RESEARCH ARTICLE

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Factors affecting population dynamic and preference of *Acyrthosiphon pisum* Harris in winter pea lines

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ABSTRACT

Population dynamics and preference of *Acyrthosiphon pisum* Harris on six winter pea lines in field conditions were studied. Aphid number was recorded on marked plants by counting. The impact of several factors on the population growth and pea aphid choice during the period 2016–2018 was tracked. Weather condition strongly affected on the population dynamics of the pea aphid. The highest positive and significant effect on pea aphid infestation had the temperature (r = 6.748) followed by humidity (r =1.992). It was found that a peak in the aphid population density was reached in the sensitive stage of pod formation, as well as at flowering under a favorable weather condition. Among winter pea lines, lines 6 and 12A stood out with a significantly lower aphid density, nitrogen concentration and shorter generative period. They were much less preferred by aphids and defined as tolerant. Also, the interaction between aphid density and generative period as well as total nitrogen was positive and significant.

Key words: winter pea lines, *Acyrthosiphon pisum*, dynamics, preference

Introduction

Aphids, which are the largest group of phloem-feeding insects, are major agricultural pests causing extensive damage to the crop, garden and wild plants (Foyer et al., 2009). A considerable annual variation in aphid population numbers has been frequently observed. The population size of species is changing in time and space due to different reasons, such as fluctuations in environmental conditions, availability of resources and impact of enemies. Environmental factors (temperature, rainfall, humidity) and food availability greatly affect the population dynamic of aphids (Saljoqi, 2009; Abbas et al., 2015).

A number of authors have studied the impact of abiotic factors on aphid density, but the results were varied. According to several authors, the temperature and rainfall had a positive and significant effect on aphid population, while humidity played a negative role (Ahmad et al., 2016). Others reported that environmental conditions rainfall and especially the high temperature had adverse effects on aphid reproduction (Vickers, 2011; Abbas et al., 2015). Perhaps one of the reasons was the location of the geographic area in a different climate zone.

Not only climatic conditions but also varietal host tolerance affected the population growth and dynamic of aphids (Vassilev & Lecheva, 2003; Ahmad et al., 2016). Plants employ morphological, phenological traits and chemical compounds against insect herbivory in different varieties (Smith & Clement, 2012). Data such as these may provide valuable background data for predictions of possible associations between plants and aphids and expressed tolerance against the species. Therefore, the identification and improvement of key traits that determine the susceptibility of the plant species to aphid infestation play an important role in breeding programs as a possible source of resistance (Meradsi, 2009).

The aim of this study was to identify factors affecting the population dynamic and preference of *Acyrthosiphon pisum* such as weather condition, chemical component and plant phenology in winter pea lines.

Materials and Methods

During the 2016–2018 period in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria (43° 23.312' N; 24° 34.856' E; altitude 230 m), a study was conducted on the population dynamics of *Acyrthosiphon pisum* Harris (Hemiptera, Aphididae) in six hybrid lines (number 6, 14, MR, 13, PL and 12A) of winter forage pea. The field trial was conducted using a long-plot design with a sowing rate of 120 germinating seeds m^2 in three replications, a plot of 6 m^2 and a natural background of soil supply with the major nutrients. No pesticides were applied. Aphid number was recorded twice a week by counting on 15 marked plants per repetition (from April to June). Aboveground biomass samples were taken in the flowering stage to determine the nitrogen concentration (AOAC, 2001). In dry plant samples, total nitrogen content by Kjeldahl (as a percentage of absolute dry matter) in the aboveground mass was determined. Institute of Meteorology, Pleven affiliate provided the meteorological data.

The mathematical processing of the data used a one-way ANOVA, the mean being compared by a Tukey test in 5% probability ($P \leq 0.05$). Relationships between aphid variable and certain traits as a continuance of the generative period and nitrogen content were tested using linear quadratic regression models and multiple regression analysis. The statistical processing of experimental data was conducted using the Statgraphics Plus software program and PBSTAT program.

