Harris) is the most important leafhopper species [69]. Even though aphids are recognized as a regular part of entomofauna of soybean, we did not record them. Several species of aphids are known to attack soybean crops. The most important species in North America is *Aphis gyicines* Matsumura [70, 71]. This species is not registered in Europe [60]. Some other species of aphids that are members of the fauna of Europe [60] and Croatia [72, 73] successfully colonize and reproduce parthenogenetically on soybean [71]: *A. craccivora* Koch, *Aulacorthum solani* (Kaltenbach) and *Aphys gossypii* Glover.

Out of four families of the order Coleoptera that were identified, one represents mainly predaceous species (family Coccinelidae). Some species of the family Coccinelidae are reported as the members of arthropod fauna on soybean in Croatia and Serbia [9, 21]. Species *Epilachna varivestris* Mulsant is known as a soybean pest in USA [48]. Three species of the phytophagous genus *Epilachna* are present in Europe, including Croatia [60] but only in Dalmatia where soybean cultivation is not common. Individuals from the genus *Corticaria*, family Latridiidae were the most numerous. Adults and larvae of this family feed on the conidia of fungi and *Myxomycetes* [74]. All found species from the order Coleoptera were previously listed as potential members of soybean fauna in Croatia [9] but, due to the low populations, their potential to be significant pests or predators is not very high. We did not employ any method for sampling soil dwelling insects or underground fauna. Therefore we did not collect the species which belong to families Elateridae and Scarabaeidae that are known as polyphagous soil pests that could cause damage on soybean crops [15, 21].

Only 22 individuals from **order Lepidoptera** were collected. Painted Lady (*Vanessa cardui*) was the only identified species. Other specimens were classified into the family Noctuidae. *V. cardui* was previously recorded to significantly damage soybean in Croatia [9, 25, 27, 29] and in neighboring countries [20, 23]. The outbreak of this pest is periodical. Higher population could be expected in weedy soybean fields because females are attracted by pollen sources and heavy plant density [25]. There are many of species from the family of Noctuidae and from the other families, members of the order Lepidoptera which could cause the damage but, until now, serious damage in Croatia was reported only by *V. cardui*. In USA, the most important lepidopterous species are *Anticarsia gemmatalis* (Hubner), *Pseudoplusia includens* (Walker), *Trichoplusia ni* (Hubner), *Platypena scabra* F., *Heliothis zea* Boddie, *Heliothis virescens* (Fabricius) and *Heliothis* (=*Helicoverpa*) *armigera* (Hubner) [46, 47, 75]. Except *T. ni* and *H. armigera,* other species are not distributed in Europe [60]. *H. armigera* is often mentioned as one of the potentially very dangerous species. Because of its invasive nature this

pest is currently placed on Annex I A II of Council Directive 2000/29/EC, indicating that it is considered to be relevant for the entire EU and that phytosanitary measures are required when it is found on any plants or plant products. Some countries made pest risk analyses [76]. Damages caused by this species were reported on soybeans in Vojvodina Province of Serbia and in Montenegro in the very warm summer of 2003 [77] when 85.3% of the soybean pods were injured in August. *H. armigera* is a serious pest on outdoor crops in Portugal and Spain, predominantly on tomato crops as well as on cottom and maize. It developed resistance to many groups of insecticides [78]. We did not find caterpillars of limabean pod borer (*Etiella zinckenella* Treitschke) even though this species was reported as soybean pest in Croatia [9] and in neighboring countries [6, 14, 21]. In Southern Europe and in Central and South America *E. zinckenella* is only damaging pod borer species in soybean.

Order Diptera was represented by 17 individuals that were not indentified to the species. The pest species from the order Diptera reported in the literature are *Delia platura* Meigen [6, 58], *Liriomyza congesta* Becker and *Clinodiplosis trotteri* Kief. [21]. Larvae of *D. platura* could cause damage on soybean seed during the emergence. Larvae of *L. congesta* are damaging leaves [21] and larvae of *C. trotteri* are damaging plant stem [21]. Some of Diptera species in soybean could be natural enemies, for example predaceous species *Acarolestes tetranychorum* feed on mites [21].

We established one species from **order Prostigmata** (infraclass Acari). This was the species *T. urticae* which was established only by leaf inspection. This species is the most important pest of soybean in the whole region [6, 8-10, 13, 17, 19, 29]. The pest outbreaks are occurring in dry and warm years in which farmers must apply control measures. Besides *T. urticae*, soybean in Croatia [9] and neighboring countries [16, 17, 19] is often attacked by *T. atlanticus* (= *turkestani*). Both species have similar thermal requirements but, *T. atlanticus* prefers extremely dry conditions [9]. Some differences were established in their response to host plant nutrient status [79]. The development of *T. urticae* is positively influenced by potassium content in the plant host, while *T. atlanticus* is positively influenced by content of phosphorus. According to the data obtained from Meteorological and Hydrological Service of Republic of Croatia, somewhat lower temperatures and higher amount of rainfalls in July and August in 2010, comparing to the average were recorded. That could cause the absence of *T. atlanticus* in experimental field and relatively low population of *T. urticae.*

The dominance indices of the insect orders established in total capture are shown in Figure 3.

