Bulletin of Entomological Research

cambridge.org/ber

Research Paper

Cite this article: Radonjić A, Jovičić I, Lalićević I, Petrović-Obradović O (2023). Factors affecting host plant selection in alfalfa aphids. *Bulletin of Entomological Research* 1–10. https://doi.org/10.1017/S0007485323000093

Received: 7 April 2022
Revised: 30 September 2022
Accepted: 13 February 2023

Keywords:
Acyrthosiphon pisum; alfalfa; aphids; *Aphis craccivora*; competition; host plant selection; *Therioaphis trifolii*

Author for correspondence:
Andja Radonjić, Email: avucetic@agrif.bg.ac.rs

Factors affecting host plant selection in alfalfa aphids

Andja Radonjić[1], Ivana Jovičić[2], Ivana Lalićević[3] and Olivera Petrović-Obradović[1]

[1]Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia; [2]Institute of Pesticides and Environmental Protection, Banatska 31b, 11080 Belgrade, Serbia and [3]Tamiš Research and Devalopment Institute, Ltd. Novoseljanski put 33 Street, 26000 Pančevo, Serbia

Abstract

Alfalfa (*Medicago sativa* L.) hosts several species of aphid, *Acyrthosiphon pisum* (Harris), *Aphis craccivora* Koch and *Therioaphis trifolii* (Monell). The preference of the aphids of alfalfa plants for dense assemblies or individual plants, as well as for healthy or infested plants, was investigated in the field as in the laboratory. Years of field research have revealed the specific preferences of all three species of aphid. *A. pisum* and *T. trifolii* are most commonly found in alfalfa crops, while *A. craccivora* is mostly found on alfalfa weeds. Also, a single species of aphid alone is usually present on a plant. In order to determine the reason for this clear preference and to establish whether at the very beginning, i.e. at the stage of choosing a host, aphid species distance themselves from each other, we tested the effect of the volatiles of healthy and infested plants on their attractiveness to aphids. *A. craccivora* is repelled by the volatiles of dense crops and plants previously infested with one of the other two species. *A. pisum* and *T. trifolii* choose a dense assembly of plants, repelled by the volatiles of plants previously infested with *A. craccivora*. *A. pisum* displays the weakest competitive traits, and *A. craccivora* the strongest. This research showed that competition between aphid species does not occur only when they find themselves on the same plant at the same time, fighting for resources, but also in the choice of plant, in order to avoid later competition.

Introduction

Under natural conditions, one plant species often hosts several species of aphids that occupy the same or different niche at the same or different times (Gianoli, 2000; van Veen *et al.*, 2009; Liu *et al.*, 2020). Insect species that feed on one plant share the resources of that plant even when they are located on different plant organs or their parts. While feeding in the phloem, aphids release salivary secretions that are easily dispersed via the plant juices, so the whole plant, as well as the insects present on it, receive information about the presence and feeding of the aphids (Petersen and Sandström, 2001). In addition, plant resources are limited, so the aphid species that develop on one plant are most often in some form of competition, but it is not excluded that they have a neutral or positive effect on each other (Müller and Steiner, 1991; Petersen and Sandström, 2001). Insect feeding not only leads to a change in the nutritional value of a plant but is also reflected in a change in volatile organic compounds (VOCs) (Arimura *et al.*, 2009). However, it is not only the feeding of insects that causes changes in VOCs. Abiotic factors (Gouinguené and Turlings, 2002), as well as other biotic factors such as the communal life of plants (Ninkovic *et al.*, 2013), bring about changes in the odor profiles of plants. All these changes are registered by aphids and generate a reaction. Sometimes such changes increase the attractiveness of plants to the aphids (Rajabaskar *et al.*, 2013), and sometimes they reduce it (Dahlin *et al.*, 2015).

Alfalfa is the most important forage legume in the world (Michaud *et al.*, 1988) and one of the most commonly grown crops in Serbia (Katić *et al.*, 2005). Aphids can be serious pests in alfalfa fields (Blackman and Eastop, 2000). In addition to fields, alfalfa plants can be found as ruderal weeds alongside roads and ditches, on urban green areas, in parks as well as volunteer alfalfa in other crops (Mueller, 2004). Such plants can also host aphids. Three aphid species, *Acyrthosiphon pisum* (Harris), *Aphis craccivora* Koch and *Therioaphis trifolii* (Monell), develop on alfalfa in Serbia and cause damages to the host plant directly by feeding and indirectly by vectoring plant-pathogenic viruses (Katis *et al.*, 2007; Bol, 2010; Jovičić *et al.*, 2016). In alfalfa fields, the most abundant species is *T. trifolii*, followed by *A. pisum*, while *A. craccivora* occurs rarely in small colonies (Petrović-Obradović and Tomanović, 2005; Jovičić *et al.*, 2016). All three species can be found in alfalfa crops throughout the growing season, but it was noticed that the highest population density of *T. trifolii* in alfalfa crops in Serbia was recorded in summer, *A. pisum* is dominant in spring and *A. craccivora* is the most abundant in July (Jovičic *et al.*, 2016). *T. trifolii* is most often found on the underside of middle leaves (Jovičić *et al.*, 2017a), *A. pisum* infests the growing tips of lucerne stems (Ryalls *et al.*, 2013), and *A. craccivora* develops predominantly on the stem, most often in the lower-to-middle portions, while its appearance on the petioles or leaves is rare (Berberet *et al.*, 2009).

