

Aphids (Hemiptera: Aphididae) on Alfalfa and their Coccinellid Predators in Serbia: Seasonal Abundance

Ivana Jovičić, Anđa Radonjić & Olivera Petrović-Obradović

Institute for Phytomedicine, University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Zemun, Serbia;
E-mails: mizuljak@gmail.com, avucetic@agrif.bg.ac.rs, petrovic@agrif.bg.ac.rs

Abstract: Population dynamics and flight activity of alfalfa aphids were documented at two locations in Serbia in 2011 and 2012. Additionally, the abundance of coccinellid predators was tested for correlation with aphid abundance. In order to verify the potential presence of the invasive *Acyrtosiphon kondoi*, aphids were collected from 44 alfalfa fields. Two sampling methods were used: stem samples and water traps. There were two seasonal peaks for aphids and coccinellids during both years, the end of May and the end of August. The most abundant aphid species was *Therioaphis trifolii*. Eight aphidophagous coccinellids were recorded. The invasive *Harmonia axyridis* (Pallas) was one of the three most abundant coccinellids. Pearson's product-moment correlation showed a significant correlation of coccinellid predators associated with aphids on alfalfa. Pearson's product-moment correlation between the number of aphids on alfalfa stem and in traps was found significant only for *T. trifolii*, which was the most numerous species in the traps. More *Aphis craccivora* were collected with water traps as compared to alfalfa stems, whereas more *Acyrtosiphon pisum* specimens were collected from alfalfa stems than from traps. The invasive species *A. kondoi* was not recorded in Serbia during this study.

Key words: *Acyrtosiphon pisum*, *Acyrtosiphon kondoi*, *Aphis craccivora*, *Therioaphis trifolii*, *Harmonia axyridis*

Introduction

Alfalfa (*Medicago sativa* L) is the oldest and the most important forage legume (MICHAUD *et al.* 1998, KATIĆ *et al.* 2005). Normally, it is cut five or six times per year and is usually grown for hay or silage. A reduction in the yield and quality of this crop was observed in the last ten years in Serbia (BULAJIĆ *et al.* 2010).

Aphids are one of the most important alfalfa pests because of their rapid reproductive rates and short life cycles. Worldwide there are four aphid species that are pests on alfalfa: *Acyrtosiphon pisum* (Harris), *Acyrtosiphon kondoi* Shinji, *Therioaphis trifolii* (Monell) and *Aphis craccivora* Koch (BERBERET *et al.* 1983, PONS *et al.* 2009, RAKHSHANI *et al.* 2009, ZUMOFFEN *et al.* 2012, XIMENEZ-EMBUN *et al.* 2014). *Acyrtosiphon kondoi* is an invasive species in Europe which currently has a restricted distribution: it has been found only in Greece and France (COEUR D'ACIER *et al.* 2010).

During vegetation three aphid species live on alfalfa in Serbia: *A. pisum*, *T. trifolii* and *A. craccivora*. Earlier studies on alfalfa aphids found that in the Pannonian area *A. pisum* was more abundant than the two other species (TOMANOVIĆ *et al.* 1996).

Aphids cause damage to their host plants directly by feeding and indirectly by vectoring plant-pathogenic viruses (KATIS *et al.* 2007). Alfalfa aphids are vectors of Alfalfa mosaic virus (AMV) and many other plant viruses which can trigger severe economic damage in growing the culture (BOL 2010, ZUMOFFEN *et al.* 2012). AMV is the most destructive and frequent alfalfa virus, worldwide and in Serbia (BOL 2010, BULAJIĆ *et al.* 2010). Role in its transmission have aphids feeding on alfalfa and winged aphids which fly from other fields (JONES & FERRIS 2000).

Several aphid predators, including Coccinellidae, prey upon aphids on alfalfa (PONS *et al.* 2009, RAKHSHANI *et al.* 2009, XIMENEZ-EMBUN

et al. 2014). The invasive ladybird *Harmonia axyridis* (Pallas) has been used as a biological control agent of aphids and has become one of the dominant coccinellid species in Europe (KOCH 2003, ROY & MIGEON 2010). In many parts of Western Europe it is now the most abundant coccinellid species (KINDLMANN *et al.* 2010), while in Serbia it was identified only recently, in 2008 (THALJI & STOJANOVIĆ 2008).

