

# Flight activity of aphids as potential vectors of viral infection of alfalfa in Serbia

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## SUMMARY

Flight activity of aphids as potential vectors of viral infection of alfalfa plants were monitored in Serbia for the first time in Europe. Research was conducted at the location Progar (Srem) for two years using six yellow water traps. A total of 1626 individual winged aphids were collected. The collected specimens were classified into 49 different taxa. During the two-year study, maximum population density of aphids and maximum potential vector activity were noted at the beginning of June, during the second alfalfa intercut. More than 65% of the collected specimens were potential vectors of the most important alfalfa viruses, *Alfalfa mosaic virus* (AMV) and *Cucumber mosaic virus* (CMV). The most numerous winged aphid species on alfalfa were *Aphis craccivora*, *Aphis fabae*, *Aphis gossypii*, *Aphis pomi/spiraecola* and *Therioaphis trifolii*. The Morisita-Horn similarity index was used to calculate similarities in species composition among the traps. High values of this index showed no significant differences among the aphids in traps. It indicates that one trap alone could provide good insight into the abundance, aphid diversity and number of potential vector species in small alfalfa fields.

**Keywords:** Aphididae; Vectors; Plant viruses; Alfalfa

## INTRODUCTION

Three aphid species (Hemiptera, Sternorrhyncha, Aphididae): *Acyrtosiphon pisum* (Haris), *Aphis craccivora* Koch and *Therioaphis trifolii* (Monell) live on alfalfa plants in Serbia and cause direct damage to crops by feeding (Jovičić et al., 2016). Many winged aphid species fly over from other plants, making short feeding probes on alfalfa, while searching for a host plant. They have an important role in transmission of plant viruses and cause indirect damage to alfalfa (Katis et al., 2007).

*Alfalfa mosaic virus* (AMV, genus *Alfamovirus*, family *Bromoviridae*) and *Cucumber mosaic virus* (CMV, genus *Cucumovirus*, family *Bromoviridae*) are the most frequent alfalfa viruses worldwide, including Serbia. Both viruses belong to a group of nonpersistent viruses (Bol, 2010; García-Arenal & Palukaitis, 2010).

At least 15 aphid species are known to transmit AMV in a nonpersistent manner (Bol, 2010). Aphid species occurring in Serbia are: *Acyrtosiphon pisum* (Haris), *Aulacorthum solani* (Kaltenbach), *Aphis craccivora* Koch, *Aphis fabae* Scopoli, *Aphis gossypii* Glover, *Aphis*

*medicaginis* Koch, *Macrosiphum euphorbiae* (Thomas), *Myzus persicae* (Sulzer), *Myzus ligustri* (Mosley), *Nearctaphis bakeri* (Cowen), *Phorodon cannabis* Passerini and *T. trifolii* (Petrović-Obradović, 2003). Furthermore, AMV can be transmitted through alfalfa seeds (Bol, 2010). Over 80 species of aphids have been reported to transmit CMV (García-Arenal & Palukaitis, 2010). The most important vectors are *A. pisum*, *A. craccivora*, *A. fabae*, *A. gossypii*, *Aphis glycines* Matsumura, *Aphis spiraeicola* Patch, *M. euphorbiae*, *M. persicae*, *N. bakeri*, *Rhopalosiphum maidis* (Fitch) and *T. trifolii* (Gildow et al., 2008; García-Arenal & Palukaitis, 2010). Seed transmission of CMV has been reported in many plant species (García-Arenal & Palukaitis, 2010).

Monitoring the flight activities of aphids in alfalfa, using yellow water traps, is a reliable way to obtain data on potential plant viral vectors, the number of specimens of each species, as well as the moment when aphids fly into the field and the dynamics of each species' flight (Nault et al., 2004; Vučetić et al., 2013).