By observing and monitoring the appearance of alfalfa aphids, it is possible to notice that certain species, in addition to preferring a particular part of a plant, also prefer plants that live under certain conditions. Alfalfa is grown in dense assemblies, but it is a very common ruderal plant that can be found along roadsides or as a weed amongst other crops. In order to determine whether there is regularity in the appearance of different aphid species on cultivated or weed alfalfa, the aim of our research was to collect as many samples as possible of aphids from alfalfa plants from several localities over the course of several years. In addition, our goal was to determine whether the smell of non-infested plants, as well as that of infested plants, is a factor affecting the presence of aphids on alfalfa. Do aphids decide to land on an alfalfa plant depending on the environment in which the plant lives, as an individual (as a weed) or as a dense (as cultivated) assembly of plants, and is there inter- or intraspecies competition when choosing a host plant, were questions we aimed to answer.

Materials and methods

Field research

In order to examine the presence of the three aphid species (*A. craccivora*, *A. pisum* and *T. trifolii*) on alfalfa plants, sampling was done at different intervals over 10 years (2011–2020). Two categories of alfalfa plants were selected: cultivated plants and alfalfa weeds. The occurrence of aphids in alfalfa fields was studied at 60 localities (82 samples) in the major growing areas in Serbia and at 41 localities (52 samples) with the presence of alfalfa as a volunteer or ruderal weed. Aphids were collected directly from plant stems and leaves and placed in plastic tubes with 70% ethanol. Identification was based on morphological characters examined using a stereomicroscope (Bio-Optica, Type 100), and the keys of Blackman and Eastop (2000).

Laboratory studies

Growing of plants for laboratory research

Alfalfa plants were produced in climatic chambers maintained at 23°C, relative humidity 60%, light 35,000 lx, with a light regime of L16:D8. Alfalfa variety K28 (Institute for Forage Crops, Kruševac, Republic of Serbia) was used for the experiment.

Plants grown for the experiment, as well as those grown to maintain the aphids, were sown in plastic pots ($8 \times 8 \times 8$ cm^3), ten plants per pot. To test the effect of sparse sowing on the attractiveness of plant aphids, two plants per pot were sown. The pots were uncovered until germination of the plants.

Maintenance of aphid colonies

A laboratory population of *A. craccivora*, *A. pisum* and *T. trifolii* reared on alfalfa plants at insectary of Faculty of Agriculture – University of Belgrade since 2017. In the insectary, the ambient temperature was 23°C, relative humidity 60%, with a light regime of L16:D8.

Each aphid species was kept in a different chamber.

Infestation of plants for olfactory studies

Infestation of plants for the experiment was done by applying ten adult aphids per pot. When the average number of aphids per pot was 100 ± 20, the plants were ready for the experiment. Plants infested by different aphid species were kept in separate chambers to prevent plant–plant interaction of volatiles during the pre-experimental period.

For all experiments, alfalfa plants in stage 5 (early flowering) were used.

Olfactory bioassay

To study the behavioral responses of aphids to the volatiles of non-infested and infested plants, a two-way olfactometer consisting of two stimulus zones, arms (length 4 cm) directly opposite to each other connected by a neutral central zone (2.5×2.5 cm^2) separating them, was used (Ninkovic *et al.*, 2013). Both ends of the olfactometer were connected by plastic tubes to containers holding test plants. Airflow was provided by a vacuum pump and circulated over non-infested and infested plants, carrying their odor into the olfactometer arms and further on into the central zone, which was connected by a tube to the vacuum pump. Airflow in the olfactometer was set to 180 ml min^{-1}.

Apterous viviparous females of all three species were taken from the colonies by random sampling and transferred to Petri dishes. To prevent the dehydration of individuals, Petri dishes were lined with damp filter paper. They were left to adjust to the conditions in the laboratory for 2 h before the start of the experiment. Individual aphids were inserted into the olfactometer through an opening in the upper side. After a 10-min adaptation period, the movement of the aphids in the arena of the olfactometer was monitored and their positions were recorded every 3 min during the 30 min of the experiment (ten positions). The number of repetitions for each test was 18–20 (one aphid = one repetition). Prior to each insect test, the olfactometer was rotated by 180° to avoid positional bias. After each test, the olfactometer was cleaned with 96% ethanol. The experiments were conducted in a dark room with the discrete light above olfactometer.

The response of *A. craccivora*, *A. pisum* and *T. trifolii* was tested on a combination of non-infested and infested plants, plants infested by different aphid species, as well as on individual plants and grouped plants. Twenty-one different treatment arrangements were designed.

We compared the following aphid preferences for: (i) individual plants (two potted plants) vs. a plant in a group (ten potted plants), (ii) non-infested plants vs. plants infested with *A. craccivora*, (iii) non-infested plants vs. plants infested by *T. trifolii*, (iv) non-infested plants vs. plants infested with *A. pisum*, (v) plants infested with *A. pisum* vs. plants infested with *T. trifolii*, (vi) plants infested by *A. craccivora* vs. plants infested with *T. trifolii*, and (vii) plants infested with *A. pisum* vs. plants infested with *A. craccivora*.

Data analysis

Wilcoxon's test for paired samples (StatSoft, 2011), with a significance level of $P \leq 0.05$, was used to compare the number of aphid visits to each olfactometer arm.

Results

Field research

Presence of aphids in cultivated alfalfa

In the period from 2011 to 2020, a total of 82 samples were collected from 60 localities with alfalfa grown in Serbia. A single species of aphid was found in 47 samples (57.32%). *A. pisum* alone was found in the largest number (24 samples or 29.27%), in slightly fewer samples, *T. trifolii* (20 samples or 24.39%), while *A. craccivora* alone was found in three samples (3.66%).

Two species of aphid were found in a total of 26 samples (31.71%). *A. pisum* + *T. trifolii* in 15 samples (18.29%), *A. pisum* + *A. craccivora* in five samples (6.09%), and *A. craccivora* + *T. trifolii* in six samples (7.32%).