The aims of this study were to study population dynamics of the alfalfa aphids and their coccinellid predators and analyse the relationship between aphids and the density of the coccinellid predators on alfalfa. Flight activity of alfalfa aphids has been studied. Correlation between the number of aphids on stems and winged aphids of alfalfa has been calculated. Special attention has been devoted to the study of *H. axyridis*, its occurrence on alfalfa and its relative number compared to other coccinellid species. This is the first study in Serbia on the occurrence of *H. axyridis* on herbaceous cultivated plants during the growing season.

Materials and Methods

During the vegetation period, the occurrence and population dynamics of three aphid species, *A. pisum*, *T. trifolii* and *A. craccivora*, as well as coccinellid predators were studied in two insecticide-free alfalfa fields (cultivar Banat NS). The two fields had different ages: one-year-old young field and an old field (four-year-old). They were situated in the plains of Serbia (Pannonian area). We visited two locations: Ovča – South Banat (the young field) and Progar – Srem (the old field) in April – October 2011 and March – October 2012. In order to study their population dynamics, samples were taken every ten days from both locations. Aphids and predators were collected directly from ten plant stems in each of the one m² plots, 20 at Ovča and 16 at Progar. Aphids were placed in plastic tubes in 70% ethanol. Coccinellid predators (adults only) were placed separately in plastic boxes. Identification was based on morphological characters using stereomicroscope (Bio-optica, Type 1000). Aphid and coccinellid abundances were calculated as the total number per 100 stems.

The alfalfa fields were at 75–85 m a.s.l., with an average rainfall of 400 mm and a mean temperature during the alfalfa growing season of c. 20°C. In Serbia, summers 2011 and 2012 were the warmest ones since the beginning of weather records (http://www.hidmet.gov.rs/latin/meteorologija/klimatologija_godisnjaci.php).

We sampled during five alfalfa intercrops, i.e. the plant growth period between cutting (except Progar 2012: alfalfa was cut three times per year due to poor crop condition caused by long periods of drought): the 1st intercrop corresponding to the period from the start of the growing season to the 1st cutting, the 2nd intercrop to the period from the 1st to the 2nd cutting, etc. The flight activities of alfalfa aphids were monitored at Progar using six yellow water traps. They were 17 x 17 x 11 cm, with holes below the upper edge, one-third filled with water, with the addition of liquid detergent. At the beginning of each cut, traps were placed on the ground and during plant growth they were elevated in order to be visible for aphids. The fluid from the traps was sieved every ten days and the insects were packed into plastic boxes with 75% alcohol. In the laboratory, alfalfa aphids were separated from the other insects and conserved in 75% alcohol.

In order to examine the potential presence of the species *A. kondoi*, aphid sampling was done at different intervals for four years from 44 fields in the major alfalfa growing areas in Serbia.

We have calculated Pearson's product-moment correlation between the number of aphids and the number of predators for each year separately and for both years together; between the number of the three aphid species which are feeding on alfalfa plant stems and the number of these aphids in traps, for both years. The Pearson product-moment correlation coefficient is a measure of the linear correlation. Its value ranges between +1 and -1, where 1 is total positive correlation, 0 is no correlation, and -1 is total negative correlation. Furthermore, we have calculated a 95% confidence interval and tested the hypothesis that the correlation is zero.

Results

Aphids on alfalfa

From 44 alfalfa fields studied, three aphid species were recorded: *Acyrtosiphon pisum*, *Aphis craccivora* and *Therioaphis trifolii*. *Acyrtosiphon kondoi*, an invasive aphid species in Europe, was not recorded during the present study.

Coccinellids on alfalfa

Eight aphidophagous coccinellid species were recorded: *Adalia bipunctata* (L.), *Coccinella septempunctata* L., *Harmonia axyridis* (Pallas), *Hippodamia apicalis* Casey, *Hippodamia heideni* Wse, *Hippodamia variegata* (Goeze), *Hippodamia tredecimpunctata* (L.) and *Propylea quatuordecimpunctata* (L.). The multicoloured Asian ladybird beetle *H. axyridis* is an invasive species (ROY & MIGEON 2010).

