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EFFECTS OF TEMPERATURE ON *ACYRTHOSIPHON PISUM* AND *THERIOAPHIS TRIFOLII* (HEMIPTERA: APHIDIDAE) ABUNDANCE IN ALFALFA CROPS: A CASE STUDY IN NORTHERN SERBIA

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Abstract: Populations of the most abundant alfalfa aphids, *Acyrthosiphon pisum* and *Therioaphis trifolii*, have periodic fluctuations, and many factors affect their dynamics. In the present study, we examined the impact of daily air temperatures on the abundance of two alfalfa aphids in field conditions. The numbers of these two aphids on alfalfa were documented at two locations in a representative alfalfa growing area in Serbia during a three-year field study. Based on the records of aphid abundance and daily air temperatures during the whole study, it was found that a correlation between the sum of optimal daily air temperatures for aphid development, the sum of maximum daily air temperatures and the number of recorded aphid peaks was significant and can therefore be considered for the detection of suitable temperature conditions to increase aphid abundance. The study shows that the highest correlations were between a high density of *A. pisum* and the sum of optimal daily air temperatures for its development (Ck=0.569) and between a high density of *T. trifolii* and the sum of maximum daily air temperatures (Ck=0.595). The length of time required for the growth of populations of the two alfalfa aphids differed: 30 days for *A. pisum* and 5 days for *T. trifolii*. The association of temperature data to alfalfa aphid abundance enables a projection of their population behavior in changed future climate conditions. This study suggests increased population sizes of *T. trifolii* and decreased population sizes of *A. pisum* on alfalfa under the warmer conditions that are expected to prevail in the future.

Key words: alfalfa aphids, abundance, *Medicago sativa*, temperature conditions, climatic changes.

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Introduction

Over the past decades, climate change has accelerated worldwide, bringing increases in temperature, changes in precipitation regimes and more frequent and severe extreme events, such as droughts and heatwaves. Changes in climate conditions and their variability are among the most important threats to agriculture and biodiversity. Plants are more frequently exposed to heat and/or water stress, pests and diseases, which significantly influence the quality and quantity of yields and, consequently, food availability (IPCC, 2014).

Insect population dynamics depend on many factors, such as host-plant quality, biological forces (predators and parasitoids) and, in particular, climate conditions (Kindlman and Dixon, 2010). Insect herbivores and the plants involved in their development are dependent on many climatic factors: temperature, solar radiation, precipitation and relative humidity. Current knowledge suggests that climate change through temperature increases, changes in precipitation regime and more frequent extreme events can alter an insect's development (Bale et al., 2002; Bale and Hayward, 2010; Colinet et al., 2015; Castex et al., 2018). Several studies have shown that temperature directly affects insect population abundance, development, survival, the number of generations, distribution and invasion by exotic insect pests (Bale, 2002; Satar et al., 2005). Since insects are poikilothermic organisms, their body temperature depends on the external environment and is highly sensitive to temperature variability (Bale and Hayward, 2010).

Of all environmental factors that influence aphid abundance, the temperature is of major importance. Aphids can develop over a limited range of temperatures (Dixon et al., 2009). Because of their short generation time and high reproductive capacity, they have the potential to respond rapidly to temperature increases (Harrington et al., 2007).

Three aphid species develop on alfalfa in Serbia: the pea aphid *Acyrthosiphon pisum* (Harris), the cowpea aphid *Aphis craccivora* Koch and the spotted alfalfa aphid *Therioaphis trifolii* (Monell) (Tomanović et al., 1996; Petrović-Obradović and Tomanović, 2005; Jovičić et al., 2016). Alfalfa aphids can reduce crop yields directly through their feeding (Lykouressis and Polatsidis, 1990; Pons et al., 2009; Ryalls et al., 2013; Jovičić et al., 2016) and indirectly through the transmission of the plant-pathogenic viruses such as *Alfalfa mosaic virus* (AMV) and *Cucumber mosaic virus* (CMV) (Bol, 2010; García-Arenal and Palukaitis, 2010). Recent research into aphid population dynamics on alfalfa in Serbia has shown that *T. trifolii* is the most abundant species, followed by *A. pisum* (Jovičić et al., 2017). Earlier studies found that three decades ago, in the Pannonian area, *A. pisum* was more abundant than the two other species (Tomanović et al., 1996). *Aphis craccivora* is a comparatively minor pest of alfalfa and rarely occurs in high numbers on this plant (Berberet et al., 2009; Ryalls et al., 2013; Jovičić et al., 2016).