The total number of samples in which all three species of plant aphid were found was nine (10.96%) (table 1).

Alfalfa as a volunteer or ruderal weed

Out of a total of 52 samples from alfalfa weeds, *A. craccivora* alone was found in 49 (94.23%). The other two species were not found on their own. *T. trifolii* was found together with *A. craccivora* in three samples, which is 5.77% of the total number of infested weed alfalfa plants (table 2).

Laboratory bioassay

Test of aphid preferences according to the density of plant assembly

Tests of species preference for plants growing in dense or thinned assemblies showed that *A. craccivora* prefers a thinned assembly ($Z = 2.18$, $P = 0.029$, $N = 17$), while *A. pisum* and *T. trifolii* prefer a dense assembly of plants ($Z = 3.147$, $P = 0.0016$, $N = 17$; $Z = 2.38$, $P = 0.017$, $N = 16$) (fig. 1).

Olfactory response of A. craccivora *to non-infested and infested plants*

Non-infested plants and plants previously infested with its own species were chosen equally by *A. craccivora* ($Z = 0.166$, $P = 0.87$, $N = 17$). Also, in the attractiveness test of plants infected with *A. pisum* and *T. trifolii*, *A. craccivora* showed no statistically significant attraction to any of the infested plants ($Z = 0.26$, $P = 0.79$, $N = 19$). It showed a statistically significant preference for plants infested with its own species relative to plants infested with *A. pisum* or *T. trifolii* ($Z = 2.68$, $P = 0.007$, $N = 18$; $Z = 2.16$, $P = 0.031$, $N = 2.16$). However, between non-infested plants and plants infested by the other two species, it statistically significantly more often selected healthy plants: *A. pisum* ($Z = 2.13$, $P = 0.03$, $N = 17$) and *T. trifolii* ($Z = 3.172$, $P = 0.0015$, $N = 17$) (fig. 2).

Olfactory response of A. pisum *to non-infested and infested plants*

In the selection test between plants previously infested with *A. pisum* and non-infested plants, *A. pisum* chose more infested plants, however not statistically significantly ($Z = 1.72$, $P = 0.08$, $N = 18$). It did not differentiate between non-infested and *T. trifolii*-infested plants ($Z = 0.11$, $P = 0.91$, $N = 18$), nor between plants infested with *A. pisum* and *T. trifolii* ($Z = 1.05$, $P = 0.29$, $N = 19$). When choosing between *A. craccivora*-infested and non-infested plants, it was statistically significantly attracted more to non-infested plants ($Z = 2.07$, $P = 0.038$, $N = 16$). In all other combinations where on the one side there were plants infested with *A. craccivora*, it statistically significantly chose the other side, plants infested with *A. pisum* ($Z = 1.99$, $P = 0.046$, $N = 16$), plants infested with *T. trifolii* ($Z = 3.05$, $P = 0.002$, $N = 19$) (fig. 3).

Olfactory response of T. trifolii *to non-infested and infested plants*

In the test of choice between non-infested plants and plants previously infested with its own species, *T. trifolii* did not show a statistically significantly preference for either side ($Z = 1.29$, $P = 0.19$, $N = 16$). In all combinations in which *A. craccivora*-infested plants were on one side, it chose the other side: non-infested plants ($Z = 3.74$, $P = 0.0001$, $N = 19$), *T. trifolii* ($Z = 2.69$, $P = 0.007$, $N = 16$), *A. pisum* ($Z = 2.66$, $P = 0.007$, $N = 16$). In the selection test between a non-infested plant and one infested with *A. pisum*, non-infested plants were chosen ($Z = 2.58$, $P = 0.009$, $N = 16$), and between *T. trifolii* and *A. pisum*, it chose *T. trifolii* ($Z = 3.32$, $P = 0.0009$, $N = 19$) (fig. 4).

Discussion

Our field research indicates that the presence of three aphid species on alfalfa plants depends on the conditions in which the plants are grown (cultivated or weed), as well as on the physiological status of the plant, i.e. whether a plant is non-infested or infested by aphids. Laboratory research has shown that the reason for this specific preference may be plant odors.

Analyzing the presence of aphids on plants collected in the field, it was noticed that each of the three observed species had a clear preference for plants with either a dense assembly or for plants that grow as weeds. *A. pisum* and *T. trifolii* were predominantly found in crops. In contrast to these two species, the presence of *A. craccivora* was very rarely registered in alfalfa crops, while on ruderal alfalfa weeds it was found alone in over 94% of the infested samples. These results are consistent with several studies showing that *A. pisum* and *T. trifolii* form dense colonies in alfalfa crops and are significant pests of cultivated alfalfa (Barberet *et al*., 1983; Sunnucks *et al*., 1997; Julier *et al*., 2004; Pons *et al*., 2005; Rakhshani *et al*., 2010; Ryalls *et al*., 2013; Grez *et al*., 2014; Ximenez-Embun *et al*., 2014). Also, *A. craccivora* is often not considered a significant pest of cultivated alfalfa because it occurs in small numbers with irregular distribution in the field (Pons *et al*., 2009, 2013; Ryalls *et al*., 2013). It is interesting that *A. craccivora* is often found in the yellow water traps placed in alfalfa crops, but very rarely on plants, which means that it crosses fields, but rarely lands in dense crops (Jovičić *et al*., 2017b).

