

***Harmonia axyridis* (Coleoptera: Coccinellidae) in Serbia: its presence on aphid-infested plants and co-occurrence with native aphidophagous coccinellids**

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Received: 18 January 2021

Accepted: 3 February 2021

SUMMARY

The presence of the invasive coccinellid *Harmonia axyridis* in aphid colonies on plants, as well as its co-occurrence with native coccinellid predators was studied in the period 2011-2020. Also, its occurrence on fruit plants and grapes was monitored at harvest time. Research was conducted on 41 locations in Serbia. The feeding of this invasive species was recorded on a total of 43 aphid taxa of 25 different genera. It was most frequently present on aphid colonies of the genera *Aphis*, *Brachycaudus* and *Myzus*. The presence of *H. axyridis* in aphid colonies was recorded on 58 cultivated plant species: 10 fruit, 7 field crop, 5 vegetable and 16 ornamental species, as well as 20 non-cultivated plants. *Harmonia axyridis* feeding was also observed on mature fruit plants of cultivated blueberries, nectarine and grapes in representative production areas in Serbia. Nine native coccinellid species were detected in this study: *Adalia bipunctata*, *Adalia decempunctata*, *Coccinella septempunctata*, *Hippodamia* sp., *Hippodamia apicalis*, *Hippodamia variegata*, *Hippodamia tredecimpunctata*, *Hippodamia undecimnotata* and *Propylea quatuordecimpunctata*. Populations of *H. axyridis* and native coccinellids co-occurred in aphid colonies on many plants, i.e. in 53.45% of all samples. The results inferred that *H. axyridis* as a predator of numerous aphids on many plants is a useful coccinellid species. However, it may also be considered as a potential pest in fruit and grape production, and a threat to native aphidophagous coccinellids.

Keywords: harlequin ladybird, Coccinellidae, Aphididae, host plants, predatory insects

INTRODUCTION

Coccinellids are well-known natural enemies of aphids. About 90% of the approximately 4200 described coccinellid species are considered beneficial because of their predatory activity (Evans, 2009; Roy & Migeon, 2010). Aphidophagous coccinellids may have minor to

significant roles in biological control of aphids on many plants (Obrycki et al., 2009).

The harlequin ladybird, *Harmonia axyridis* Pallas, 1773 (Coleoptera: Coccinellidae: Coccinellini), is a well-known aphid predator with many vernacular names, including multicolored Asian lady beetle, Halloween

beetle (Koch, 2003) and harlequin ladybird (Brown & Roy, 2018). It is native to East Asia: the south-east of West Siberia, south of East Siberia, south of Russian Far East, east of Kazakhstan, north of Kyrgyzstan, Mongolia, China, North Korea, South Korea, Japan and the north of Vietnam (Orlova-Bienkowska et al., 2015).

This aphidophagous coccinellid has a long history of use as a classical biological control agent of aphids worldwide. Early introductions of *H. axyridis* in Eastern Europe (Ukraine and Belarus) occurred in the 1970s, and it was intended for aphid control on fruit trees. A decade later it was first used as a biological control agent in Western Europe: France, Belgium and the Netherlands (Brown et al., 2008). In the 1990s, *H. axyridis* was used as a biological control agent in greenhouses in northern Italy (Brown et al., 2008) and Spain (Jacas et al., 2006), and in citrus-growing areas of Portugal and Greece (Katsoyannos et al., 1997). Brown et al. (2008) reported that *H. axyridis* was spreading across Europe at a very fast rate, particularly since 2002. The species was observed for the first time in Serbia in 2008, and it was in the Fruška Gora National Park (Thalji & Stojanović, 2008). Its presence has also been reported in neighboring countries: Hungary (Merkl, 2008), Croatia (2008) (Mičetić Stanković et al., 2011), Romania (Marko & Pozsgai, 2009), Bulgaria (Tomov et al., 2009), Bosnia and Herzegovina (Kulijer, 2010), Albania (2012) (Ibrahimi et al., 2016), North Macedonia (2015) (Kulijer, 2016). Although the species has not been reported in Montenegro as yet, it is highly possible that the invasion has spread to that territory also.

This voracious predator is associated with trees and semi-arboreal plants in natural and agricultural settings where prey is available, and it may occur less often on various herbaceous plants (Koch et al., 2006). Whilst primarily an aphid-feeder, it can also feed on many other soft-bodied insects (coccids, adelgids, psyllids, and eggs and larvae of many other insects) and aphidophagous coccinellids (Koch, 2003). Through intraguild predation – i.e. the killing and eating of species that use similar and often limited resources (competitors), this predator can cause serious impact on native coccinellids in areas of introduction (Aebi et al., 2011). Studies in many of European countries have shown that certain aphidophagous species may be harmed by *H. axyridis* (Burgio et al., 2002; Adriaens et al. 2010; Brown et al., 2011; Brown & Roy, 2018). This coccinellid can also

be considered as a pest in orchards and particularly in vineyards. *Harmonia axyridis* uses different types of plant resources as alternative food: mature fruit, grapes, pollen and nectar (Koch, 2003).

Considering that studies of *H. axyridis* have been missing in Serbia and there is little data of its predatory activity, the aim of this paper was to provide information on the incidence of this invasive species and its feeding on aphid colonies, association with aphid-infested plants (cultivated and non-cultivated) and co-occurrence with native aphidophagous coccinellids in aphid colonies, as well as its presence and damage caused to fruits and grapes at harvest time.