Field studies

The entire sampling effort yielded a total of 9889 aphids and 758 adult coccinellids. The maximum population density of aphids on alfalfa stems was recorded at the end of August in 2012 at the Ovča site (543 individuals per 100 alfalfa stems) and in 2012 at the Progar site (463 individuals per 100 alfalfa stems; Graph. 2 and 4). The minimum population density was recorded in June (2-20 individuals per 100 alfalfa stems) during both years at both locations (Graph. 1-4).

The most abundant aphid on the alfalfa stems was *T. trifolii* followed by *A. pisum* (Fig. 1) at both locations during both years. *Aphis craccivora* was found in lower densities. The first individuals of *T. trifolii* were collected at Progar on 10 April 2012. The maximum population density was observed in 2012, at the end of August: 537 individuals per 100 alfalfa stems at Ovča and 388 individuals per 100 alfalfa stems at Progar. This species was the most abundant in hot and dry summer months, while the other two species were practically absent during that time.

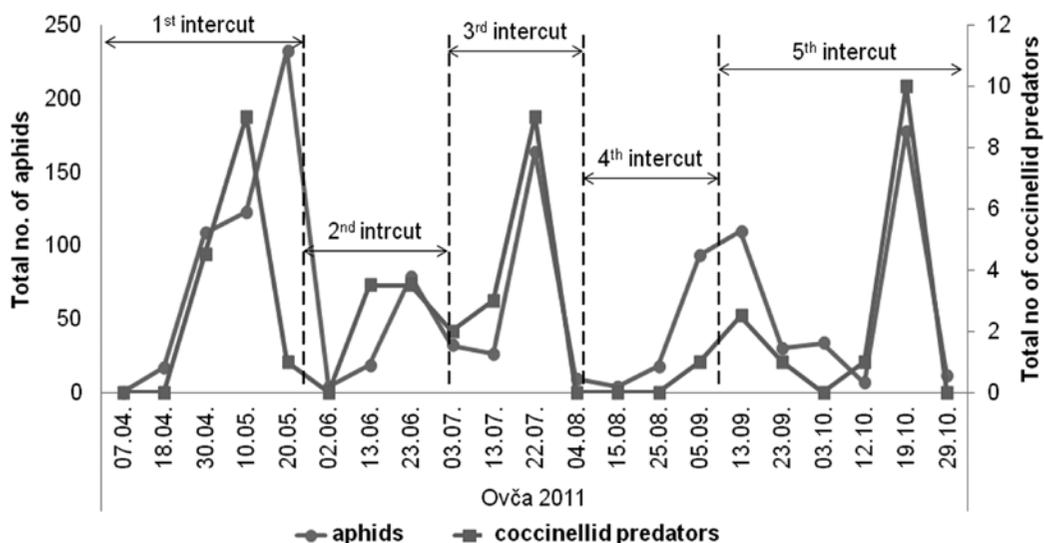
The first individuals of *A. pisum* were collected at Progar on 4 April 2011. Maximum population density (232 individuals per 100 alfalfa stems) was recorded in May 2011 at Ovča, with fast decline and low population density during the vegetation period. Another peak of this species (172 individuals per 100 alfalfa stems) was recorded at the end of October 2011 at Ovča.

Population density of *A. craccivora* was much lower than those of the other two species at both localities. *Aphis craccivora* is typically rare throughout the alfalfa growing season. A notable density of this aphid was recorded on individual alfalfa plants only in mid-July 2012 at Progar.