Over the last 50 years, the mean annual temperature in Serbia has increased with a trend of 0.36°C per 10 years. Since the 1980s, the observed warming has accelerated to a trend of 0.6°C per 10 years. The average mean annual temperature in the period 2008–2017 was about 1.5°C higher in comparison to the period 1961–1990, while the mean temperature of summer months (June, July, August) increased even more, by over 2.5°C compared to the same reference period (Djurdjevic et al., 2018).

Considering the observed temperature increases, especially pronounced during the vegetation season, and the prevalence of *T. trifolii* over *A. pisum* on alfalfa observed in Serbia in the last decade, the aim of this study was to explore the impact of air temperature on the abundance of these two alfalfa aphids in field conditions.

Material and Methods

Aphid sampling

The study was conducted in two alfalfa fields in Serbia located in Progar (the Srem region) and Ovča (South Banat) in a representative alfalfa production area. The plots were approximately 1 ha in Ovča and 0.5 ha in Progar. Aphids were monitored in order to determine the abundance of *A. pisum* and *T. trifolii* during the growing season (from April to October) in 2011–2012 in Progar and 2011–2013 in Ovča. Aphids were collected directly from plants and placed in plastic tubes containing 70% ethanol. Samples were taken every 10 days from both locations. The identification of aphids was carried out based on identification keys (Blackman and Eastop, 2000) using a stereomicroscope. The total number of *A. pisum* and *T. trifolii* per 100 alfalfa stems was counted on each sampling date.

Weather conditions

The analysis of meteorological conditions in the two alfalfa fields in Srem and Southern Banat was performed using data recorded at two nearby official meteorological stations, Surčin (near Progar) and Banatski Karlovac (near Ovča), run by the Hydrometeorological Service of the Republic of Serbia. Mean monthly temperatures in the years of the case study were compared to climatological values of the reference period 1986–2013, while daily maximum and minimum air temperatures were used in degree-day calculations.

Statistical analysis

A degree-day method was used to determine the length of time required for the growth of populations of the two alfalfa aphids. Temperature sums were calculated for a period from 1 to 45 days, with steps of 5 days, before the aphids reached their peaks: the total number of *A. pisum* or *T. trifolii* per 100 alfalfa stems was 100 or more.

The effect of temperature on *A. pisum* was calculated as a correlation between the sum of optimal daily air temperatures for its development, i.e. the accumulated degrees-day15–25ºC, the sum of maximum air temperatures and the number of recorded peaks. For *T. trifolii*, the correlation between the sum of optimal daily air temperatures for its development, i.e. the accumulated degrees-day 20–30ºC, the sum of maximum air temperatures and the number of peaks recorded for the sample was calculated. Data on the thermal requirements for the development of two alfalfa aphids were obtained from the literature (Bieri et al., 1983; Berberet et al., 1983; Lykouressis and Polatsidis, 1990; Lamb, 1992; Liu et al., 2012). The correlation coefficient C_k (where 1 is a total positive correlation, 0 is no correlation, and -1 is a total negative correlation) was calculated using a self-developed FORTRAN code.

The correlation coefficient was calculated using the following formula:

$$C_k = \frac{\sum_{i=1}^{n}[(x_{ki}-\overline{x_k})(y_i-\bar{y})]}{\sqrt{\sum_{i=1}^{n}[(x_{ki}-\overline{x_k})^2(y_i-\bar{y})^2]}} \qquad (1)$$

n – the number of samples;

x_k – the sum of temperatures t $x_k = \sum_{j=1}^{k} t_j$, for period $k=1,5,..45$ days before the aphid peak;

y – the number of aphids at their peak.