MATERIALS AND METHODS

The occurrence of *H. axyridis* in aphid colonies on plants, as well as co-occurrence with native aphidophagous coccinellids, was studied in many parts of Serbia from April to November over the period 2011-2020. The research was conducted on 41 locations (with additional 10 locations in Belgrade), and samples collected in Belgrade were the most numerous. Sampling was conducted from two categories of plants, cultivated plants (fruit, field crop, vegetable and ornamental crops) and non-cultivated plants (weeds, wild-growing plants in urban environments, ruderal plants). More than 100 plant species were checked. Locations and dates of sampling are listed in Tables 1-5.

Aphids and coccinellids were collected from different plant parts, especially terminal shoots and leaves, trunks/stems and branches. Upon collection, aphids along with parts of their host plants were transferred alive to the laboratory. Coccinellids (adults, pupae, larvae and eggs) were placed separately in plastic boxes. Most of the collected aphid samples were preserved in 70% alcohol, while some were mounted on microscope slides using standard methods (Eastop & van Emden, 1972). Aphids were identified using a stereomicroscope and identification keys (Blackman & Eastop, 2020). Coccinellids were identified using a stereomicroscope, keys and description provided by Koch (2003), Bienkowski (2018) and Biranvand et al. (2020). Identification of coccinellids was confirmed by Marc Kenis (Centre for Agricultural Bioscience International, CABI Switzerland). The samples were processed in the Laboratory of Entomology and Agricultural Zoology,

Faculty of Agriculture, University of Belgrade, and in the Laboratory of Applied Entomology, Institute of Pesticides and Environmental Protection in Belgrade. In addition, potential occurrence and feeding of *H. axyridis* on fruits and grapes at harvest time was monitored in representative production areas in Serbia over the period 2011-2020.

RESULTS

Our study showed that *H. axyridis* is widespread in Serbia. The majority of findings on aphid-infested plants were in Belgrade and in Northern Serbia (in the southern part of the Pannonian plane), in locations on altitudes of up to 300 m. Its presence was confirmed in several locations in Central, Southwestern and Southern Serbia. Furthermore, populations of *H. axyridis* were confirmed at altitudes of about 1000 m in locations Mokra Gora, Rtanj and Tara.

During the ten-year study on 41 locations, *H. axyridis* feeding was recorded on a total of 43 aphid taxa belonging to 25 genera. It was most frequently present in aphid colonies of the genera *Aphis*, *Brachycaudus* and *Myzus*.

The occurrence of this invasive coccinellid in aphid colonies was recorded on 58 different plant species (Tables 1-5). Its presence was recorded on 38 cultivated plants: 10 fruit species, 7 field crops, 5 vegetables and 16 ornamentals; and on 20 non-cultivated plants.

In studying the co-occurrence of *H. axyridis* and native coccinellids in aphid colonies, a total of nine native coccinellid species were recorded: *Adalia bipunctata* (Linnaeus, 1758); *Adalia decempunctata* (Linnaeus, 1758); *Coccinella septempunctata* Linnaeus, 1758; *Hippodamia apicalis* Casey, 1899; *Hippodamia* sp. Chevrolat, 1836; *Hippodamia variegata* Goeze, 1777; *Hippodamia tredecimpunctata* Linnaeus, 1758; *Hippodamia undecimnotata* Schneider, 1792 syn. *Ceratomegilla undecimnotata* (Schneider, 1792); and *Propylea quatuordecimpunctata* (Linnaeus, 1758) (Tables 1-5). Populations of *H. axyridis* and native coccinellids co-occurred in aphid colonies on many plant species (in 53.45% of all samples). The most numerous native coccinellid was *C. septempunctata* (present in 31% of the samples). Compared to *H. axyridis* and *C. septempunctata*, all other coccinellid species were considerably rarer. In a majority of samples, adults were the predominating coccinellid stage in aphid colonies on plants, and then larvae.

Table 1. Fruit species infested with aphids used by *H. axyridis* and native coccinellids for feeding

Fruit species	Date and location	Aphids	Coccinellids	Coccinellid stage
<i>Corylus colurna</i>	13.06.2015. Kotraža, Lučani	<i>Myzocallis coryli</i>	Ha	L, A
	19.05.2012. Barajevo, Belgrade	<i>Dysaphis plantaginea</i>	Ha	A
	07.06.2012. Radmilovac, Belgrade	<i>Aphis pomi</i>	Ha	L
<i>Malus domestica</i>	05.05.2013. Kotraža, Lučani	<i>Dysaphis</i> sp.	Ha	L, A
	03.06.2019. Mali Požarevac	<i>Dysaphis</i> sp.	Ha Ab	L, A A
	07.06.2019. Petrijеvo, Smederevo	<i>Dysaphis</i> sp.	Ha Cs	A A
	09.06.2019. Brestovik	<i>Dysaphis</i> sp.	Ha	A
<i>Prunus avium</i>	05.06.2016. Vinča, Topola	<i>Myzus varians</i>	Ha Ab	E, L, P, A A
	12.06.2018. Novi Beograd, Belgrade	<i>Hyalopterus pruni</i>	Ha Ad	L

Table 1 - continued. Fruit species infested with aphids used by *H. axyridis* and native coccinellids for feeding