In total catch the eudominant orders were Hemiptera (60.46%) and Acarina (28.6%), while subdominant were orders Thysanoptera (4.73%), Coleoptera (3.33%), Lepidoptera (1.63%) and Diptera (1.26%).

3.5. Most important phytophagous species

The significant feeding on soybean was established by two species, *N. viridula* and *T. urticae.* Therefore we will further analyze their appearance with the respect of their life cycle and possible damages that they can cause.

The dynamic of the appearance of *N. viridula* is shown in Figure 4.

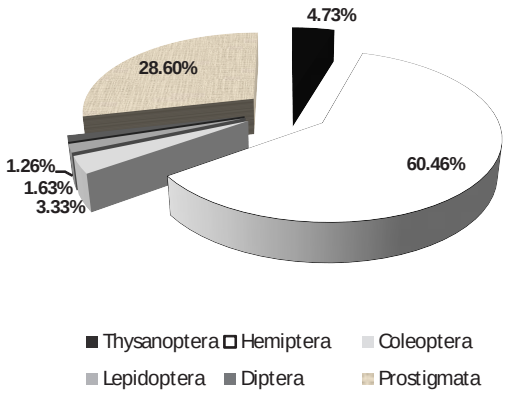

Figure 3. The dominance indices of arthropod orders established in the total capture of arthropod species on soybean in Croatia in 2010

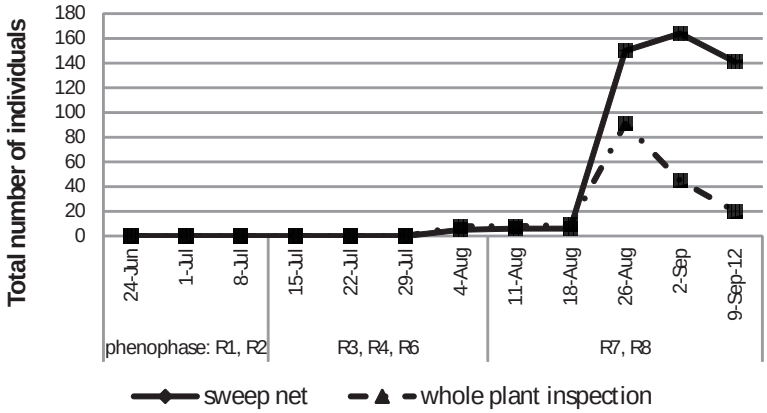

Figure 4. The dynamic of the appearance of *Nezara viridula* (L.) on soybeans in Croatia in 2010 established by two sampling methods

The southern green stink bug, *N. viridula*, is one of the most economically important soybean pests [80]. It has a worldwide distribution, occurring throughout the tropical and subtropical regions of Europa, Asia, Africa and America [49]. This pest is in constant expansion as a consequence of the increased acreage for soybean production, particularly in South America [80]. This pentatomid is highly polyphagous, attacking more than 145 species of plants (including cultivated and uncultivated species) within 32 families [49]. Life cycle of the southern green stink bug has been studied by number of authors in different parts of the world [81-85]. The biology of this pest has not been studied in Croatia jet but, some data were presented by different authors [26, 29]. From literature it is known [49] that southern green stink bug, like most pentatomids, overwinters in the adult stage under different objects that offer protection (litter, bark etc.). In the northern hemisphere [49] overwintering

adults emerge in March and first generation develops on clover. The total developmental time from eggs to adults lasts between 23 days [82] and 49 days [81]. In USA, it develops 3-5 generations per year, depending on the climate. The 3rd, 4th and 5th generations attack soybean. We found it on soybean when soybeans began to mature, in August and onward what corresponds with the data presented by Todd and Herzog [49]. Probably the first two generations developed on some other plants. Stink bugs feed primarily on the seeds of soybean. Feeding results with puncture marks on the seed coat, deformation of the seed coats and reduced seed weight and size. Adults live longer, approximately 30 to 50 days [81, 82] and they cause more damage than nymphs [86]. Croatian authors [26] proposed economic threshold of 1 bug/30 m of soybean row or 8-10 bugs/10 sweep nets what seems to be too low. It is important to point out that the threshold depends on the period when insects occur. Early infestation causes more damage than late infestation [86]. Late in the season high infestation level of 2 bugs/m^2 will not result with the damage [86]. In our investigation we established infestation of 2 bugs/plant by whole plant count method and 4-5 bugs/10 sweep nets without seeing any damage on the yield. The appearance of *N. viridula* was in literature [26] connected with higher temperatures and drought, what was not the case in our investigation. Generally, in other countries the southern green stink bug is controlled with non-selective insecticides, which belong to carbamates, the organophosphate group, or the cyclodiene group, such as endosulfan or to pyrethroids [80]. Some of the mentioned insecticides are banned in Croatia and others are not allowed for that purpose. In the case of pest outbreak farmers do not have any available option to control this pest.