Laboratory research has confirmed the predictability noted in the field and emphasizes the importance of plant odor as a signal aphids need in their search for a host plant. All plant species have a specific VOC that they emit under natural conditions (Ahmed *et al*., 2019). Plants release a variety of different volatile compounds that provide aphids with information that allows them to discriminate between host and non-host plants (Webster, 2012). However, when growing together, plants change their odor profiles as they adapt to each other, so the smell of a plant differs depending on whether it lives alone or in a community with other individuals. These are small differences, but the aphids can detect them (Ninkovic *et al*., 2019), and our research confirms this theory. But what could be the reason for one species of aphid to prefer alfalfa plants that live in a dense assembly, while another prefers solitary plants? We can look for reasons in the biology of the species. Under the climatic conditions of Serbia, *A. pisum* and *T. trifolii* are holocyclic, while *A. craccivora* is predominantly anholocyclic. All three are monoecious. Perhaps the fact that a sexual generation must be formed and eggs laid directs *T. trifolii* and *A. pisum* toward dense assemblies of plants where the possibility of finding individuals of the same species and laying eggs to overwinter is greater than on solitary plants.

Herbivore-induced plant volatiles (HIPVs), which the plant emits when an insect is feeding, are different from basic VOCs.

https://doi.org/10.1017/S0007485323000093 Published online by Cambridge University Press

Table 1. Location of the sampling sites, geographical coordinates, dates of sampling, and collected aphid species on cultivated alfalfa (Serbia, 2011–2020)

Locality	GPS	Date	Aphids
Ovča, Belgrade	44°52′49″N, 20°32′13″E	18.04.2011	*Ap*
Progar, Belgrade	44°43′36″N, 20°07′03″E	12.05.2011	*Ap*
Ovča, Belgrade	44°51′40″N, 20°32′52″E	13.07.2011	*Ap* + *Ac* + *Tt*
Štitar, Šabac	44°47′18″N, 19°35′20″E	24.07.2011	*Ac* + *Tt*
Progar, Belgrade	44°43′22″N, 20°07′42″E	03.08.2011	*Tt*
Ovča, Belgrade	44°52′02″N, 20°32′11″E	03.10.2011	*Ap* + *Tt*
Progar, Belgrade	44°43′31″N, 20°07′25″E	04.10.2011	*Ap* + *Tt*
Kotraža, Lučani	43°41′57″N, 20°14′12″E	17.04.2012	*Ap*
Šatra, Kuršumlija	43°05′25″N, 21°12′23″E	22.08.2012	*Tt*
Aleksandrovac	43°27′44″N, 21°37′02″E	27.04.2013	*Ap* + *Tt*
Kotraža, Lučani	43°41′48″N, 20°14′45″E	05.05.2013	*Ap*
Leskovac	43°01′07″N, 21°54′52″E	07.05.2013	*Tt*
Pertate, Lebane	42°57′21″N, 21°15′11″E	07.05.2013	*Tt*
Rača	44°13′22″N, 21°01′14″E	27.05.2013	*Tt*
Vranjska Banja	42°33′12″N, 21°59′19″E	19.07.2013	*Ac* + *Tt*
Goračići, Lučani	43°46′41″N, 20°19′11″E	25.07.2013	*Tt*
Togočevce, Lebane	42°56′22″N, 21°51′11″E	09.08.2013	*Ac*
Mladenovac	44°27′29″N, 20°42′09″E	02.09.2013	*Tt*
Gornja Šatornja Topola	42°12′03″N, 20°34′11″E	02.09.2013	*Tt*
Konjevići, Čačak	43°54′02″N, 20°23′50″E	02.09.2013	*Tt*
Vranje	42°32′11″N, 21°53′28″E	21.04.2014	*Ap* + *Tt*
Rimski Šančevi, Novi Sad	45°19′39″N, 19°50′31″E	29.04.2014	*Ap*
Novi Slankamen, Inđija	45°07′18″N, 20°13′40″E	11.05.2014	*Ap*
Rimski Šančevi, Novi Sad	45°19′10″N, 19°50′22″E	25.05.2014	*Ap*
Donja Šatornja, Topola	41°11′11″N, 20°33′09″E	08.06.2014	*Ac*
Belosavci, Topola	44°20′31″N, 20°40′58″E	08.06.2014	*Tt*
Ovča, Belgrade	44°52′49″N, 20°32′13″E	12.06.2014	*Ap* + *Ac*
Rusko Selo, Kikinda	45°45′16″N, 20°33′47″E	16.06.2014	*Ap* + *Ac* + *Tt*
Progar, Belgrade	44°43′36″N, 20°07′03″E	21.06.2014	*Ap* + *Ac* + *Tt*
Surčin, Belgrade	44°47′13″N, 20°16′20″E	21.06.2014	*Ap* + *Ac* + *Tt*
Boljetin, Majdanpek	44°32′39″N, 22°01′31″E	21.06.2014	*Ac* + *Tt*
Kotraža, Lučani	43°41′57″N, 20°14′12″E	23.06.2014	*Ap* + *Ac*
Grab, Lučani	43°81′92″N, 20°27′12″E	23.06.2014	*Ap*
Radenković, S. Mitrovica	44°54′53″N, 19°30′12″E	28.06.2014	*Ap* + *Ac* + *Tt*
Vranje	42°32′01″N, 21°53′03″E	06.07.2014	*Ac* + *Tt*
Suva Banja, Vranje	42°34′23″N, 21°59′20″E	06.07.2014	*Ap* + *Tt*
Rimski Šančevi, Novi Sad	45°19′48″N, 19°50′55″E	08.07.2014	*Ap*
Svrljiške Planine	43°16′51″N, 22°22′43″E	20.07.2014	*Ap* + *Tt*
Toponica, Bela Palanka	43°16′20″N, 22°14′01″E	20.07.2014	*Ac* + *Tt*
Šomrda, Majdanpek	44°32′39″N, 22°01′38″E	25.07.2014	*Ap* + *Ac* + *Tt*
Kotraža, Lučani	43°41′48″N, 20°14′45″E	05.08.2014	*Ac* + *Tt*
Predvorica, Šabac	44°41′10″N, 19°48′21″E	10.08.2014	*Ap*
Požarevac	44°37′19″N, 21°09′48″E	01.10.2014	*Ap* + *Ac* + *Tt*