Results and Discussion

Field studies

The maximum population densities of *A. pisum* on alfalfa were recorded in spring at both locations in all years, and in the autumn of 2011 at Ovča. The maximum population densities of *T. trifolii* at both locations were recorded during the summer (2011–2013).

The peaks of *A. pisum* (100 individuals per 100 alfalfa stems or more) were recorded in mid-May at Progar during 2011–2012, and at the end of April (2011), in May (2011–2013), mid-June (2013) and mid-October (2011) at Ovča (Table 1).

The peaks of *T. trifolii* were recorded at the end of August and at the beginning of September (2012) at Progar, and at the end of July and August (2011–2013) and at the beginning of September (2012) at Ovča (Table 1).

Table 1. Dates of recorded peaks and the number of recorded individuals of *A. pisum* and *T. trifolii* per 100 alfalfa stems at the Progar locality in 2011–2012 and at the Ovča locality in 2011–2013.

Aphid *A. pisum*				*T. trifolii*			
Locations	Progar	Ovča		Progar		Ovča	
Dates of sampling and the number of recorded aphid individuals per 100 alfalfa stems (in peaks)							
2011							
12 May	104	30 April 10 May 20 May 19 October	208 222 232 171	/		22 July	164
2012							
19 May	114	18 May	235	18 August 7 September 18 September	338 106 1123	24 July 3 August 24 August 3 September	114 369 537 164
2013							
	/	10 May 17 June	227 108	/		29 July 19 August 29 August	131 179 129

Temperature conditions

Based on the data from the Serbian Hydrometeorological Service (weather stations Banatski Karlovac and Surčin), the mean monthly air temperatures recorded for the three-year study period in Ovča and the two-year study period in Progar were compared to the 1986–2013 reference period (Figures 1 and 2). The mean monthly air temperatures recorded for the study period deviated from the climatological normal, particularly in 2012.

Temperatures at both locations in 2011 were within the climatological normal values, except in September, when the mean monthly air temperatures were above the long-term average.

The year of 2012 was one of the hottest years on record since the start of meteorological measurements in Serbia. The summer was especially hot, with extreme heat and drought conditions prevailing for months. In July of 2012, the mean monthly air temperature ranged from 24.6ºC in Ovča up to 26.5ºC in Progar, surpassing the maximum mean air temperatures recorded since 1986. At the same time, February of 2012 was the coldest month recorded in the study, with mean monthly air temperatures of -4ºC in Progar and -5.1ºC in Ovča. The mean monthly air temperatures at the Ovča site in 2013 were within the long-term normal values.

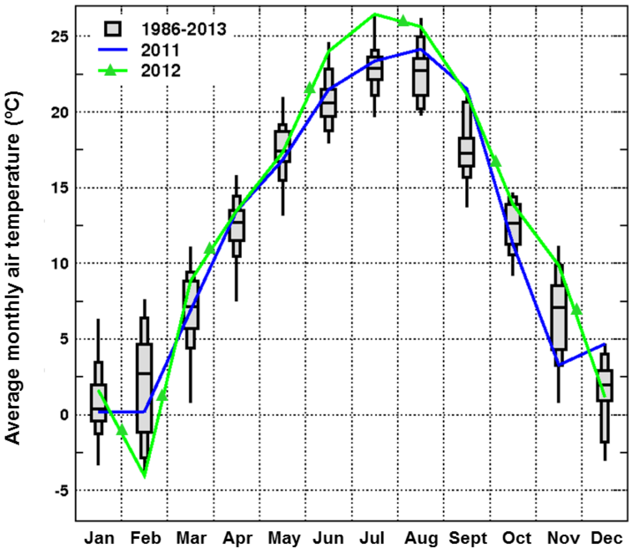

Figure 1. Average monthly air temperatures (ºC) in the period 1986–2013 and mean monthly air temperatures during the two-year study (2011 and 2012) at the Progar locality (Weather station Surčin).