The second species which was recorded in high population density was *T. urticae*. The infestation with *T. urticae* started somewhat earlier that the attack of *N. viridula*. The dynamic of the appearance of *T. urticae* is shown in Figure 5.

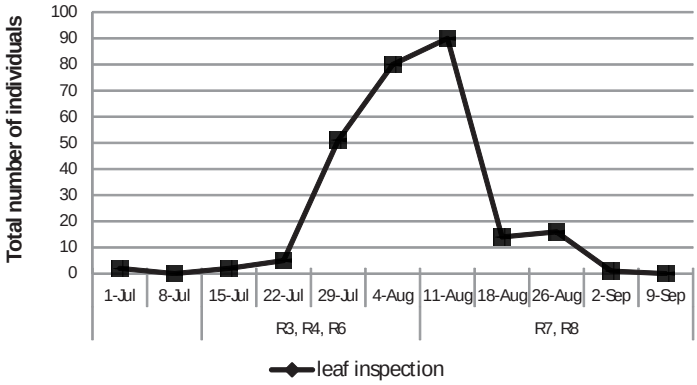

Figure 5. The dynamic of the appearance of *Tetranychus urticae* on soybeans in Croatia in 2010

The maximal infestation of *T. urticae* was recorded on August 11th and it was 2.25 mites/leaf. This infestation is considered as very weak to weak infestation [87]. After that date, the number of mites significantly decreased without the use of acaricides. The reason of the decrease of the population is the period in August in which strong rain occurred. Strong rain probably caused washing up the spider mites from the leaves as it was mentioned by some authors [29, 87]. *T. urticae* injure soybean by feeding on the green foliage and pods. By their needle-like chelicerate mouthparts that are used to puncture individual plant tissue cells and consume the entire cytoplasmic contents, they are leaving and empty irreversible damaged cell. The presence of numerous empty cells results in the yellow or brown spots on mite-injured leaves. Extensive feeding by large numbers of mites causes the leaves to appear yellow or brown [37]. Complete defoliation due to mite feeding can reduce pod set and seed yield. Under the favorable conditions mites have very short developmental time, between 8-20 days [9]. That enables them to develop several generations in a very short time and to increase population up to the economic threshold very fast. Therefore permanent monitoring of the pest population is needed. No acaricides are allowed for the control of *T. urticae* in soybean crops at the moment in Croatia. Even though there are some mite resistant cultivars in USA [88], they are not registered in Croatia.

3.6. Most important zoophagus species

Total of 73 predaceous species are collected in the investigation. Family Nabidae was represented by one specius, *N. ferus*. Members of the family Nabidae are confirmed predators of different kind of insects [89]. Most types of insect prey of nabids are plant-feeding species, but nabids sometimes attack predaceous insects, including members of their own species. The polyphagous feeding habits of the nabids make them less effective than species-specific predators against specific prey species [89]. Altogether 55 individuals of *N. ferus* were counted. The dynamic of the appearance of *N. ferus* is shown in Figure 6.

Nabis ferus is a common, widespread species in the Palearctic region [89]. It was reported as predatory species on *Trialeurodes vaporariorum* Westwood [90], *Oulema melanopus* L. [91], *Sitobion avenae* F. [92] and other aphid species [65], *N. viridula* [93] and leafhoppers in all stages [89]. Species *N. ferus* overwinters in the adult stage [65]. Adults emerge from the soil and migrate to field of various crops in April and May according to the weather. They mate, lay eggs and the nymphs appear between late May and June and are present until July [65]. There is a second generation with nymphs in July-August and adults in August-October. The dynamic of the appearance of *N. ferus* in experimental field corresponds with the data on life cycle of this species [65]. In mid-late July we collected adults of the first generation and nymphs were collected in August. Kereši [94] stated that zoophagous *Nabis* species develop one generation per year in soybeans. She mentioned adult appearance at the end of July and maximal larval appearance at the end of August. It remains unknown in which crop species is developing the first generation. Due to its preference to prey aphids which are abundant in wheat fields, it could be that the first generation is developing in wheat fields. The experimental field in our investigation was surrounded by wheat fields what could influence high population of *N. ferus*.