The aims of this study were to determine the population dynamics of aphids on alfalfa, as well as the number of potential viral vectors. In addition, the study investigated a similarity observed in aphid composition among six traps and the optimal number of traps for monitoring aphid flight activity in alfalfa crops.

## MATERIAL AND METHODS

Aphid flight in alfalfa was monitored by using yellow water traps at Progar, Srem district, Serbia (N 44° 42' 60", E 20° 08' 13") in 2011 and 2012. The dishes, size 22x22x11 cm with holes below upper lids, were one-third filled with water, and liquid detergent was added. Six traps were set at a distance of 70 m in the middle of each 1 ha plot in an alfalfa field. Yellow water traps were placed in the field immediately after the emergence of alfalfa. At the beginning of each intercut, they were placed on the ground, and then elevated to 1 m above ground during vegetation in order to be visible to insects. Insect specimens caught in the traps were collected once in ten days during five intercrops (April-October). The liquid from the traps was sieved and the insects were packed into plastic boxes with 75% alcohol. In the laboratory, aphids were separated from other insects and preserved in 75% alcohol. Identification of all sampled aphids was carried out based on identification keys (Taylor, 1984; Jacky & Bouchery, 1988; Remaudiere & Seco Fernandez, 1990).

The Morisita-Horn similarity index was used to calculate similarities in aphid composition among the traps. This index takes into account the composition and richness of fauna and successfully compares samples of different sizes (Magurran, 2004). The higher the Morisita-Horn index value, the greater the similarity between examined samples. The maximum value of this index is 1. Cluster analysis was conducted based on Morisita-Horn index after unweighted pair group analysis (UPGA).

## RESULTS

During the two-year study, 1626 aphid specimens were collected in six yellow water traps. The total number of aphids caught in 2012 was 1132. A much lower number of aphids (494 specimens) were recorded in 2011. A total of 49 different aphid taxa were identified (Table 1).

In 2011, the first specimens of winged aphids were caught in the yellow water traps at the beginning of May. Maximum population density in the traps was observed during the second alfalfa intercut at the beginning of June (155 specimens in 6 traps), while population density was low during the summer. Another peak (55 aphids in 6 traps) was recorded in mid-September (Figure 1).

The first specimens in 2012 were collected at the end of April. Maximum population density (395 specimens in 6 traps) was recorded during the second alfalfa intercut at the beginning of June. The second peak (137 aphids in 6 traps) occurred in mid-September, as in the previous year (Figure 2).

About 65% of all collected aphid specimens in the yellow water traps were potential vectors of two most important viruses on alfalfa, AMV and CMV. Population dynamics of the potential vector species are shown in Figures 1 and 2. Maximum population density of the vector species in traps was recorded during the second alfalfa intercut at the beginning of June in both seasons.

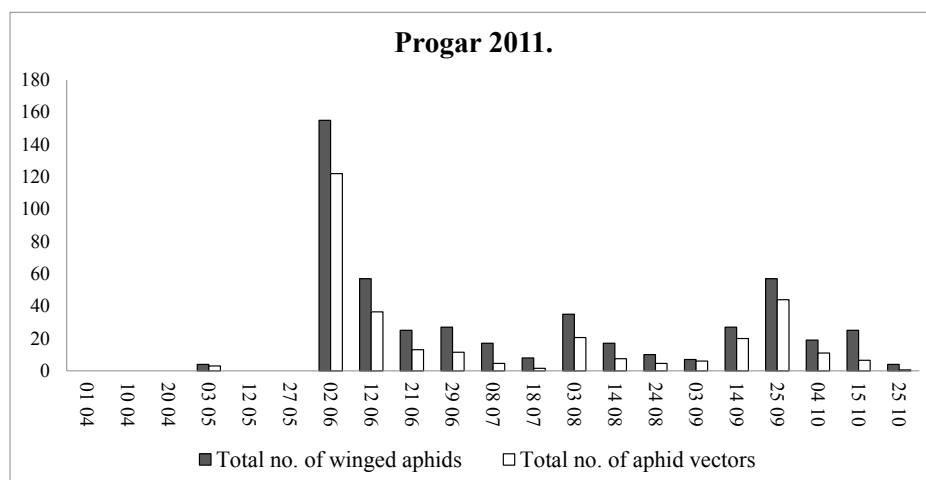
The most abundant species were *T. trifolii* and *A. craccivora*, both infesting alfalfa as a host plant, and the polyphagous species- *A. fabae*, *A. gossypii* and *A. pomi/spiraeicola* (Table 2).