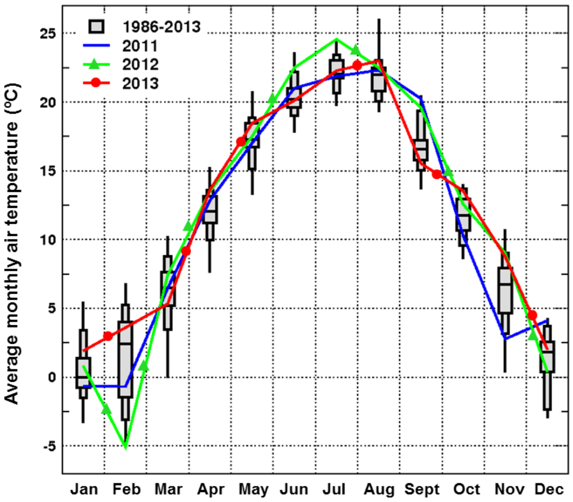

Figure 2. Average monthly air temperatures (ºC) in the period 1986–2013 and mean monthly air temperatures during the three-year study (2011, 2012 and 2013) at the Ovča locality (Weather station Banatski Karlovac).

Temperature effects on alfalfa aphids

Climate change is happening, and there is a need for a constant monitoring of its impact on pests such as aphids. Climate conditions, especially air temperature, have an effect on aphid population dynamics, and it is clear that climatic changes such as global warming are very likely to strongly affect their pest status (Harrington et al., 2007; Petrović-Obradović et al., 2018). Extreme air temperatures may affect insect development positively or negatively (Castex et al., 2018).

Aphids are the most destructive insect pests of alfalfa, causing production losses of 25% worldwide (He and Zhang, 2006). Heavy infestations of *A. pisum* and *T. trifolii* were reported in the main alfalfa growing regions in Serbia. The most abundant aphid on alfalfa, *T. trifolii*, is adapted to warmer temperatures. The second one, *A. pisum*, is adapted to cooler temperatures. Previously recorded results of Tomanović et al. (1996) clearly showed that, three decades ago, *A. pisum* was dominant in comparison to *T. trifolii* on alfalfa in Serbia. In the past 20–30 years, the warmer climate and changes in the rainfall regime have significantly influenced the previously established occurrences of *A. pisum* and *T. trifolii*.

Temperature effects on the abundance of *A. pisum*

In this study, a correlation was found between the maximum population density (peaks) of *A. pisum* and the sum of optimal and maximum daily air temperatures.

In the series of calculated correlations for different periods, the highest correlation (Ck=0.569) was observed between the sum of the optimal daily air temperatures in the range of 15–25ºC and the maximum population density of *A. pisum*, 30 days before this aphid reached its peaks (Table 2, Figure 3). The next high correlation (*Ck*=0.542) was noted between the sum of maximum daily air temperatures and the maximum population density of *A. pisum*, also 30 days before it reached its peaks (Table 2, Figure 3).

Table 2. Correlations between the sums of air temperatures for every five days and the maximum population density of *A. pisum*.

	The number of days before the peak								
	5	10	15	20	25	30	35	40	45
Sum Topt	-0.182	0.337	0.482	0.470	0.541	0.569	0.480	0.386	0.318
Sum Tx	-0.130	0.213	0.406	0.459	0.514	0.542	0.460	0.293	0.219

(Sum Topt – the sum of optimal daily air temperatures in the range of 15–25ºC; Sum Tx – the sum of maximum daily temperatures).

A high variation between correlation values in the series of calculated correlations was found. A negative correlation coefficient between the sums of optimal and maximum daily air temperatures and maximum *A. pisum* population density was found five days before its peaks. The correlation coefficient values gradually increased in the next steps (10 to 30 days), and in the 30- to 45-day periods, the values gradually decreased (Table 2).