Fruit species	Date and location	Aphids	Coccinellids	Coccinellid stage
<i>Prunus cerasus</i>	07.06.2012. Radmilovac	<i>Myzus cerasi</i>	Ha Pp	L A
	27.05.2013. Zemun, Belgrade	<i>Myzus cerasi</i>	Ha Ab	E, L, P, A A
	06.05.2013. Čačak	<i>Myzus cerasi</i>	Ha	A
<i>Prunus domestica</i>	22.06.2012. Zemun, Belgrade	<i>Hyalopterus pruni</i>	Ha	L, P
	05.05.2013. Kotraža, Lučani	<i>Hyalopterus pruni</i> <i>Phorodon humuli</i>	Ha	A
	06.05.2013. Čačak	<i>Hyalopterus pruni</i> <i>Phorodon humuli</i>	Ha Cs	A A
	29.4.2012. Prnjavor, Gornji Milanovac	<i>Hyalopterus pruni</i>	Ha	A
	13.08.2020. Mokra Gora	<i>Hyalopterus pruni</i>	Ha Ab	L, A A
<i>Prunus persica</i>	25.06.2012. Ovča	<i>Brachycaudus schwaritzi</i>	Ha	A
	31.07.2012. Kotraža, Lučani	<i>Brachycaudus schwaritzi</i>	Ha Cs	A L, P, A
	05.05.2013. Kotraža, Lučani	<i>Myzus varians</i>	Ha	L, A
	31.05.2020. Vinča, Topola	<i>Myzus persicae</i>	Ha Ab	A A
<i>Prunus persica var. nucipersica</i>	22.05.2020. Sremska Mitrovica	<i>Myzus persicae</i>	Ha Cs Pp	L, A A A
<i>Ribes rubrum</i>	06.05.2013. Kotraža, Lučani	<i>Aphis schneideri</i> <i>Cryptomyzus ribis</i>	Ha	A
<i>Rubus idaeus</i>	11.06.2015. Ivanjica	<i>Aphis idaei</i>	Ha Cs	A A
<i>Rubus caesius</i>	13.06.2015. Kotraža, Lučani	<i>Amphorophora rubi</i>	Ha Cs	A A

Ab - *Adalia bipunctata*, Ad - *Adalia decempunctata*, Cs - *Coccinella septempunctata*, Ha - *Harmonia axyridis*, Pp - *Propylea quatuordecimpunctata*, E - eggs, L - larvae, P - pupae, A - adults

Harmonia axyridis was recorded as feeding on 14 aphid taxa (9 genera) hosted by a total of 10 fruit species. The most frequent findings were in *Dysaphis* sp. colony on apple (*Malus domestica*), in *Myzus cerasi* colony on sour cherry (*Prunus cerasus*), in *Hyalopterus pruni* and *Phorodon humuli* colonies on plum (*Prunus domestica*), and in *Brachycaudus schwaritzi*, *Myzus varians* and *Myzus persicae* colonies on peach (*Prunus persica*) (Table 1).

Populations of *H. axyridis* and native coccinellids (*A. bipunctata*, *A. decempunctata*, *C. septempunctata*, *P. quatuordecimpunctata*) on aphid-infested fruit plants co-occurred in 52% of all samples. The most numerous native coccinellids were *C. septempunctata* (present in 24% of all samples in this category of plants) and *A. bipunctata* (20%). The occurrence of *H. axyridis* and others coccinellids in aphid colonies on fruit plants was most frequently recorded in May and June (Table 1).

Table 2. Field crops infested with aphids used by *H. axyridis* and native coccinellids for feeding

Field crops	Date and location	Aphids	Coccinellids	Coccinellid stage
<i>Brassica napus</i> var. <i>oleifera</i>	28.05.2013. Ledine, Belgrade	<i>Brevicoryne brassicae</i>	Ha	A
<i>Helianthus annuus</i>	29.06.2012. Progar, Belgrade	<i>Brachycaudus helichrysi</i>	Ha	P
	02.08.2012. Kačarevo, Pančevo	<i>Aphis fabae</i>	Ha	A
	02.07.2020. Golubinci, Stara Pazova	<i>Aphis fabae</i>	Ha Pp	L, A A
	09.05.2012. Progar, Belgrade	<i>Acyrtosiphon pisum</i> , <i>Therioaphis trifolii</i>	Ha Cs	A A
<i>Medicago sativa</i>	24.08.2012. Ovča, Belgrade	<i>Therioaphis trifolii</i>	Ha	L, P, A
			Cs	A
			Hap	A
			Hsp	A
			Hv	A
			Ht	A
	17.06.2012. Kotraža, Lučani	<i>Acyrtosiphon pisum</i> , <i>Aphis craccivora</i> , <i>Therioaphis trifolii</i>	Ha	E, L, P, A
			Cs Hv Pp	L, P, A A A
	22.08.2012. Šatra, Kuršumljija	<i>Therioaphis trifolii</i>	Ha	A
			Cs Hv	A A
18.08.2012. Prnjavor, Gornji Milanovac	<i>Aphis craccivora</i>	Ha	A	
		Cs	A	
	29.06.2013. Cerje, Ušće	<i>Aphis craccivora</i>	Cs	A
			Ha	A
	28.06.2014. Radenković, Sremska Mitrovica	<i>Acyrtosiphon pisum</i> , <i>Aphis craccivora</i> , <i>Therioaphis trifolii</i>	Cs	L, A
			Ha	A
	16.06.2014. Rusko selo, Kikinda	<i>Aphis craccivora</i> , <i>Therioaphis trifolii</i>	Ha	A
			Cs	A
	23.06.2014. Zablaće, Čačak	<i>Aphis craccivora</i>	Ha	L, A
			Cs	A
Hv			A	
06.06.2015. Čortanovci, Indija	<i>Acyrtosiphon pisum</i> , <i>Aphis craccivora</i>	Ha	A	
23.06.2017. Kovilovo, Belgrade	<i>Acyrtosiphon pisum</i>	Ha	L, A	
		Cs	A	
27.06.2017. Sopot, Piroć	<i>Aphis craccivora</i>	Ha Pp	A A	
31.06.2018. Susek, Beočin	<i>Acyrtosiphon pisum</i> , <i>Therioaphis trifolii</i>	Ha	A	