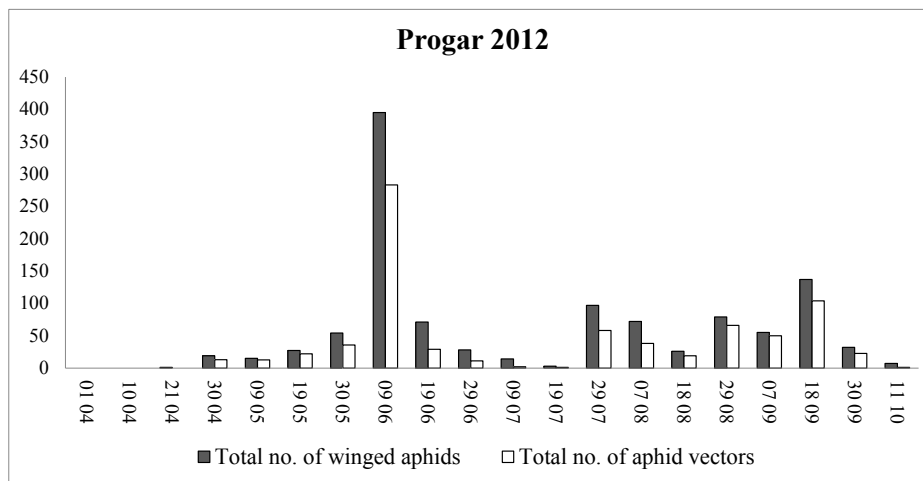
The Morisita-Horn similarity indexes for 2011, 2012 and collectively for both years were high (0.71-0.98) (Table 3). The index showed no significant differences in aphid composition among the six traps.

A cluster analysis based on this index was carried out (Figures 3, 4 and 5), and the traps were grouped as having low levels of difference.

**Table 1.** Aphid taxa identified after retrieval from six yellow water traps set in an alfalfa field during 2011 and 2012 (\* vectors of AMV and/or CMV)

<i>*Acyrtosiphon pisum</i> (Haris)	<i>Macrosiphoniella</i> spp.
<i>Amphorophora</i> spp.	<i>Macrosiphum rosae</i> (L.)
<i>Anoecia corni</i> (F.)	<i>Macrosiphum</i> spp.
Aphididae	<i>Megourella purpurea</i> Hille Ris Lambers
<i>*Aphis craccivora</i> Koch	<i>*Myzus persicae</i> (Sulzer)
<i>*Aphis fabae</i> Scopoli	<i>Ovatus</i> spp.
<i>*Aphis gossypii</i> Glover	Pemphiginae
<i>Aphis nerii</i> Boyer de Fonscolombe	<i>Pemphigus</i> spp.
<i>*Aphis pomi</i> De Geer / <i>spiraecola</i> Patch	<i>Rhopalomyzus poae</i> (Gill)
<i>Aphis sambuci</i> L.	<i>Rhopalosiphoninus</i> spp.
<i>Aphis</i> spp.	<i>Rhopalosiphoninus staphyleae</i> (Koch)
<i>Aphis</i> ( <i>Protaphis</i> ) spp.	<i>Rhopalosiphum insertum</i> (Walker)
<i>Brachycaudus helichrysi</i> (Kaltenbach)	<i>*Rhopalosiphum maidis</i> (Fitch)
<i>Brachycaudus</i> sp.	<i>Rhopalosiphum padi</i> (L.)
<i>Brevicoryne brassicae</i> (L.)	<i>Rhopalosiphum</i> spp.
<i>Capitophorus</i> spp.	<i>Sipha elegans</i> del Guercio
<i>Capitophorus horni</i> Börner	<i>Sipha maydis</i> Passerini
<i>Chaitophorus</i> spp.	<i>Sipha</i> spp.
<i>Dysaphis</i> spp.	<i>Sitobion avenae</i> (Fabricius)
<i>Eucallipterus tiliae</i> (L.)	<i>Tetraneura</i> spp.
<i>Euceraphis</i> spp.	<i>Therioaphis</i> spp.
<i>Hyadaphis polonica</i> Szelegiewicz	<i>*Therioaphis trifolii</i> (Monell)
<i>Hyadaphis</i> spp.	<i>Tribosiphonaphis polygonifoliae</i> (Shinji)
<i>Hyalopterus pruni</i> (Geoffroy)	<i>Uroleucon</i> ( <i>Uroleucon</i> ) spp.
<i>Lipaphis erysimi</i> (Kaltenbach)	