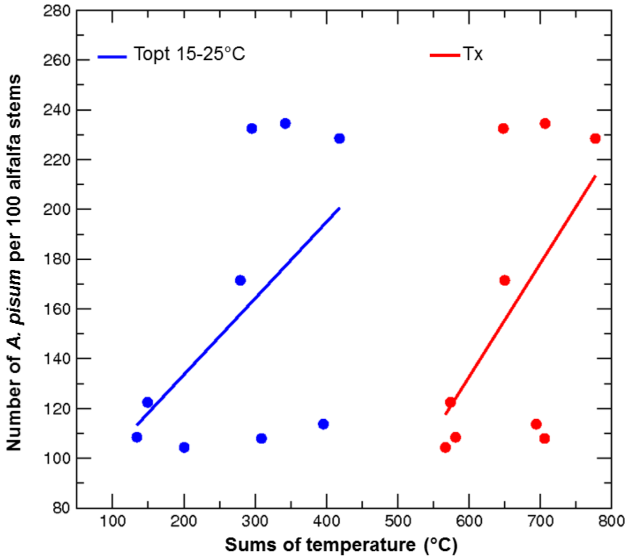

Figure 3. Correlations between the abundance of *A. pisum* and sums of optimal daily air temperatures in the range of 15–25ºC (Topt) and sums of maximum daily air temperatures (Tx) 30 days before its peak.

Aphid populations continuously adapt to alterations in their environments, such as increases in air temperature. Compared to other insects, aphids have a low developmental temperature threshold (around 4°C), developmental optimum (around 25°C) and a short generation time (Harrington et al., 2007). Temperatures primarily affect the abundance of *A. pisum*. The optimum temperature range for its population growth is 15–25°C (Berberet et al., 1983; Lamb et al., 1987; Lamb, 1992). The results of *A. pisum* population fluctuations in Serbia clearly showed the existence of two periods, spring and autumn, when development may occur under the prevailing air temperatures. The maximum population density of *A. pisum* (100 individuals per 100 alfalfa stems or more) has been recorded at the end of April, in May and mid-June (2011–2013) at both locations and in mid-October (2011) in Ovča. The mean monthly air temperature in this period ranged between 15°C and 20°C. Correlations between the sums of the optimal daily air temperatures, maximum daily temperatures and the maximum population density of *A. pisum* on alfalfa were Ck=0.569 (Sum Topt) and Ck=0.542 (Sum Tmax). The air temperatures during spring and autumn have large oscillations, and cooler conditions cause slower heat accumulation. Consequently, the length of time required for the growth of a population of *A. pisum* was 30 days. During this period, aphids accumulated temperatures for their development.

Cold-adapted species such as *A. pisum* are adversely affected by warming. The low population density of this species during the summer months is connected with the adverse effects of high temperatures. The mean monthly air temperature in the summer months (2011–2013) was around 25°C. This temperature is the upper limit in the optimum temperature range for *A. pisum* population growth. Thus, maximum daily air temperatures of 35°C or higher during the summer may be detrimental to its development. The extremely high summer temperatures in 2012 at both locations limited *A. pisum* development up to the end of the growing season. Based on these results, we assume that higher air temperatures have a negative influence on the survival and reproduction of *A. pisum* populations. Similar effects of temperature on aphids were recorded in other studies. High temperatures over 30°C were detrimental to the survival of *Metopolophium dirhodum* (Walker) on cereals in central Europe (Ma et al., 2004). Satar et al. (2005) reported that the optimal temperature range for the population growth of *Brevicoryne brassicae* (L.) on white cabbage was about 25°C, while temperatures of 30–35ºC were lethal to the early larval stages. Furthermore, the effects of temperature on insect herbivores can be direct through impacts on their physiology and behavior, or indirect through impacts on their host plant (Bale et al., 2002). Barton and Ives (2014) have shown that drought in alfalfa fields and the deterioration of plant quality lowered the population growth rate of *A. pisum.* The deterioration of alfalfa plant quality recorded in the summer of 2012 at both locations was also detrimental to *A. pisum* development.

Temperature effects on the abundance of *T. trifolii*

A correlation was found between the maximum population density (peaks) of *T. trifolii* and the sums of optimal daily air and maximum daily temperatures.

In the series of calculated correlations for different periods, the highest correlation (Ck=0.595) was found between the sum of maximum daily air temperatures and the maximum population density of *T. trifolii*, 5 days before it reached its peaks (Table 3, Figure 4). The next high correlation (Ck=0.546) was observed between the sum of optimal daily air temperatures and the maximum population density of *T. trifolii*, also 5 days before it reached its peaks (Table 3, Figure 4).