Table 2 - continued. Field crops infested with aphids used by *H. axyridis* and native coccinellids for feeding

Field crops	Date and location	Aphids	Coccinellids	Coccinellid stage
<i>Medicago sativa</i>	25.08.2018 Platičevo, Ruma	<i>Acyrtosiphon pisum</i> , <i>Therioaphis trifolii</i>	Ha Cs	L, A A
	27.08.2020. Donja Lakošnica, Leskovac	<i>Therioaphis trifolii</i>	Ha	A
<i>Nicotiana tabacum</i>	11.08.2019. Golubinci, Stara Pazova	<i>Myzus persicae</i>	Ha	E, L, P, A
<i>Triticum vulgare</i>	21.05.2012. Galenika, Belgrade	<i>Shyzaaphis graminum</i>	Ha Cs	A A
	21.05.2015. Ledine, Belgrade	<i>Sitobion avenae</i>	Ha Cs	A A
<i>Trifolium pratense</i>	02.10.2020. Sremska Mitrovica	<i>Aphis craccivora</i>	Ha	A
<i>Zea mays</i>	02.08.2012. Banatsko Novo Selo, Pančevo	<i>Sipha</i> sp.	Ha Hv	L, A A
	17.06.2013. Ovča, Belgrade	<i>Aphis fabae</i> <i>Sitobion avenae</i>	Ha	A

* Cs - *Coccinella septempunctata*, Ha - *Harmonia axyridis*, Hap - *Hippodamia apicalis*, Hv - *Hippodamia variegata*, Hsp - *Hippodamia* sp., Ht - *Hippodamia tredecimpunctata*, Pp - *Propylea quatuordecimpunctata*, E-eggs, L-larvae, P-pupae, A-adult

Harmonia axyridis was recorded in association with 10 aphid taxa (9 genera) on 7 field crops. Very few specimens of this coccinellid were recorded in field crops, except in alfalfa, in colonies of *Acyrtosiphon pisum*, *Aphis craccivora* and *Therioaphis trifolii*. Most findings on alfalfa were made during the summer months (Table 2).

Six native coccinellids were found on aphid-infested field crops: *C. septempunctata*, *H. apicalis*, *H. variegata*, *Hippodamia* sp., *H. tredecimpunctata* and *P. quatuordecimpunctata*. Co-occurrence of *H. axyridis* with other aphidophagous coccinellids was recorded in 64% of the samples. *C. septempunctata* (present in 52% of all samples in this category of plants) and *H. variegata* (20%) were the most numerous native coccinellid species in field crops (Table 2).

The presence of *H. axyridis* was less frequent on aphid-infested vegetable plants. Its feeding was recorded on 5 aphid species (3 genera) on a total of 5 vegetable species. Its presence was detected on polyphagous and economically significant aphid pests of vegetables: *Aphis gossypii*, *Aphis fabae*, *Aphis craccivora* and *M. persicae*. *H. axyridis* was most frequently found in colonies of *A. fabae* and *A. craccivora* on beans (*Phaseolus vulgaris*) during June and July (Table 3).

Harmonia axyridis and the native coccinellids *C. septempunctata*, *H. variegata* and *P. quatuordecimpunctata* on aphid-infested vegetable plants co-occurred in 71.43% of all samples. Native coccinellids were equally represented in aphid colonies on vegetables (Table 3).

Table 3. Vegetable species infested with aphids used by *H. axyridis* and native coccinellids for feeding

Vegetable species	Date and location	Aphids	Coccinellids	Coccinellid stage
<i>Citrullus vulgaris</i>	01.06.2020. Ruma	<i>Aphis gossypii</i>	Ha	L, A
<i>Cucurbita pepo</i>	05.07.2012. Slanci, Belgrade	<i>Aphis gossypii</i>	Ha Cs	L, P, A A
	05.07.2012. Slanci, Belgrade	<i>Aphis fabae</i>	Ha Cs	L, A L, A
<i>Phaseolus vulgaris</i>	26.06.2014. Zemun, Belgrade	<i>Aphis fabae</i> <i>Aphis craccivora</i>	Ha Hv	A A
	07.06.2016. Kotraža, Lučani	<i>Aphis fabae</i>	Ha Pp	A A
<i>Pisum sativum</i>	26.06.2020. Sivac, Kula	<i>Acyrtosiphon pisum</i>	Ha Hv	L, A A
	20.10.2013. Kotraža, Lučani	<i>Myzus persicae</i>	Ha	A

* Cs - *Coccinella septempunctata*, Ha - *Harmonia axyridis*, Hv - *Hippodamia variegata*, Pp - *Propyrea quatuordecimpunctata*, E-eggs, L-larvae, P-pupae, A-adult

Table 4. Ornamental plants infested with aphids used by *H. axyridis* and native coccinellids for feeding