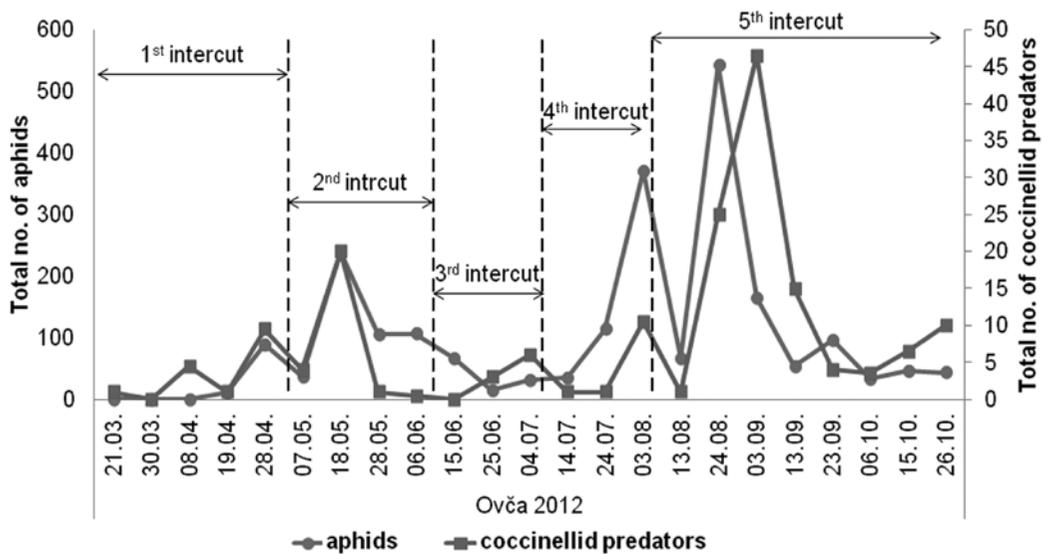
Aphidophagous coccinellid species were present on alfalfa throughout the growing season. The first individuals of coccinellid predators were collected in April before collecting the first aphid individuals. We found eight aphidophagous coccinellid species in both locations and years. A total of 758 adults of coccinellids was collected. *Hippodamia variegata* (38.52%), *C. septempunctata* (34.96%) and *H. axyridis* (23.76%) were three of the most numerous coccinellid predators (Fig. 2). Other coccinellids were present in low densities (*A. bipunctata* 0.13%, *H. apicalis* 0.13%, *H. heideni* 0.23%, *H. tredecimpunctata* 0.40%, *P. quatuordecimpunctata* 2.25%).

Harmonia axyridis was very numerous on alfalfa. We registered three forms: *succinea*, *conspicua* and *spectabilis*. The prevalent form was *succinea*, present in 85.31% of the samples, while the forms *spectabilis* (11.30%) and *conspicua* (3.39%) were less numerous.

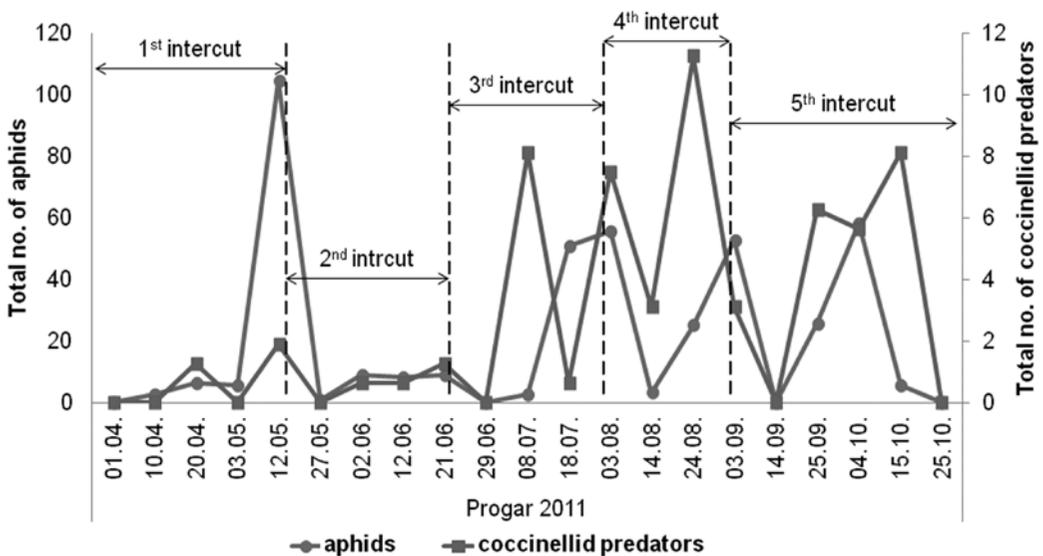
The Pearson's product-moment correlation between the number of aphids and the number of predators in 2011 was $cor=0.48$, 95% CI (0.20, 0.68). The test confirmed that the correlation was statistically different from zero ($t = 3.45$, $df = 40$, $p\text{-value} = 0.001$). The Pearson's product-moment correlation between the number of aphids and the number of predators in 2012 was $cor=0.63$, 95% CI (0.41, 0.78). The test confirmed that the correlation was statistically different from zero ($t = 5.23$, $df = 42$, $p\text{-value} \approx 0.001$). The Pearson's product-moment correlation between the pooled number of aphids and the number of predators of the two years was $cor=0.62$, 95% CI (0.47, 0.74) and was statistically different from zero ($t = 7.29$, $df = 84$, $p\text{-value} = 0.000$).