(*Continued*)

https://doi.org/10.1017/S0007485323000093 Published online by Cambridge University Press

Table 1. (*Continued.*)

Locality	GPS	Date	Aphids
Sremski Karlovci	45°11′39″N, 19°56′43″E	01.06.2015	*Ap*
Čortanovci	45°09′43″N, 19°59′30″E	06.06.2015	*Ap*
Mali Požarevac	44°55′64″N, 20°66′59″E	04.09.2016	*Ap* + *Tt*
Belosavci, Topola	44°20′31″N, 20°40′58″E	11.06.2017	*Tt*
Ovča, Belgrade	44°52′49″N, 20°32′13″E	23.06.2017	*Ap*
Kovilovo, Belgrade	44°90′87″N, 20°45′28″E	23.06.2017	*Ap*
Sirogojno, Zlatibor	43°67′63″N, 19°86′12″E	02.08.2017	*Tt*
Stari Slankamen, Inđija	45°16′52″N, 20°21′86″E	15.05.2018	*Ap* + *Tt*
Novi Slankamen, Inđija	45°07′35″N, 20°14′00″E	22.05.2018	*Ap*
Golubinci, Stara Pazova	44°97′32″N, 20°53′36″E	22.05.2018	*Ap*
Begeč, Novi Sad	45°24′77″N, 19°59′70″E	23.05.2018	*Ap*
Ribari, Šabac	44°60′22″N, 19°46′24″E	24.05.2018	*Tt*
Dublje, Bogatić	44°77′26″N, 19°56′16″E	24.05.2018	*Ap*
Kukujevci, Šid	45°07′06″N, 19°31′57″E	25.05.2018	*Ap* + *Ac*
Jazak, Irig	45°10′10″N, 19°07′73″E	25.05.2018	*Ap* + *Ac* + *Tt*
Vučkovica, Lučani	43°68′29″N, 20°24′64″E	27.05.2018	*Ap* + *Ac*
Preljina, Čačak	43°92′64″N, 20°41′06″E	27.05.2018	*Ap*
Manđelos, Sr. Mitrovica	45°10′35″N, 19°60′11″E	31.05.2018	*Ap* + *Tt*
Susek, Beočin	45°22′22″N, 19°54′48″E	31.05.2018	*Ap* + *Tt*
Ovča, Belgrade	44°53′17″N, 20°32′60″E	05.06.2018	*Ap* + *Ac* + *Tt*
Susek, Beočin	45°22′26″N, 19°54′11″E	06.06.2018	*Ap* + *Ac*
Belosavci, Topola	44°20′31″N, 20°40′58″E	06.06.2018	*Tt*
Lipolist, Šabac	47°70′57″N, 19°54′01″E	07.06.2018	*Ap*
Čelarevo, Bačka Palanka	45°28′25″N, 19°52′48″E	11.06.2018	*Tt*
Novi Slankamen, Inđija	45°08′11″N, 20°14′32″E	12.06.2018	*Ap*
Sopot, Pirot	43°22′80″N, 22°56′46″E	27.06.2018	*Ac*
Gornja Šatornja, Topola	42°12′03″N, 20°34′11″E	01.07.2018	*Ap*
Velika Krsna, Mladenovac	44°46′02″N, 20°78′16″E	31.07.2018	*Tt*
Grab, Lučani	43°81′56″N, 20°27′20″E	11.08.2018	*Ap*
Platičevo, Ruma	44°83′23″N, 19°77′18″E	25.08.2018	*Ap* + *Tt*
Vinča, Topola	44°22′15″N, 20°60′36″E	26.08.2018	*Ap* + *Tt*
Virovo, Arilje	43°77′12″N, 20°11′32″E	09.09.2018	*Ap* + *Tt*
Raška	43°26′94″N, 20°60′01″E	04.08.2019	*Tt*
Kikinda	45°81′96″N, 20°49′25″E	22.08.2019	*Ap* + *Tt*
Bačka Topola	45°80′73″N, 19°61′51″E	04.09.2019	*Tt*
Ležimir, Sr. Mitrovica	45°11′26″N, 19°57′01″E	28.04.2020	*Ap*
D. Lakošnica, Leskovac	43°10′40″N, 21°97′01″E	28.04.2020	*Tt*
Sremska Mitrovica	45°00′18″N, 19°63′18″E	02.06.2020	*Ap* + *Tt*
Bavanište, Pančevo	44°82′36″N, 20°84′14″E	14.08.2020	*Tt*

Ap, *A. pisum*; *Ac*, *A. craccivora*; *Tt*, *T. trifolii*.

When insects feed on plants, the production of VOCs increases, and qualitative changes occur. The host plant selection of herbivores varies depending on quantitative and qualitative changes in the odor of the host plants (Ahmed *et al.*, 2019).