Ornamental plants	Date and location	Aphids	Coccinellids	Coccinellid stage
<i>Acer</i> sp.	05.06.2012. Zemun, Belgrade	<i>Periphillus</i> sp.	Ha Ad	A A
	28.09.2013. Radenković, Sremska Mitrovica	<i>Aphis nerii</i>	Ha Hv Pp	L, A L, A A
<i>Cotonaster dameri</i>	19.08.2013. Karaburma, Belgrade	<i>Aphis pomi</i>	Ha	L, P, A
<i>Crataegus oxyacantha</i>	31.05.2012. Zemunski kej, Belgrade	<i>Aphis pomi</i>	Ha	A
<i>Corylus colurna</i>	13.06.2015. Kotraža, Lučani	<i>Myzocallis coryli</i>	Ha	A
<i>Fraxinus</i> sp.	31.07.2014. Dušanovac, Belgrade	<i>Prociphilus</i> sp.	Ha	L, A
	18.06.2012. Karaburma, Belgrade	<i>Aphis gossypii</i>	Ha	A
<i>Hibiscus syriacus</i>	13.06. 2018. Čačak	<i>Aphis gossypii</i>	Ha Cs	A L, A
	15.06.2019. Zemun, Belgrade	<i>Aphis gossypii</i>	Ha	A
	15.07.2020. Mali Požarevac	<i>Aphis gossypii</i>	Ha Pp	L, A A
<i>Jasminum fruticans</i>	31.05.2012. Zemunski kej, Belgrade	<i>Aphis fabae</i>	Ha	A

Table 4 - continued. Ornamental plants infested with aphids used by *H. axyridis* and native coccinellids for feeding

Ornamental plants	Date and location	Aphids	Coccinellids	Coccinellid stage
<i>Jasminum officinale</i>	13.05.2012. Kotraža, Lučani	<i>Aphis fabae</i>	Ha	A
	01.06.2019. Toplola	<i>Aphis fabae</i>	Ha	L, A
<i>Prunus cerasifera</i>	06.06.2012. Karaburma, Belgrade	<i>Hyalopterus pruni</i>	Ha	L
	15.07.2020. Mali Požarevac	<i>Hyalopterus pruni</i>	Ha Cs	L, A L, P, A
<i>Pyracantha</i> sp.	23.08.2012. Novi Beograd, Belgrade	<i>Aphis spiraeicola</i>	Ha	E, L, A
<i>Rosa</i> sp.	29.06.2012. Novi Beograd, Belgrade	<i>Macrosiphum rosae</i>	Ha	L, A
	06.05.2013. Kotraža, Lučani	<i>Macrosiphum rosae</i>	Ha	L
	06.05.2013. Čačak	<i>Macrosiphum rosae</i>	Ha	A
<i>Spiraea media</i>	28.04.2012. Novi Beograd, Belgrade	<i>Aphis spiraeicola</i>	Ha Cs	A A
	31.05.2012. Zemunski kej, Belgrade	<i>Aphis spiraeicola</i>	Ha	A
	23.04.2014. Zemun park, Belgrade	<i>Aphis spiraeicola</i>	Ha Cs	A A
	02.5.2018. Dušanovac, Belgrade	<i>Aphis spiraeicola</i>	Ha	L, A
<i>Salix</i> sp.	17.04.2012. Kotraža, Lučani	<i>Aphis farinosa</i>	Ha	A
	22.04.2018. Kotraža, Lučani	<i>Aphis farinosa</i>	Ha Cs Pp	A A A
<i>Tecoma radicans</i>	03.06.2012. Karaburma, Belgrade	<i>Aphis gossypii</i>	Ha Ab	L, A A
	19.08.2012. Gornja Bukovica, Valjevo	<i>Aphis gossypii</i>	Ha Pp	L A
<i>Tamarix</i> sp.	02.11.2012. Niška Banja	<i>Brachyunguis tamaricis</i>	Ha Cs	L, P, A A

* Ab - *Adalia bipunctata*, Ad - *Adalia decempunctata*, Cs - *Coccinella septempunctata*, Ha - *Harmonia axyridis*, Hv - *Hippodamia variegata*, Pp - *Propylea quatuordecimpunctata*, E - eggs, L - larvae, P - pupae, A - adult

Harmonia axyridis feeding was recorded on 12 aphid taxa (7 genera) of 16 ornamental plants. The most frequent findings were in colonies of the polyphagous aphids *A. gossypii* (on *Hibiscus syriacus*) and *Aphis spiraeicola* (on *Spiraea media*), and on *Macrosiphum*

rosae (on *Rosa* sp.) It was found on several trees and ornamental plants infested by aphids in urban habitats of Belgrade and in gardens at several locations in Central, Southwestern and Southern Serbia (Table 4).

Native coccinellids - *A. bipunctata*, *A. decempunctata*, *C. septempunctata*, *H. variegata* and *P. quatuordecimpunctata*, and the invasive *H. axyridis* co-occurred in aphid colonies on 39.29% of all samples of ornamental plants. The most represented native coccinellid in aphid colonies on ornamentals was *C. septempunctata* (present in 21.49% samples) (Table 4).