**Figure 1.** Total number of winged aphids, including vector species, caught in 6 yellow water traps in alfalfa at Progar in 2011



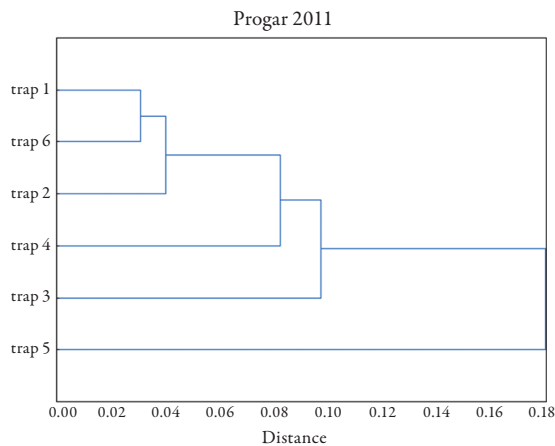
**Figure 2.** Total number of winged aphids, including vector species, trapped in 6 yellow water traps in alfalfa at Progar in 2012

**Table 2.** Most represented aphid species in yellow water traps in alfalfa (Progar, 2011 and 2012)

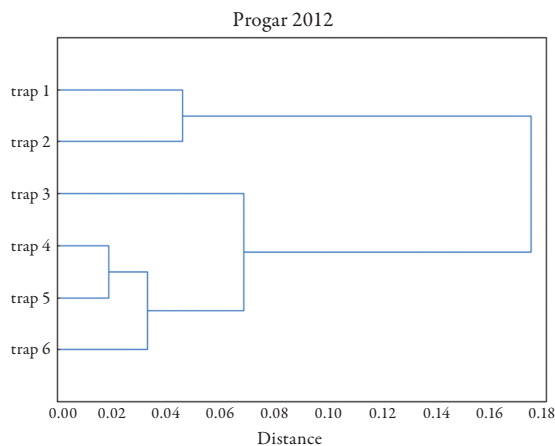
2011	%	2012	%
<i>Therioaphis trifolii</i>	16.6	<i>Aphis fabae</i>	24.5
<i>Aphis craccivora</i>	14.4	<i>Therioaphis trifolii</i>	21.2
<i>Aphis fabae</i>	14.4	<i>Aphis pomi/spiraecola</i>	10.6
<i>Aphis gossypii</i>	8.9	<i>Aphis craccivora</i>	9.8
<i>Aphis pomi/spiraecola</i>	6.7	<i>Aphis gossypii</i>	3.6
$\Sigma$	61	$\Sigma$	69.7