The results of our field research clearly show that different species of aphid avoid each other in most cases, i.e. they are most often found alone on plants. This is particularly pronounced in *A. craccivora*, which was rarely found in cultivated crops in

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Table 2. Location of the sampling sites, geographical coordinates, dates of sampling, and collected aphid species on alfalfa as a volunteer or ruderal weed (Serbia, 2011–2020)

Locality	GPS	Date	Aphids
Zelenilo, Belgrade	44°49′50″N, 19°35′20″E	31.08.2011	*Ac*
Kotraža, Lučani	43°41′48″N, 20°23′22″E	10.09.2011	*Ac*
Ledine, Belgrade	44°47′62″N, 20°21′02″E	07.04.2012	*Ac*
Prnjavor, Rudnik	44°03′37″N, 20°35′58″E	16.08.2012	*Ac*
Cerje, Ušće	43°29′59″N, 20°36′50″E	29.06.2013	*Ac*
Novi Pazar	43°09′56″N, 20°29′08″E	14.07.2013	*Ac*
Lisa, Ivanjica	43°37′13″N, 20°11′11″E	09.08.2013	*Ac*
Tatarski Vis, Golubac	44°37′15″N, 21°57′43″E	21.06.2014	*Ac*
Boljetin, Majdanpek	44°32′39″N, 22°01′31″E	21.06.2014	*Ac* + *Tt*
Braničevo, Golubac	44°41′54″N, 20°32′29″E	21.06.2014	*Ac*
Zablaće, Čačak	43°50′20″N, 20°26′18″E	23.06.2014	*Ac*
Bresnica, Vranje	42°33′52″N, 21°58′23″E	06.07.2014	*Ac*
Korbevac, Vranje	42°35′13″N, 22°01′53″E	06.07.2014	*Ac*
Togočevce, Lebane	42°56′31″N, 21°51′05″E	17.07.2014	*Ac*
Bela Palanka	43°14′07″N, 22°19′38″E	20.07.2014	*Ac*
Kotraža, Lučani	43°42′11″N, 20°14′03″E	11.08.2014	*Ac*
Kotraža, Lučani	43°42′23″N, 20°14′45″E	07.09.2014	*Ac*
Kotraža, Lučani	43°42′40″N, 20°14′10″E	07.06.2016	*Ac*
Bežanija, Belgrade	44°81′95″N, 20°39′36″E	24.06.2017	*Ac*
Akmačići, Nova Varoš	43°52′53″N, 19°87′50″E	29.07.2017	*Ac* + *Tt*
Prijepolje, Mileševa	43°37′09″N, 19°71′15″E	29.07.2017	*Ac*
Ušće, Belgrade	44°82′10″N, 20°44′13″E	13.05.2018	*Ac*
Studentski grad, Belgrade	44°82′27″N, 20°39′97″E	23.05.2018	*Ac*
Jurija Gagarina, Belgrade	43°80′15″N, 20°77′21″E	23.05.2018	*Ac*
Čelarevo, Bačka Palanka	45°28′23″N, 19°52′17″E	23.05.2018	*Ac*
Lipolist, Šabac	47°70′14″N, 19°54′10″E	24.05.2018	*Ac*
Gibarac, Šid	45°08′81″N, 19°27′00″E	25.05.2018	*Ac*
Preljina, Čačak	43°92′59″N, 20°41′11″E	27.05.2018	*Ac*
Novi Slankamen, Inđija	45°07′03″N, 20°14′14″E	28.05.2018	*Ac*
Ovča, Belgrade	44°51′44″N, 22°32′70″E	05.06.2018	*Ac*
Studentska, Belgrade	44°82′72″N, 20°40′28″E	07.06.2018	*Ac*
Vlaško Polje, Mladenovac	44°48′72″N, 20°65′45″E	09.06.2018	*Ac*
Zemunski Kej, Belgrade	44°83′53″N, 20°41′88″E	10.06.2018	*Ac*
D. Kamenica, Knjaževac	43°48′70″N, 20°41′11″E	12.06.2018	*Ac*
H. Jugoslavija, Belgrade	44°82′93″N, 20°42′12″E	31.06.2018	*Ac*
Vinča, Topola	44°22′21″N, 20°60′33″E	01.07.2018	*Ac* + *Tt*
Manojlovci, Rudnik	44°18′59″N, 20°55′47″E	01.07.2018	*Ac*
Kotraža, Lučani	43°42′33″N, 20°14′17″E	15.07.2018	*Ac*
Sava Kovačević, Zemun	44°84′24″N, 20°38′99″E	18.08.2018	*Ac*
Tekeriš, Cer	44°55′48″N, 19°52′60″E	25.08.2018	*Ac*
Manojlovci, Topola	44°18′61″N, 20°55′49″E	26.08.2018	*Ac*
Konjarnik, Belgrade	44°78′43″N, 20°48′93″E	01.09.2018	*Ac*

(*Continued*)

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Table 2. (*Continued.*)

Locality	GPS	Date	Aphids
Ušće, Belgrade	44°82′12″N, 20°44′16″E	08.09.2018	*Ac*
Karaburma, Belgrade	44°81′67″N, 20°49′96″E	09.09.2018	*Ac*
Karaburma, Belgrade	44°81′52″N, 20°49′51″E	05.10.2018	*Ac*
Medak, Belgrade	44°77′49″N, 20°50′63″E	07.10.2018	*Ac*
Vlasotince	42°96′51″N, 22°05′31″E	30.08.2019	*Ac*
Senta	45°90′66″N, 20°08′49″E	04.09.2019	*Ac*
Ečka, Zrenjanin	45°31′95″N, 20°43′92″E	08.10.2019	*Ac*
Zmajevo, Vrbas	45°44′98″N, 19°71′05″E	07.05.2020	*Ac*
Bežanijska Kosa, Belgrade	44°81′59″N, 20°37′19″E	11.08.2020	*Ac*
Bavanište, Pančevo	44°82′11″N, 20°84′56″E	14.08.2020	*Ac*

Ap, *A. pisum*; *Ac*, *A. craccivora*; *Tt*, *T. trifolii*.