Table 5. Non-cultivated plants infested with aphids used by *H. axyridis* and native coccinellids for feeding

Non-cultivated plants	Date and location	Aphids	Coccinellids	Coccinellid stage
<i>Ambrosia artemisifolia</i>	27.06.2012. Zemun, Belgrade	<i>Aphis spiraeicola</i>	Ha	L, A
Apiaceae Weed	30.06.2012. Rtanj	<i>Aphis fabae</i>	Ha Cs Hv	L, A A A
<i>Arctium lappa</i>	31.07.2012. Kotraža, Lučani	<i>Brachycaudus</i> sp.	Ha	E, L, P, A
<i>Artemisia</i> sp.	27.06.2013. Deliblatska peščara	<i>Cryptosiphum artemisiae</i> , <i>Macrosiphoniella artemisiae</i>	Ha	L
<i>Artemisia vulgaris</i>	01.06.2015. Fruška Gora	<i>Aulacorthum solani</i>	Ha	L, A
<i>Capsella bursa pastoris</i>	25.08.2018 Platičevo, Ruma	<i>Aphis craccivora</i>	Ha	L, A
<i>Carduus acanthoides</i>	07.06.2012. Ledine, Belgrade	<i>Aphis fabae</i>	Ha Cs	A A
<i>Centaurea</i> sp.	08.06.2012. Račanska šljivovica, Tara	<i>Uroleucon jaceae</i>	Ha Pp	E, L, A A
<i>Chenopodium album</i>	25.06.2014. Ledine, Belgrade	<i>Aphis fabae</i> , <i>Aphis sambuci</i>	Ha Cs	A A
	07.06.2012. Ledine, Belgrade	<i>Aphis fabae</i>	Ha Hv	A A
	28.05.2013. Ledine, Belgrade	<i>Uroleucon cirsii</i>	Ha	A L
	27.06.2013. Ovča, Belgrade	<i>Aphis fabae</i> , <i>Brachycaudus cardui</i>	Ha	L, P, A
	07.05.2014. Novi Beograd, Belgrade	<i>Aphis fabae</i>	Ha	A
	22.05.2014. Bežanijska kosa, Belgrade	<i>Aphis fabae</i> , <i>Brachycaudus cardui</i>	Ha Cs	A A
<i>Cirsium arvense</i>	25.06.2014. Ledine, Belgrade	<i>Aphis fabae</i>	Ha Cs	A L, A
	01.06.2015. Fruška gora	<i>Aphis fabae</i> , <i>Brachycaudus cardui</i>	Ha	A
	01.06.2015. Čortanovci, Indija	<i>Aphis fabae</i>	Ha	L, A
	23.06.2017. Kovilovo, Belgrade	<i>Brachycaudus cardui</i>	Ha Cs	L, P, A A
	29.06.2018. Kosmaj	<i>Aphis fabae</i>	Ha	L

Table 5 - continued. Non-cultivated plants infested with aphids used by *H. axyridis* and native coccinellids for feeding

Non-cultivated plants	Date and location	Aphids	Coccinellids	Coccinellid stage
<i>Cirsium arvense</i>	25.06.2020. Sremska Mitrovica	<i>Aphis fabae</i>	Ha Pp Cs	L, P, A A L
<i>Cornus sanguinea</i>	21.04.2012. Progar, Belgrade	<i>Anoecia corni</i>	Ha	A
<i>Cornus mas</i>	08.09.2014. Zemun, Belgrade	<i>Anoecia corni</i>	Ha Ab	E, A A
<i>Mycelis muralis</i>	01.06.2015. Fruška Gora	<i>Uroleucon</i> sp.	Ha	A
<i>Prunus spinosa</i>	25.06.2017. Ašanja, Pećinci	<i>Hyalopterus pruni</i>	Ha Cs Pp	A A A
<i>Robinia pseudoacacia</i>	27.06.2012. Zemun, Belgrade	<i>Aphis craccivora</i>	Ha Cs	A L, A
<i>Sinapsis arvensis</i>	27.06.2012. Zemun, Belgrade	<i>Brevicoryne brassicae</i>	Ha Cs Hv	A L, A A
<i>Sorghum halepense</i>	30.08.2012. Galenika, Belgrade	<i>Rhopalosiphum padi</i>	Ha Hv	A A
<i>Sambus nigra</i>	08.04.2014. Šumice, Belgrade	<i>Aphis sambuci</i>	Ha	A
	23.04.2014. Zemunski kej, Belgrade	<i>Aphis sambuci</i>	Ha Pp	A A
<i>Stenactis annua</i>	01.06.2015. Fruška Gora	<i>Brachycaudus helichrysi</i>	Ha	A
<i>Tragopogon dubius</i>	13.05.2014. Kvantaš, Belgrade	<i>Brachycaudus tragopogonis</i>	Ha Cs	A A

* Ab - *Adalia bipunctata*, Cs - *Coccinella septempunctata*, Ha - *Harmonia axyridis*, Hv - *Hippodamia variegata*, Pp-*Propylea quatuordecimpunctata*, E-eggs, L-larvae, P-pupae, A-adult

Harmonia axyridis was recorded in association with 18 aphid taxa (10 genera) on 20 non-cultivated plants (weeds, wild-growing urban plants, ruderal plants). The most frequent findings were noted in colonies of *A. fabae*, *Brachycaudus cardui* and *Uroleucon cirsi* on the weed species *Cirsium arvense*. It was frequently found on aphid-infested weeds in suburban and agricultural areas around Belgrade (Table 5).

Co-occurrence of *H. axyridis* and native coccinellids (*A. bipunctata*, *C. septempunctata*, *H. variegata* and *P. quatuordecimpunctata*) in aphid colonies was recorded in 51.61% of all samples from non-cultivated species. The species *C. septempunctata* (present in 35.48%

of all samples in this category of plants) was the most numerous native coccinellid on non-cultivated plants (Table 5).