Results and Discussion

The population dynamics of the pea aphid during the vegetation period had a characteristic course, but the environmental factors (temperature, rainfall, and relative humidity) largely affected the aphid density. Due to the cool and dry weather in April of 2017 (lower temperatures and less rainfall of 3.1°C and 35.6 mm, as well as 4.7°C and 21.9 mm, compared to 2016 and 2018, respectively), plant growth slowed down and aphids appeared late, at the end of the month – Table 1. Weather conditions in April of 2016 and 2018 favored the emergence of *A. pisum* at the beginning of the month, but lower temperatures with 1.6°C combined greater rainfall with 21.9 mm in 2016 led to a lower density. April of 2018 was characterized by an optimal combination of a higher average daily temperature and an equal distribution of the amount of rainfall. These conditions determined the earliest occurrence and a high number of the pea aphid.

In May of 2017, when plants were in the sensitive stage of flowering, cool and rainy weather suppressed aphid population growth and generation development and as a result, the density occupied low values. Particularly indicative in this case was the third ten days of May when heavy rainfall from 106.5 mm washed the aphid population but the aphid population reached at peak when rainfall was at a normal rate in June. The relatively higher temperature of 3.3°C and the lack of heavy rainfall in May of 2018 compared to 2016 resulted in a considerably higher density and aphid outbreak. Weather conditions in June 2018 favored the generation development, and when plants approaching the technical maturity stage aphid density started reducing. Subsequently, aphids moved on other leguminous.

Considering the later development of plants in 2017, the sensitive stage of pod formation became in June. The combination of high temperature and a moderate and equal rainfall distribution resulted in outbreaks of *A. pisum*. On the other hand, the low temperature (19.2°C) and high amount of rainfall (46.3 mm) during the first ten days of June 2016 suppressed aphid population growth.

The results of carrying out analysis showed that the linear component in the regression of insect density with respect to the meteorological conditions was significant (Table 2A). From the complex study of the traits was obtained model (1) which demonstrated the complicated character of the change of density depending on the variation of weather conditions.

The common type of the obtained equation of regression was:

$$Y = -367.611 + 6.748 * X1 + 1.991 * X2 - 0.011 * X3 \quad (1)$$

$Y -$ *Acyrthosiphon pisum* density,
$X1 -$ average daily temperature,
$X2 -$ relative daily humidity,
$X3 -$ rainfall.

According to Gottwald (1970), the seasonal population peaks were determined by air temperature and humidity as they occurred during periods of high temperature and during or just after periods of high relative humidity. In the present study, a similar situation was also observed. The higher temperature and humidity, as well as lower rainfall amount determined the considerably higher density of the pea aphid in the sensitive stage of plant growth. That statement was

Table 1. *Meteorological conditions and aphid density.*

Ten days	Temperature, °C			Relative humidity, %			Rainfall, mm			*Acyrthosiphon pisum* density		
	2016	2017	2018	2016	2017	2018	2016	2017	2018	2016	2017	2018
01-10 April	16.3	11.6	14.1	61	60	62	1.5	10.1	19.5	3.3	0.0	27.3
11-20 April	16.7	11.5	16.9	67	64	68	43.3	22.9	21.5	15.0	0.0	152.5
21-30 April	13.0	13.5	19.8	70	60	65	28.3	4.5	10.2	39.3	10.5	81.7
Average	**15.3**	**12.2**	**16.9**	**66**	**61**	**65**	**73.1**	**37.5**	**51.2**	**19.2**	**3.5**	**86.2**
01-10 May	13.5	15.6	20.1	75	79	61	37.7	42.4	6.6	32.8	52.2	85.7
11-20 May	16.4	17.6	18.3	70	66	72	17.6	6.1	40.1	86.7	18.8	138.3
21-31 May	19.1	17.7	20.3	70	73	62	31.2	106.5	1.0	64.2	34.5	94.2
Average	**16.3**	**17.0**	**19.6**	**72**	**73**	**65**	**86.5**	**155.0**	**47.7**	**61.2**	**35.2**	**106.1**
01-10 June	19.2	21.1	23.4	72	74	62	46.3	21.6	0.4	19.0	138.2	49.0
11-20 June	24.1	20.9	22.7	66	65	72	6.5	21.6	39.1	9.0	95.5	6.5
21-30 June	25.7	27.0	19.4	63	54	72	0.0	1.6	115.7	0.0	6.7	0.0
Average	**23.0**	**23.0**	**21.8**	**67**	**64**	**68**	**52.8**	**44.8**	**155.2**	**9.3**	**80.1**	**18.5**