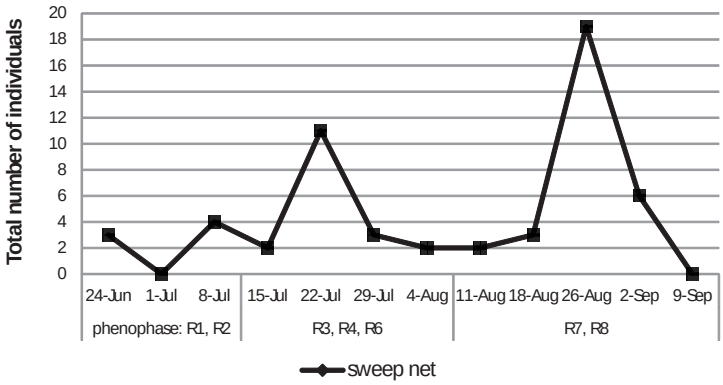

Figure 6. The dynamic of the appearance of *Nabis ferus* on soybeans in Croatia in 2010

4. Conclusions

Literature reports that soybean crops in the region where Croatia belongs (Croatia, Hungary, Serbia, Romania, Bulgaria and Bosnia and Herzegovina) are attacked by over 180 pests (150 insects and 30 species from other animal classes). However, by literature review from Croatia, Serbia and Bosnia and Herzegovina we established that 52 species (or genus) of arthropods are reported to be associated with soybean crops. Out of these 52 species, seven species are zoophagous, 44 species are phytophagous and one species is myceliophagous. Additionally, we have found data on 43 species of phytoparasitic nematodes that can be find in soybean fields but without causing significant damages and literature also reports on three species of rodents that could cause significant damage on soybean fields.

In our investigations the number of established species was lower than the number obtained by literature review. Total of 1357 individuals were collected and classified into the five orders from the class of Insects and one order from the class of Arachnida (infraclass Acari). 1232 individuals were classified in 15 species or genus, 58 individuals were classified into the six families while 67 individuals were classified into the orders. Phytophagous arthropods were more abundant than zoophagous. The ratio between phytophagous and zoophagous specimens was 94.63% : 5.37%.

Based on the results of the literature review and of the research conducted, it could be concluded that significance of the arthropod pest fauna connected with soybean has changed over the time. Nowadays, soybean production in Croatia could be endangered by four phytophagous arthropod species: *N. viridula* L., *V. cardui* L., *T. urticae* and *T. turkestani*.

N. viridula is attacking soybean pods and seed causing the loss in yield quantity and quality. The early infestation is very dangerous. The population of this pest has been increasing in the past few years. This could be connected with the increase of soybean cultivation area. Obtained results indicate that the increase in pest population has occurred recently and that

one existing species became significant pest of soybean. The life cycle of this pest as well as other issues related to sampling procedure, economic threshold and control possibilities have not been studied jet in Croatian agro-ecological conditions. In the future, investigations should be carried out with the aim to collect more data on this pest and to be able to implement IPM principles in its control.

V. cardui is causing defoliation of the soybean plants. As periodical pest it appears from time to time in certain areas causing significant damage. Sampling procedure for this pest is established but, research should be conducted in order to determine economic threshold and the efficacy of environmentally acceptable insecticides (*B.t.k.*, spinosad, neem, IGRs, avermectins).

Phytophagous mites, *T. urticae* and *T. turkestani* as soybean pests are well known to Croatian farmers. Due to their feeding on soybean leaves they are causing defoliation. Their infestation is related to the climatic condition. In warm and dry years these pests cause more severe damage than in "normal" years. Sampling procedure for these pests is established but, due to the lack of registered acaricides, their control is not possible. The future research should be focused on finding appropriate ecologically acceptable acaricide for the control of this pest.

The critical period for the infestation by all four species is from flowering through maturity in which period all four pests should be monitored and sampled on a regularly basis in order to ensure the proper information about the need for control measure.

Some other pests that were found in our investigation are capable of becoming key pests if environmental conditions and population of their natural control agents are disrupted by unnecessary application of insecticides. One of these species belongs to *Piezodorus* sp. which is world widely recognized as very important soybean pest. Thus the future systematic and intensive study of the arthropod fauna associated with soybean in Croatia has to be continued. It will allow us to monitor the changes in the pest population and to prepare strategies for the control of the new pests that could arise over the time.

The main zoophagous species found on soybeans was *Nabis ferus*. The role of this species in soybean ecosystems, including its varying feeding strategies, needs much additional attention.

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