Harmonia axyridis was present in aphid colonies on plants throughout the growing season. The first specimens (adults) were collected at Progar (Belgrade) on 21 April 2012 in a colony of *Anoecia corni* on *Cornus sanguinea* (Table 5) and at Kotraž (Lučani) on 22 April 2018 in a colony of *Aphis farinosa* on *Salix* sp. (Table 4). In the spring, its presence was recorded on a wide range of plant species, but most often on fruit (Table 1) and ornamental plants (Table 4). Its occurrence on aphid-infested plants during summer was less frequent, except

on alfalfa (Table 2) and the weed *Cirsium arvense* (Table 5). The last finding in the vegetation season was at Niška Banja on 2 November 2012 in a colony of *Brachyunguis tamaricis* on *Tamarix* sp. (Table 4).

The feeding of *H. axyridis* on fruit species in Serbia at harvest time was recorded on: cultivated blueberries (*Vaccinium corymbosum*) on the location Vlaško polje near Mladenovac in June 2018, and Rogača near Sopot in June 2019; and nectarine (*Prunus persica* var. *nucipersica*) on the location Sremska Mitovica in August 2020. Its occurrence on grapevine (*Vitis vinifera*) was recorded in representative growing areas: Fruška Gora in September 2015, Topola in September 2016, Donja Lakošnica near Leskovac in August 2020. Damage was observed on the surface of mature fruits and grapes in the exocarp zone. Damage due to feeding is negligible; the major problem is contamination of mature fruits and grapes. When adults are disturbed they release a yellow fluid which creates an unpleasant odor and taste.

DISCUSSION

After its initial detection in northern Serbia in 2008 (Thalji & Stojanović, 2008), *H. axyridis* spread rapidly across the country. Our results led to a conclusion that this coccinellid is widespread, and its populations established at altitudes up to 1000 m. Its presence was confirmed in several locations but most findings were made in the City of Belgrade and Northern Serbia, and less frequently in Central, Southwestern and Southern parts of the country. We also expect *H. axyridis* to be already present in several other parts of Serbia which were not included in this study. Brown et al. (2011) calculated that *H. axyridis*, as the fastest-spreading invasive alien insect in Europe, was spreading at a maximum rate of approximately 200 km annually, so that it is now regarded as an established species in most European countries, including countries neighboring on Serbia.

Harmonia axyridis is a predator of numerous aphid species. It should be noted that aphids are arguably the most significant ecological predictor of dispersal of this aggressive predator (Brown et al., 2011). In the current study, the species was found to feed on 43 aphid taxa on all observed plants in Serbia. It represents more than 10% of the species in total aphid fauna (383 species) in Serbia (Petrović-Obradović et al., 2020). This coccinellid

was found to occur in association with: 14 aphid taxa infesting 10 fruit species, 10 aphid taxa infesting 7 field crops, 5 aphid species infesting 5 vegetable species, 12 aphid taxa infesting 16 ornamentals, and 18 aphid taxa infesting 20 non-cultivated plants. During spring and early summer, *H. axyridis* dominated on aphid-infested fruits and ornamentals. The species was found to feed on several economically significant aphids, such as *Dysaphis* sp. on apple; *M. persicae* and *H. pruni* on stone fruit; and the polyphagous species *A. fabae*, *A. spiraeicola* and *A. gossypii* on vegetables and ornamental plants. In hot summer days and in the autumn, the species preferred feeding on aphids in alfalfa crops, namely *A. pisum*, *A. craccivora* and *T. trifolii*, and on the weeds *C. arvense*, *A. fabae*, *B. cardui* and *U. cirsi*.

In Serbia, *H. axyridis* was detected in aphid colonies on 58 plant species. Vandereycken et al. (2012) showed in a review paper that *H. axyridis* had been observed on 106 infested and non-infested plant species in different countries. Koch et al. (2006) reported that *H. axyridis* was present on 48 aphid-infested plants (trees and shrubs) in South America. Its presence was confirmed on 38 cultivated plant species (10 fruit species, 7 field crops, 5 vegetable species and 16 ornamentals) infested by aphids. Regarding fruit species, it was most frequent found on aphid-infested apple, sour cherry, plum and peach trees. Stone fruit and pome fruit plants are affected by high aphid populations, especially in the spring (Petrović-Obradović et al., 2009; Vučetić et al., 2010), and are therefore attractive to *H. axyridis*. However, the presence of this coccinellid in orchards at the time of summer and autumn harvest can be harmful. While *H. axyridis* acts as an efficient aphid predator, it is also a potential pest in fruit production and processing. In Serbia, damage caused by its feeding has been observed on mature fruit: cultivated blueberries and nectarine; and grapes in representative growing areas. Koch and Galvan (2008) reported that *H. axyridis* feeds on grapes, apples, peaches, plums, pears and raspberries. This coccinellid contaminates grapes and causes significant problems in vineyards and in wine production. When these ladybirds are massively collected along with grapes at harvest and crushed, alkaloids that *H. axyridis* contains taint the flavor of the resulting wine (Ratcliffe, 2002; Ejbich, 2003).

Harmonia axyridis aggregates in fields where aphids are abundant. Very few samples of *H. axyridis* were found on aphid-infested vegetables, except on

beans. The incidence of *H. axyridis* in aphid colonies in field crops in Serbia was lower, except in alfalfa. In a study of *H. axyridis* abundance in various field crops in Belgium, Vandereycken et al. (2013) showed that it was frequent on corn and broad beans, and to a much lesser extent in wheat and potato. In our study, *H. axyridis* was more frequently found in alfalfa fields, especially in summer. Aphids are abundant in alfalfa crops over the entire vegetation season (Jovičić et al., 2016) when they are mostly absent from other cultivated plants. Jovičić et al. (2016) showed that after arriving in Serbia this invasive species has become one of the most numerous coccinellid predators of alfalfa aphids.