Table 2A. *Regression analysis (ANOVA) of Acyrthosyphon pisum density in regard to the meteorological conditions.*

Dispersion	df	SS	MS	F-Ratio	P-value
Model	3	9465.08	3155.03	1.54	0.035
Residual	20	40992.2	2049.61		
Total (Corr.)	23				

Table 2B. *Regression coefficients of Acyrthosyphon pisum density in regard to the meteorological conditions.*

Factors	Coefficients	Standard Error	t Stat	P-value	Lower Limit	Upper Limit
Intercept	-367.611	119.136	-3.085	0.027	-673.861	-61.3603
Temperature	6.748	1.770	3.809	0.012	2.19475	11.3022
Humidity	1.992	1.833	1.086	0.032	-2.72207	6.70523
Rainfall	-0.011	0.596	-0.018	0.985	-1.54426	1.5221

supported by the applied analysis in Table 2B. It showed that on pea aphid infestation, the highest positive significant influence had the temperature (r = 6.748) followed by humidity (r = 1.992). The rainfall amount had a considerably weaker negative influence.

The present finding on the effect of weather factors on the aphid population was strengthened by Melesse & Singh (2012) and they observed a significant positive correlation between maximum temperature with aphid population (r = 0.71). Inversely, rainfall was negatively correlated with aphid population (r = -0.98) and rainfall decreasing was related to a significant population increase. Similarly, based on daily values of temperature and relative humidity, Narjary et al. (2013) reported that between humid thermal ratio and aphid population had a positive correlation.

Pea aphid population levels did not follow a consistent pattern over the years and peaks of each of the three years occurred at a different time. The first individuals in winter pea crop were established at the beginning of April in the button stage at line 13 and PL (Table 3). At the beginning of flowering in the second ten days of April, aphid density was low because of the large amount of rainfall. Sudden changes in the weather conditions result in very poor survival of aphids. The numbers ranged from 4.0 to 27 individuals/plant. In line 6 a minimum aphid number per plant with significant differences to others was observed. Slightly infected with a significantly lower number were also lines MR and 12A. At the end of April and the beginning of May, in the full flowering stage, differences in pea aphid preference were more strongly pronounced. Line 13 had the highest density with a significant difference to others while significantly lower numbers and poorly infected were plants of lines 6 and 12A. Primarily, in the sensitive stage of pod formation in the second ten-day period of May, a peak in aphid population was recorded, and it remained high in the third ten days of the month. The favorable impact of higher temperature and optimum relative humidity in comparison to the previous period resulted in a maximum density. Line 13 followed by PL were the most preferred, exceeding significantly 100 individuals/plants in the period 11-31 May while at a significantly lower level in lines 6 and 12A the density was maintained. Despite the reported low number of species in June a similar trend was observed.

In general, considering the lowest average daily temperature in the sensitive stages of the plant development in 2016, *A. pisum* outbreak was strongly hindered. The aphid infestation was markedly weakest.

Despite the unfavorable conditions, established phenological differences between studied lines influence the aphid choice (Table 4). Sensitive stages of flowering and pod formation had a different duration, such as the longest one was observed in lines 13 and PL (60 and 63, respectively). The prolonged period of generative organ formation greatly favored the population growth of pea aphids, which was one of the reasons for the highest density in those lines. The other lines had an almost twice less generative duration.

In 2017, a peak in the pea aphid population in a later vegetation period was exhibited. Because of the lower temperature and relative humidity in April than 2016 and 2018, the emergence of the species was late after the beginning of flowering at the end of April. There was a significant tendency of preference to line 13 although aphid density was low. *Acyrthosiphon pisum* number was non-linearly increasing through the pod formation stage in May, and a stable position with the highest numbers occupied line 13. Differences in comparison to others were statistically significant. The other lines a similar aphid density was characterized in the exception of lines 6 and 12A which in most cases had a significantly lower number. A large amount of rainfall in May did not allow strong population growth and outbreaks of *A. pisum*.