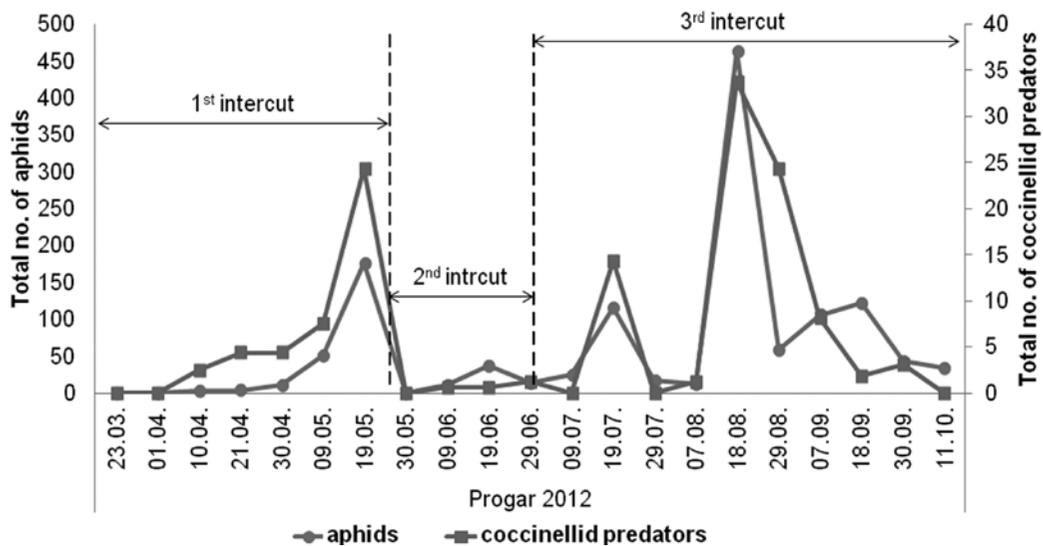
Graph. 1. Total number of aphids and coccinellid predators collected from 100 alfalfa stems at Ovča locality in 2011



Graph. 2. Total number of aphids and coccinellid predators collected from 100 alfalfa stems at Ovča 2012



Graph. 3. Total number of aphids and coccinellid predators collected from 100 alfalfa stems at Progar locality in 2011



Graph. 4. Total number of aphids and coccinellid predators collected from 100 alfalfa stems at Progar locality in 2012

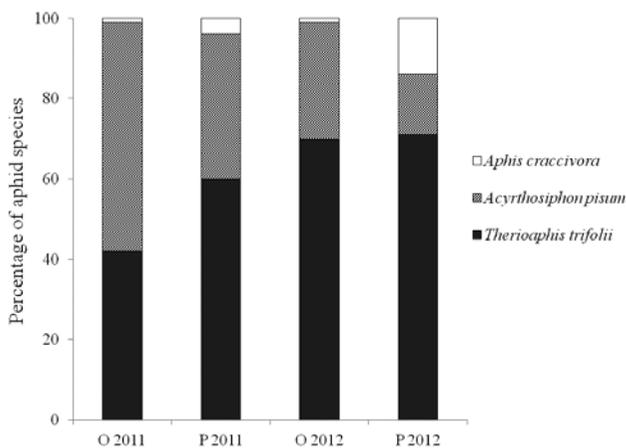


Fig. 1. Percentage of aphid species on two alfalfa fields Ovča (O) and Progar (P) during 2011 and 2012