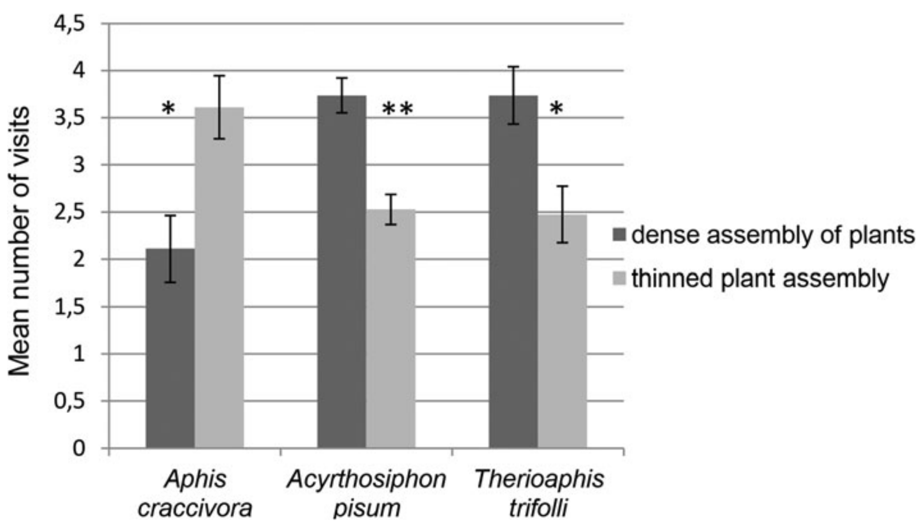

Figure 1. Aphid olfactory responses to volatiles of thinned or dense assemblies of alfalfa plants. Asterisks indicate significant preferences. $^{*}P < 0.05$, $^{**}P < 0.01$, Wilcoxon's matched pair test.

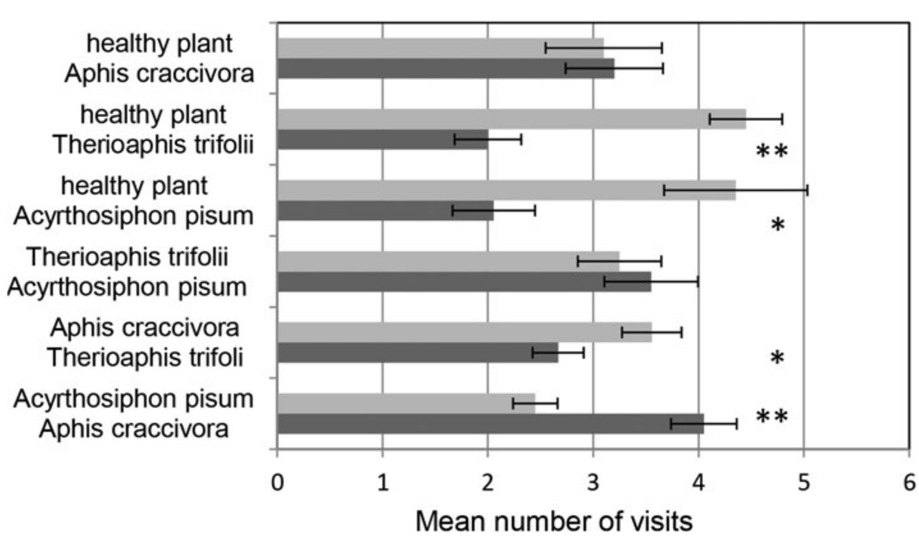

Figure 2. *A. craccivora* olfactory responses to volatiles of non-infested (healthy) or infested alfalfa plants. Asterisks indicate significant preferences. $^{*}P < 0.05$, $^{**}P < 0.01$, Wilcoxon's matched pair test.

combination with one of the other two species, and in weed alfalfa it was almost always alone. Only in slightly more than 5% of the samples was it found in combination with *T. trifolii*. A single species of aphid was found in cultivated alfalfa in more than half of the analyzed samples. Two species together were found in 31% of cases, of which the dominant combination was *A.*

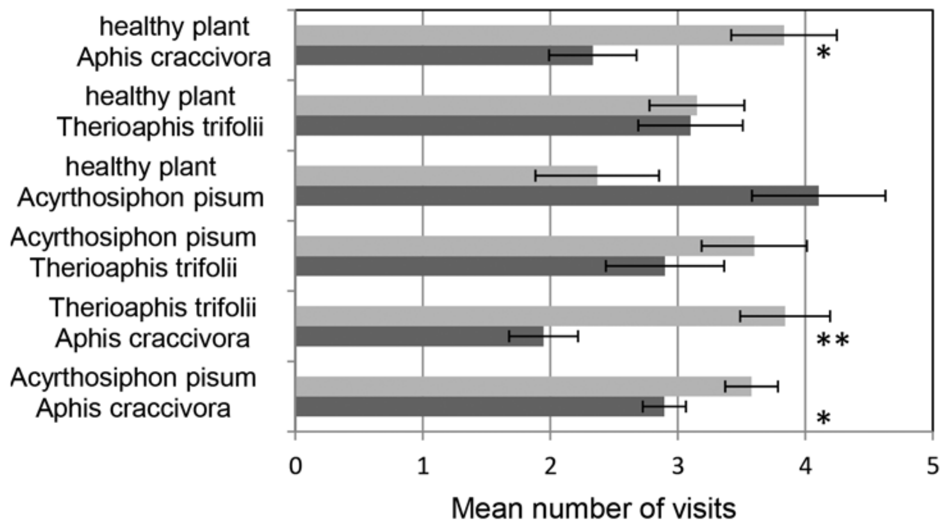

Figure 3. *A. pisum* olfactory responses to volatiles of non-infested (healthy) or infested alfalfa plants. Asterisks indicate significant preferences. *$P < 0.05$, **$P < 0.01$, Wilcoxon's matched pair test.