Higher temperatures and relative humidity in June resulted in a peak in the pea aphid population in the first ten days of the month which also remained high during the second period. The most active reproduction and aphid's colony formation were recorded in line 13, followed by PL. Significant density differences between them and the other lines were observed. Plants of lines 14 and MR occupied an intermediate position. Plants of lines 14 and MR occupied an intermediate position

Table 3. *A number of individuals of Acyrthosiphon pisum Harr. per plant in winter pea lines.*

2016

Period /lines	01-10 April	11-20 April	21-30 April	1-10 May	11-20 May	21-31 May	01-10 June	11-20 June	21-30 June
№ 6	0 a	4 a	13 b	12 a	17 a	9 a	8 a	0 a	0
№ 14	0 a	18 c	52 d	27 b	43 c	44 c	11 b	0 a	0
№ MR	0 a	10 b	46 c	32 b	60 d	21 b	17 c	0 a	0
№ 13	12 b	27 e	67 e	76 d	210 f	108 d	42 e	37 c	0
№ PL	8 b	23 d	53 d	38 c	159 e	192 e	24 d	17 b	0
№ 12A	0 a	8 b	5 a	10 a	31 b	11 a	12 b	0 a	0
$F_{5,29}$	4.121	3.081	4.154	5.307	9.534	7.710	3.00	2.765	
P =	<0.001	0.001	<0.001	0.010	0.034	0.001	<0.001	<0.001	

2017

Period / lines	21-30 April	1-10 May	11-20 May	21-31 May	01-10 June	11-20 June	21-30 June
№ 6	7 b	7.5 a	18 bc	16 a	32 a	9 a	0 a
№ 14	6 ab	42.5 b	12 b	36 b	41 b	19 b	0 a
№ MR	12 c	38 b	17 bc	38 b	134 c	95 c	0 a
№ 13	21 d	94 d	43 d	57 c	427 e	304 e	27 b
№ PL	15 c	82 c	20 c	39 b	161 d	134 d	13 a
№ 12A	2 a	49 b	3 a	21 a	34 a	12 a	0 a
$F_{5,29}$	4.574	11.629	7.822	10.142	8.186	5.743	4.153
P =	<0.001	0.023	0.001	0.013	<0.001	<0.001	<0.001

2018

Period /lines	01-10 April	11-20 April	21-30 April	1-10 May	11-20 May	21-31 May	01-10 June	11-20 June
№ 6	17 ab	92 a	38 a	35 a	57 b	48 a	24 b	0 a
№ 14	34 cd	124 b	96 c	92 c	135 c	83 b	28 b	0 a
№ MR	21 ab	174 c	59 b	72 b	162 d	124 d	43 c	1 a
№ 13	43 d	234 e	148 d	154 e	249 f	162 e	100 e	25 c
№ PL	26 bc	205 d	105 c	127 d	187 e	92 c	84 d	13 b
№ 12A	13 a	86 a	44 a	34 a	43 a	56 a	15 a	0 a
$F_{5,29}$	10.046	9.382	11.598	8.824	6.544	7.002	5.572	4.416
P =	0.026	0.010	0.042	0.001	<0.001	0.023	<0.001	<0.001

Table 4. *Phenological development of winter pea lines.*

Days between plant stages / Lines	№ 6	№ 14	№ MR	№ 13	№ PL	№ 12A
2015-2016						
Start of flowering/ full flowering	10	8	8	14	21	8
Start of flowering / technical maturity	30	31	31	60	63	32
Vegetation period	223	218	221	234	228	221
2016-2017						
Start of flowering/ full flowering	6	8	8	15	13	3
Start of flowering / technical maturity	29	36	29	60	59	27
Vegetation period	225	218	225	232	229	221
2017-2018						
Start of flowering/ full flowering	5	12	5	10	5	5
Start of flowering / technical maturity	36	20	49	49	52	34
Vegetation period	216	218	225	232	228	215

whereas weak infection and a significantly lower number had lines 6 and 12A.