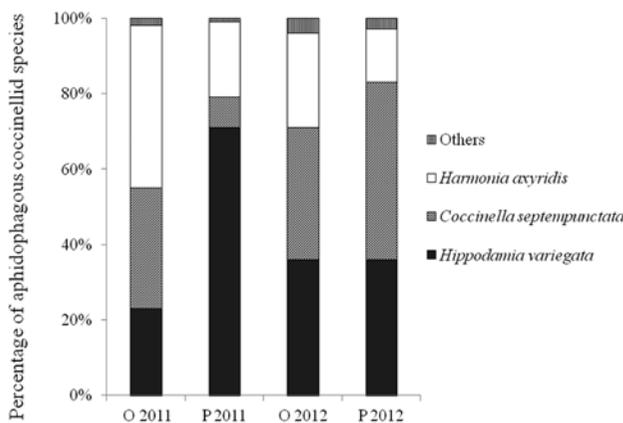


Fig. 2. Percentage of aphidophagous coccinellid species on two alfalfa fields Ovča (O) and Progar (P) during 2011 and 2012

Flight activities of alfalfa aphids

During the two-year study in Progar, a total of 541 alfalfa aphids were collected in six yellow water traps. The most numerous aphid species were *T. trifolii* followed by *A. craccivora*. A low percent of *A. pisum* was also present (Table 1).

On alfalfa stems at Progar, the first individuals of *A. pisum* and *T. trifolii* were found on April 10th. The first individuals of *A. craccivora* on alfalfa stems were collected on June 8th. We have found no correlation between the number of *A. pisum* and *A. craccivora* on alfalfa stems and the number of these aphids in traps. Significant correlation between the number of aphids on alfalfa stems and the number of these aphids in traps was found only for *T. trifolii* (Table 1).

Discussion

During this study three alfalfa aphids, *A. pisum*, *A. craccivora* and *T. trifolii*, were found in Serbia. They are vectors of many plant viruses, includ-

ing AMV (BLACKMAN & EASTOP 2000, BOL 2010). *Acyrthosiphon kondoi*, an invasive aphid species for Europe (COEUR D'ACIER *et al.* 2010), was not found despite it being a common alfalfa pest worldwide.

Our results regarding population dynamics demonstrated that *T. trifolii* was the most abundant aphid on alfalfa plant stems in the studied fields (Ovča and Progar) and in traps. Our results are not consistent with previously recorded population densities of this species, which was only occasionally present in alfalfa fields in this area two decades ago (TOMANOVIĆ *et al.* 1996). Maximum population density of this species was observed during warm summer days. In the present work, the prevalence of *T. trifolii* might be related to climate change. BERBERET *et al.* (1983) reported that high temperatures and also dry weather are optimal for increase of the abundance of *T. trifolii*. Furthermore, it is likely the abundance of this aphid on alfalfa throughout Central Europe might increase. The most favourable temperature for reproduction of this species is about 30°C (LUI 2012). Two decades ago the cool-season species *A. pisum* (BERBERET *et al.* 1983) was the most abundant aphid on alfalfa in the Pannonian area (TOMANOVIĆ *et al.* 1996). In this study, maximum population density of *A. pisum* on alfalfa plant stems was recorded in May, with fast decline and low population density during vegetation periods. High temperatures and quality deterioration of alfalfa as a host plant is not suitable for the development of this aphid (BERBERET *et al.* 1983). Another peak was recorded at the end of October in 2011 at the Ovča site. The pattern of occurrence of *A. pisum* and *T. trifolii* seems to be common in the Mediterranean region (LYKOURESSIS & POLATSIDIS 1990, PONS *et al.* 2013). *Aphis craccivora* rarely occurs in Serbia during the alfalfa growing season and very little information is available for this species. Low population levels and erratic distribution of *A. craccivora* is recorded for alfalfa crops in several countries (BERBERET *et al.* 2009, RAKHSHANI *et al.* 2009, PONS *et al.* 2013). Similar results are obtained by using different methodologies for collecting alfalfa aphids (RAKSHANI *et al.* 2009, PONS *et al.* 2013). During the last intercut in 2012 of older alfalfa at Progar, a higher number of this aphid compared to 2011 and the other location was recorded.