**Table 3.** Morisita-Horn similarity indexes (minimum and maximum) for 6 traps (Progar, 2011 and 2012)

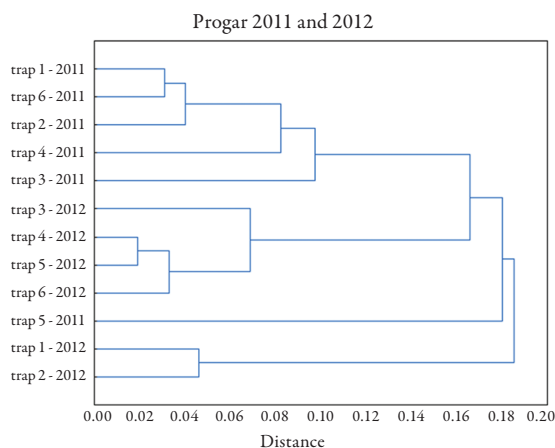
Year	Morisita-Horn index (min)	Morisita-Horn index (max)
2011	0.78	0.97
2012	0.71	0.98
2011 and 2012	0.71	0.98



**Figure 3.** Dendrogram showing similarity between traps, constructed based on Morisita-Horn similarity index (Progar, 2011)



**Figure 4.** Dendrogram showing similarity between traps, constructed based on Morisita-Horn similarity index (Progar, 2012)



**Figure 5.** Dendrogram showing similarity between traps, constructed based on Morisita-Horn similarity index (Progar, 2011 and 2012)

## DISCUSSION

This is the first investigation of aphid flight activity in alfalfa in Europe. The entire sampling effort yielded a total of 1626 aphid specimens. A total of 49 aphid taxa were identified. As a result of similar monitoring of aphid flight activities in alfalfa, beans and cabbage by yellow water traps in western New York (fields were located in Genesee, Niagara, and Orleans Counties), a total of 28 different aphid taxa were collected in alfalfa (Nault et al., 2004).

The flight activity of winged aphids depends on many factors, such as climatic conditions and plant nutrients (Kindlmann & Dixon, 2010). Rainfall, low or high temperature and airflow influence the seasonal dynamics of alate aphids (Morgan, 2000). One of the reasons why aphid abundance was higher in traps in 2012 was optimal weather for the development of some species. High temperatures and dry weather in the summer of 2012 provided optimal conditions for population increase of *T. trifolii* (Ryalls et al., 2013), which was one of the most abundant species in traps.

First aphid specimens were collected at the beginning of May in 2011, and at the end of April in 2012. Maximum population density of winged aphids in traps was observed during the second alfalfa intercut at the beginning of June of both years. The second alfalfa intercut is usually used for seed production. Both most important alfalfa viruses, AMV and CMV, are transmittable through alfalfa seed (Bol, 2010; García-Arenal & Palukaitis, 2010). Another peak was recorded in mid-September. In Serbia, maximum aphid flight activities together with maximum vector activities on seed potato, coinciding with maximum vector activities, occur at the end of May and beginning of June (Vučetić et al., 2013). In monitoring of winged aphid populations on apricot and plum in western Serbia, two spring peaks (middle May-June) and two autumn peaks (August-September) were noted (Jevremović et al., 2016).

Yellow water traps were a good sampling tool for monitoring aphid flight activity and may potentially assist in monitoring aphid-vectored viruses as well (Vučetić et al., 2013). Several species collected in this study (*A. pisum*, *A. craccivora*, *A. fabae*, *A. gossypii*, *M. persicae* and *T. trifolii*) are potential vectors of AMV, the most destructive alfalfa virus worldwide and in Serbia (Bol, 2010; Bulajić et al., 2010). *Acyrtosiphon pisum*, *A. craccivora*, *A. fabae*, *A. gossypii*, *A. spiraeicola*, *M. persicae*, *R. maidis* and *T. trifolii* are potential vectors of CMV (Gildow et al., 2008). About 65% of the aphid specimens collected in this study belong to species that are known vectors of these two most important viruses on alfalfa. However, the total number of vector species is much higher. For many of