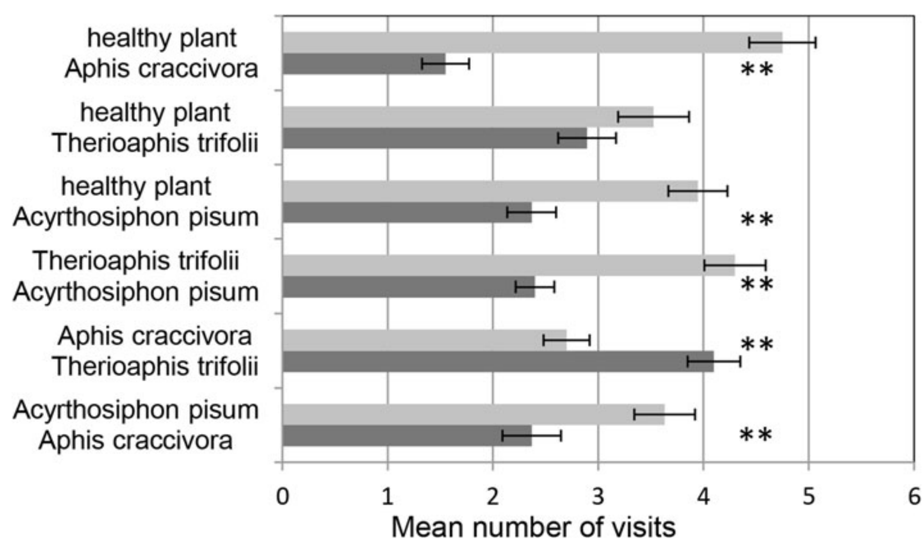

Figure 4. *T. trifolii* olfactory responses to volatiles of non-infested (healthy) or infested alfalfa plants. Asterisks indicate significant preferences. *$P < 0.05$, **$P < 0.01$, Wilcoxon's matched pair test.

pisum + *T. trifolii*. Laboratory research is consistent with fieldwork because it has shown that each species responds in a specific way to changes in the VOCs caused by one of the three species tested. HIPV are species-specific (McCormick *et al.*, 2012), so all three species, in most cases, avoided plants previously infested with another species. All three species always showed a statistically significantly preference for non-infested plants over plants infested with another species. Between non-infested plants and plants infested with their own species, the aphids made no distinction. It is as if induced plant volatiles of their own species are not recognized as a change in the scent of the host plant. Interestingly, *A. pisum* did not react to the changes caused by the feeding of *T. trifolii*. Although it is known that the harmfulness of *T. trifolii* on alfalfa is increased through the secretion of toxic saliva, which strongly affects the physiology of the plant (Berg and Boyd, 1984) and thus changes in its odor profile, *A. pisum* did not recognize these changes. On the other hand, *T. trifolii* avoided plants already infested with *A. pisum*. As we have already mentioned, *A. pisum* is dominant during spring, while *T. trifolii* is most abundant during summer (Jovičić *et al.*, 2016). *T. trifolii* is a species that inhabits plants at a time when *A. pisum* is already present and tries to avoid already infested plants. In contrast, *A. pisum*, as the species already established on plants when *T. trifolii* arrives, seems to have no cause to recognize odors that arise after *T. trifolii* infestation.

The most pronounced competitive traits were shown by *A. craccivora*, which in all combinations avoided plants previously infested with one of the other two species, while the other two species avoided plants infested with *A. craccivora*. *A. craccivora* is present on plants throughout vegetation but is most numerous during the summer (Jovičić *et al.*, 2016), which means that the time of its most intensive development on plants coincides with the time when the other two species appear in large numbers. Although it is a very polyphagous species (Mehrparvar *et al.*, 2012), it recognizes the odors of healthy host plants (Pettersson *et al.*, 1998), even different varieties of the same plant species (Diabate *et al.*, 2019), so it is not surprising that it recognizes the odor of infested plants and avoids them. On the other hand, it has a huge reproductive potential that is reflected in the fact that in alfalfa under optimal conditions one generation develops in 6–9 days, and a single female produces over 80 larvae (Berberet *et al.*, 2009). The feeding of such dense colonies of *A.*

craccivora leads to a change in the odor profile of plants, which is confirmed by studies of the behavior of ladybugs which efficiently find infested plants (Fouad, 2021). Our research shows that these changes have a repellent effect on *T. trifolii* and *A. pisum*, which is in line with research showing that changes in VOCs that are attractive to natural enemies (Vučetić *et al.*, 2014) are repellent to aphids (Dahlin *et al.*, 2015).

Competition between different species of aphid and even individuals of the same species when overbreeding occurs is known and studied (Müller and Steiner, 1991). However, most research deals with competition at the level of population development (Müller and Godfray, 1997; Petersen and Sandström, 2001; Portha and Detrain, 2004). Our research differs in that we investigated the initial phase, i.e. the search for a host plant and the reaction of different species of aphid to already infested plants. Plant odor is an equally important signal for oligophagous species such as *A. pisum* and *T. trifolii* and polyphagous species like *A. craccivora*.

Acknowledgments. This study was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant Nos. 451-03-47/2023-01/200116 and, 451-03-47/2023-01/200214 and 451-03-47/2023-01/200054).

Conflict of interest. The authors declare no conflict of interest.

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