Eight aphidophagous coccinellids were found during this study. Among them, the invasive coccinellid species *H. axyridis* was found; this species prefers to prey on a wide variety of tree-dwelling homopteran insects (ROY & MIGEON 2010). In Belgium, on agricultural crops *H. axyridis* is recorded mainly in corn and to a much lesser extent in wheat and potato crops (VANDEREYCKEN *et al.* 2013). This species was firstly found in Serbia in 2008 (THALJI & STOJANOVIĆ

Table 1. Flight activity (first record and relative abundance %) for three alfalfa aphids and Pearson's product-moment correlation (r) between the number of aphids on plants and water traps in 2011 and 2012 at the Progar locality

Aphid	2011	2012	2011	2012	r (2011 and 2012)
<i>Acyrtosiphon pisum</i>	03 May	09 May	14%	3.3%	0.18
<i>Aphis craccivora</i>	02 June	30 May	40%	30.6%	0.08
<i>Therioaphis trifolii</i>	02 June	09 May	46%	66.1%	0.52

2008) and was one of the three most numerous coccinellid predators collected on alfalfa during this study. It may impact on native aphidophagous coccinellids through intraguild predation (ROY & MIGEON 2010), thus likely reducing the impact of the other numerous coccinellids on alfalfa, *H. variegata* and *C. septempunctata*, and causing negative ecological effects.

Aphid population dynamics depend on many factors such as climatic conditions, plant nutrients and biological factors (e.g., predators). Coccinellids are responsible for aphid suppression throughout the year (KINDLMANN *et al.* 2010). During both years, a lower population density of aphids and coccinellid predators were recorded in the older alfalfa field (Progar), which was not as suitable for the development of aphids as was the younger field.

In this study, a correlation between the richness of coccinellid predators and abundance of all aphids was found. Locally, coccinellid predator densities are strongly correlated to prey availability (KINDLMANN *et al.* 2010). The Pearson's product-moment correlation between the number of aphids and the number of predators for 2011 and 2012 individually, and for both years combined, showed significant correlation. Our studies suggest that coccinellid predators could play an important role in suppressing aphid abundance during the alfalfa growing season in Serbia. However, this predatory effect was not sufficient enough to prevent crop damage.

Yellow water traps proved to be a good sampling method for monitoring aphid flight activity and their use could potentially aid in monitoring of aphid-vectored viruses as well (VUČETIĆ *et al.* 2013). The most abundant species in traps were *T. trifolii* followed by *A. craccivora*, while *A. pisum* had lower relative abundance. The first individuals of *A. pisum* and *T. trifolii* were caught by traps one month after the occurrence on plants. On the other hand, *A. craccivora* was recorded in traps more than one month before its occurrence on plants. The Pearson's product-moment correlation between the number of alfalfa aphids on stems and on traps for *A. pisum* and *A. craccivora* showed that there was no correlation. *Acyrtosiphon pisum* was more numerous on

stems in comparison with the number of individuals caught by traps. One reason for the lower abundance of this species in traps could be its body size. This aphid is rather large: its body can reach 4mm in adults (BLACKMAN & EASTOP 2000). We showed that winged forms of *A. pisum* had small role in epidemiology of AMV. In this study, *A. craccivora* was found in lower densities on stems in alfalfa fields, but this polyphagous aphid (BLACKMAN & EASTOP 2000) was very numerous in water traps. We found a correlation between the number of *T. trifolii* on alfalfa stems and the number in traps. *Therioaphis trifolii* is in the subfamily Myzocallidinae, and one characteristic of this subfamily is the production of a high number of winged forms (HEIE 1982). This can be one of the reasons for the high abundance of this species in traps. *Therioaphis trifolii*, being the most numerous species in fields and in traps, represented a good vector of viruses and very important alfalfa pest.

Conclusions

Three aphid species, *A. pisum*, *A. craccivora*, *T. trifolii*, and eight coccinellid predators were found on alfalfa in this study. The invasive *Acyrtosiphon kondoi* was not found on the territory of Serbia. The most abundant aphid was *T. trifolii*. The invasive coccinellid *H. axyridis* was one of three the most abundant coccinellid predators on alfalfa. During the two years of the study, lower population density of aphids and coccinellid predators was recorded in the older alfalfa field. Significant correlation between the richness of coccinellid predators and the abundance of all aphids was found. In the study of the flight activity of the alfalfa aphid, the most abundant species in traps was *T. trifolii*. Significant correlation between the number of aphids on alfalfa stems and in traps was found only for *T. trifolii*.

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