Many studies in Europe indicate that this species is commonly found in urban and arboreal habitats, rather than on herbaceous plants (Brown et al. 2008; Brown & Roy 2018; Honek et al., 2019). In our study, *H. axyridis* was found on several aphid-infested ornamental plants in urban habitats around Belgrade. Such findings were most frequently on *Hibiscus syriacus* and on *Spiraea media* infested with aphids. Marković et al. (2018) reported that *H. axyridis* is the dominant coccinellid species on trees in parks and tree-lined avenues of Belgrade. However, agricultural landscapes of cereals and other field crops are much larger than areas with tree stands, which make their preferred habitat (Honek et al., 2019).

In addition, *H. axyridis* was found on 20 non-cultivated plant species infested with aphids, including many weeds and ruderal plants, mostly in the family Apiaceae. The most frequent findings were in suburban and agricultural areas around Belgrade. Some aphid-infested weeds, such as *C. arvensis*, host a large number of *H. axyridis* ladybirds, and constitute important potential reserves of this coccinellid in advance of aphid invasion of crops.

A total of nine native aphidophagous coccinellids were detected in the course of this study in aphid colonies on plants in Serbia. Studies of coccinellid diversity and abundance show that all of these coccinellids are widespread in Serbia (Thalji, 1994; Jerinić-Prodanović et al., 2010; Jovičić et al. 2016; Petrović-Obradović et al., 2016; Marković et al. 2018) and across Europe (Roy & Migeon, 2010; Brown & Roy, 2018). The most numerous native coccinellid in this study was *C. septempunctata*, while other coccinellids were considerably rarer. Some coccinellids occur on a wide range of plant species, such

as *C. septempunctata* and *P. quatuordecimpunctata*, while others are specialists feeding on trees (*A. bipunctata*) or herbaceous plants (*H. variegata*). Populations of *H. axyridis* and native coccinellids co-occurred in aphid colonies on all categories of plants in different ratios: from 39.29% on ornamentals to 71.43% on vegetables.

This study indicates that *H. axyridis* is one of the dominant coccinellids on aphid-infested cultivated and non-cultivated plants in Serbia. As a predator of numerous aphid species, including significant pests, it is a useful coccinellid species. However, it is also a potential threat to native aphidophagous coccinellids. This invasive species is a striking example of a biological introduction with unintended ecological consequences. The massive occurrence of this coccinellid affects the abundance of some native coccinellid species (Burgio et al., 2002; Honek et al., 2016; Brown & Roy, 2018). Due to competition for prey and intraguild predation, this coccinellid may have a negative impact on native aphidophagous coccinellids. It should be noted that changes in coccinellidae communities can also be caused by many other factors, such as climate and habitat change through agricultural intensification (Honek et al., 2017).

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grants 451-03-68/2020-14/200116 and 451-03-68/2020-14/200214). The article is also a contribution to COST Action CA17122 Alien CSI, funded by COST (European Cooperation in Science and Technology), see www.cost.eu. The authors are very grateful to Mrs. Dragica Smiljanić (University of Belgrade, Faculty of Agriculture, Serbia), for collecting a part of the samples, and Dr. Marc Kenis (Centre for Agricultural Bioscience International, CABI Switzerland) for assisting us in identification of coccinellids.

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Harmonia axyridis (Coleoptera: Coccinellidae) u Srbiji: prisustvo na biljkama infestiranim biljnim vašima i istovremena pojava sa domaćim afidofagnim bubamarama

REZIME

Prisustvo invazivne višebojne azijske bubamare *Harmonia axyridis* u kolonijama biljnih vaši na različitim biljkama, kao i istovremena pojava sa domaćim afidofagnim bubamarama istraživana je u periodu od 2011-2020. godine. Takođe, praćeno je potencijalno prisustvo i štetnost ove bubamare na zreloom voću i grožđu. Uzorci su prikupljeni sa 41 lokaliteta na teritoriji Republike Srbije. Ishrana ove invazivne vrste zabeležena je na ukupno 43 taksona biljnih vaši svrstanih u 25 rodova. Najčešće se hranila biljnim vašima iz rodova *Aphis*, *Brachycaudus* i *Myzus*. Registrovana je u kolonijama biljnih vaši na 58 biljnih vrsta od kojih je: 38 gajenih (10 voćarskih, 7 ratarskih, 5 povrstarskih i 16 ukrasnih) i 20 korovskih i ruderalnih biljaka. Ishrana je zabeležena i na zreloom plodovima gajene borovnice i nektarine, kao i na grožđu u glavnim proizvodnim regionima u Srbiji. U istraživanju je registrovano ukupno devet vrsta afidofagnih bubamara: *Adalia bipunctata*, *Adalia decempunctata*, *Coccinella septempunctata*, *Hippodamia* sp., *Hippodamia apicalis*, *Hippodamia variegata*, *Hippodamia tredecimpunctata*, *Hippodamia undecimnotata* i *Propylea quatuordecimpunctata*. Populacije invazivne vrste *H. axyridis* i domaćih bubamara u kolonijama biljnih vaši utvrđene su u 53,45% od svih prikupljenih uzoraka. Rezultati ovih istraživanja ukazuju da *H. axyridis* ima potencijal da smanji brojnost biljnih vaši na mnogim biljkama. Međutim, ova vrste može biti štetna u proizvodnji voća i grožđa, ali i pretnja domaćim afidofagnim bubamarama.

Ključne reči: višebojna azijska bubamara, Coccinellidae, Aphididae, biljke domaćini, insekti predatori