The correlation coefficient between the sum of optimal and maximum daily temperatures and the maximum population density of *T. trifolii* for the remaining period (10 to 45 days) had low positive or negative values (Table 3).

Table 3. Correlations between the sums of air temperatures for every five days and the maximum population density of *T. trifolii*.

	The number of days before the peak								
	5	10	15	20	25	30	35	40	45
Sum Topt	0.546	0.311	-0.270	-0.122	0.062	0.255	0.320	0.309	0.283
Sum Tx	0.595	0.193	-0.285	-0.311	-0.143	0.049	0.036	-0.016	0.183

(Sum Topt – the sum of optimal daily air temperatures in the range of 20–30ºC; Sum Tx – the sum of maximum daily temperatures)

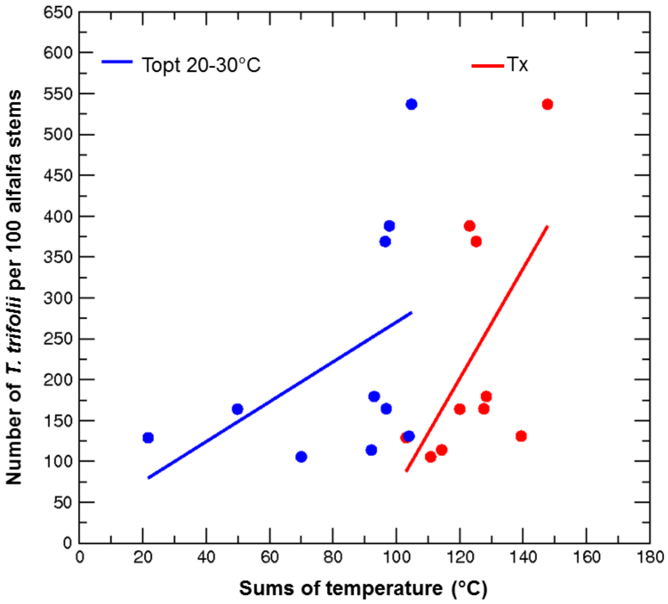

Figure 4. Correlations between the abundance of *T. trifolii* and sums of optimal daily air temperatures in the range of 20–30ºC and sums of maximum daily air temperatures 5 days before its peak.

The prevalence of *T. trifolii* on alfalfa in this study might be related to climate change. High temperatures and dry weather are optimal for increasing the abundance of *T. trifolii*. The fecundity of this species is highest at 25°C (Liu et al., 2012). The maximum population density of *T. trifolii* on alfalfa was recorded in the period July–September (2011–2013) at both locations. The mean monthly air temperature in this period ranged from 22°C to 26°C. In July 2012, at Progar and Ovča, extremely high mean daily air temperatures (about 30°C) were recorded. This temperature is the upper limit in the optimum temperature range for *T. trifolii* population growth. However, the short-term exposure of *T. trifolii* to high temperatures had a positive impact on its development. The highest number of peaks at both locations was recorded in the summer of 2012, which was one of the hottest summers since the start of meteorological measurements in Serbia (Djurdjevic et al., 2018).

The high density of this aphid on alfalfa was positively correlated with the sums of maximum daily air temperatures (Ck=0.595) and optimal daily air temperatures (Ck=0.546). The length of time required for the population growth of *T. trifolii* was 5 days. Higher temperatures tend to increase the speed of multivoltine insects' life cycle (Bale, 2002). Castex et al. (2018) have shown that warmer conditions cause faster and earlier heat accumulation, accelerating development rates and increasing the number of generations an insect can achieve in one year. Several studies showed that some aphids become more abundant with elevated temperatures. Davis et al. (2006) showed that an increase in temperature of 2.5–3.5°C correlated positively with an increase in *Myzus persicae* (Sulzer) abundance. The fecundity of *Rhopalosiphum maidis* (Fitch) on barley was higher under elevated temperatures (Xie et al., 2014). Furthermore, the higher production of winged forms that leads to increased aphid migration and a spread of plant viruses was observed (Xie et al., 2014). Also, in hot summers, *T. trifolii* has a high production of winged forms (Jovičić et al., 2017).