the species collected in this study, vector roles have not been tested yet. Furthermore, many specimens belong to the genus *Aphis*, and it is known that numerous species in that genus are vectors of viruses (Blackman & Eastop, 2000; Katis et al., 2007). High abundance of potential vector species indicates a high risk of alfalfa infection with the most important viruses (AMV and CMV). Non-persistently transmitted viruses are difficult to control because transmission can occur in a few seconds. Application of insecticides is usually ineffective because aphids are able to transmit such viruses before being disabled (Feres, 2000). Control of alfalfa viruses is possible mainly by using virus-free seeds and avoiding virus reservoir hosts. As the viruses occur naturally in many different plant species, isolation of their hosts is impossible for all practical purposes (Bol, 2010).

In the present study, five of the most abundant species – *A. craccivora*, *A. fabae*, *A. gossypii*, *A. pomi/spiraeicola* and *T. trifolii*, accounted for about 60% (2011) and 70% (2012) of the collected specimens. Alfalfa is a host plant for *T. trifolii* and *A. craccivora*. Investigating population dynamics in an earlier study, *T. trifolii* was found to be the most abundant aphid on alfalfa plant stems in Serbia, while *A. craccivora* occurred rarely (Jovičić et al., 2016). *Therioaphis trifolii*, as the most abundant species in alfalfa fields, and in the traps used in the present study, is a good potential vector of viruses in Serbia. The species *A. fabae*, *A. gossypii* and *A. pomi/spiraeicola* are considerably polyphagous (Blackman & Eastop, 2000). An increase in the abundance of the invasive *A. spiraeicola* has been observed in Serbia recently (Jevremović et al., 2016). All five of the most abundant aphid species are vectors of the most important viruses infecting alfalfa (Gildow et al., 2008; Bol, 2010).

The Morisita-Horn index takes into account the diversity of species and abundance of specimens (Magurran, 2004), and it showed that there were no great differences among the six traps. The high Morisita-Horn index values and cluster analyses indicated that the six traps had similar richness in species and number of specimens. Therefore, the use of a single trap could provide a good insight into the abundance, aphid diversity and number of potential vector species in small alfalfa fields.

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## Let biljnih vaši, potencionalnih vektora virusa, u usevu lucerke u Srbiji

### REZIME

Praćenje leta biljnih vaši, potencijalnih vektora virusa, na lucerki istraživano je na teritoriji Srbije po prvi put u Evropi. Let biljnih vaši na lucerki praćen je na lokalitetu Progar (Srem) tokom dve godine korišćenjem šest žutih lovnih klopki. Sakupljeno je ukupno 1626 krilatih jedinki biljnih vaši. Prikupljene jedinke su klasifikovane u 49 različitih taksona. Tokom dve godine praćenja leta, najveća brojnost biljnih vaši, kao i najveća aktivnost potencijalnih vektora, registrovana je početkom juna, u drugom otkosu lucerke. Više od 65% prikupljenih jedinki su potencijalni vektori dva najvažnija virusa lucerke: virusa mozaika lucerke – *Alfalfa mosaic virus* (AMV) i virusa mozaika krastavca – *Cucumber mosaic virus* (CMV). Najbrojnije biljne vaši u klopkama na lucerki bile su: *Aphis craccivora*, *Aphis fabae*, *Aphis gossypii*, *Aphis pomi/spiraecola* i *Therioaphis trifolii*. U cilju poređenja sličnosti sastava biljnih vaši u lovnim klopkama, izračunat je Morisita-Horn indeks sličnosti. Visoke vrednosti ovog indeksa ukazuju da nema značajnih razlika u sastavu afidofaune poređenih klopki. Dovoljna je samo jedna lovna klopka radi uvida u brojnost i diverzitet biljnih vaši potencijalnih vektora virusa u lucerki.

**Ključne reči:** Aphididae; Vektori; Biljni virusi; Lucerka