Studies on climate change in Europe point to an increase in temperature, changes in precipitation regimes and more frequent extremes (e.g. heat waves), particularly in the southeastern part of the continent (IPCC, 2014). According to the European Environment Agency (EEA, 2017), Serbia ranks among the countries most susceptible to the adverse effects of this change. Vuković et al. (2018) show that, by the end of the 21st century, the mean summer temperature in Serbia could be 4.5°C higher in comparison to the reference period 1986–2005, with an earlier onset and later end of the vegetation period and more frequent and intensive heat waves and drought events. In addition to other newly detected pests in Serbia, whose occurrences are confirmed as a consequence of climate change impacts (Stričević et al., 2020), the abundance of *T. trifolii* and *A. pisum* on alfalfa can also serve as climate change impact indicators. We suggest including these two alfalfa aphids in the list of proposed indicators, which are planned for continual monitoring with the purpose of enhancing adaptation capacities in agricultural production.

Conclusion

According to our results, *T. trifolii* populations on alfalfa in Serbia are well adapted to high air temperatures between 30 and 35°C. However, the high air temperature has a detrimental role in the population development of *A. pisum*. The results of this study could be used as a starting point in defining short- and long-term prognoses of aphid abundance on alfalfa in the following years, as well as enabling the timely preparations for a significant climate shift by the end of the century. Clearly, it is difficult to predict the effect of global warming on aphid populations. The direct effects of temperature on aphids need to be analyzed in a wider context, and attention should be paid to the effects interacting with other climatic factors (primarily precipitation), natural aphid enemies (predators and parasitoids) and host-plant conditions.

Acknowledgments

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UTICAJ TEMPERATURE NA BROJNOST *ACIRTHOSIPHON PISUM* I *THERIOAPHIS TRIFOLII* (HEMIPTERA: APHIDIDAE) U USEVIMA LUCERKE: STUDIJA SLUČAJA NA SEVERU SRBIJE

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Rezime

Na populacionu dinamiku dve najbrojnije vrste biljnih vaši lucerke, *Acyrthosiphon pisum* i *Therioaphis trifolii*, utiču brojni faktori. Istraživanje uticaja dnevnih temperatura vazduha na populacionu dinamiku biljnih vaši lucerke sprovedeno je u poljskim uslovima. Brojnost biljnih vaši je praćena tokom tri godine, na dva lokaliteta, u najznačajnijim proizvodnim regionima lucerke u Srbiji. Na osnovu podataka o brojnosti biljnih vaši na lucerki i vrednosti dnevnih temperatura vazduha tokom istraživanja, utvrđena je korelacija između suma optimalnih dnevnih temperatura za razvoj biljnih vaši, suma maksimalnih dnevnih temperatura vazduha i maksimuma brojnosti dve vrste biljnih vaši. Najviše vrednosti koeficijenta korelacije (Ck) bile su između maksimuma brojnosti *A. pisum* i sume optimalnih dnevnih temperatura za njen razvoj (Ck=0,569), kao i između maksimuma brojnosti *T. trifolii* i sume maksimalnih dnevnih temperatura vazduha (Ck=0,595). Za porast brojnosti populacije *A. pisum* neophodno je da odgovarajući temperaturni uslovi budu ispunjeni u trajanju od 30 dana, dok je za porast brojnosti populacije *T. trifolii* neophodno 5 dana. Nalaženje veze između temperaturnih uslova i brojnosti biljnih vaši lucerke omogućava nam predviđanje njihovog ponašanja i brojnosti u izmenjenim klimatskim uslovima koji nas očekuju u budućnosti. Ova istraživanja ukazuju da se u budućnosti, usled globalnog zagrevanja, očekuje porast brojnosti *T. trifolii* i smanjenje brojnosti *A. pisum* na lucerki.

Ključne reči: biljne vaši lucerke, brojnost, *Medicago sativa*, temperaturni uslovi, klimatske promene.

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