Plant phenology was markedly different, such as the duration of the flowering stage at lines 6 and 12A was the shortest (6 and 3 days, respectively) as well as the period to the technical maturity emergence (29 and 27 days) (Table 4). Considering the longest duration of the sensitive generative stages of plant growth (60 days) and vegetation period (232 days) in line 13, as well as the late maturity emergence, very well was expressed synchronization between the biological cycle of the pea aphid and phenological development. The trend in line PL was similar.

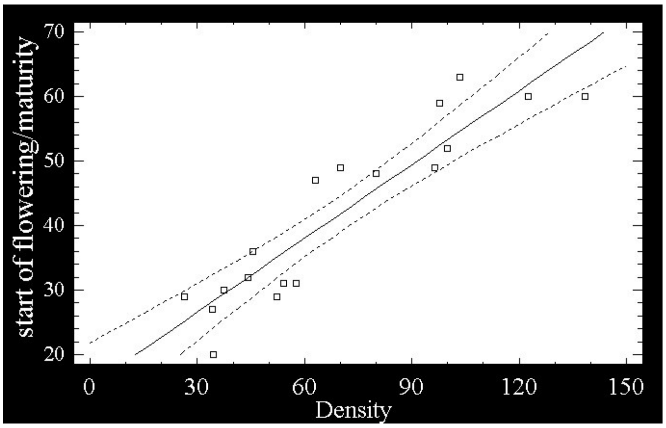

Figure 1. *Effect of the generative period on the pea aphid density.*

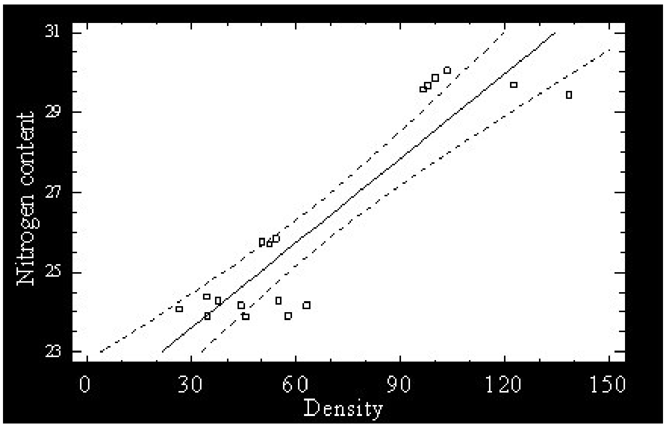

Figure 2. *Effect of the nitrogen content on the pea aphid density.*

The population density of *A. pisum* in the third experimental year was the highest and confirmed clear trends of aphid preference. The higher average daily temperature and relative humidity resulted in the earliest aphid that emerged at the beginning of April. Even then, the plant response to the infection was observed. In the second ten days of the month, was recorded a peak in the pea aphid population of 152.5 average number/plant. Strong infestation and presence of colonies on stems and leaves in most lines were observed. Nevertheless, the highest density in line 13 was recorded. Preferred lines occupied the following ascending order: 14, MR, PL and differences between them were significant. Significant lower numbers had hybrids 12A and 6. The third ten days of April and the first ten days of May were characterized by a considerable reduction of *A. pisum* density, although plants were in the sensitive flowering stage. The mutual impact of higher temperatures and lower relative humidity and rainfall compared to the previous period led to a population decrease. However, aphid choice remained unchanged.

The second peak in population density was found in the period 11-20 May, remaining high until the end of the month because of the warm and wet weather, favoring the aphid reproduction. Studied lines were strongly infested in the sensitive stage of full flowering regardless of significant differences in numbers between them. Exceptions were lines 6 and 12A with a repeatedly lower number of individuals where there was no aphid outbreak. The weak infestation on these lines was also maintained during the period 1-10 June, while the most strongly preferred and with the highest density were plants of line 13 followed by PL. Their technical maturity occurred the latest after that plants became unsuitable with the aging and the reproductive capacity of aphids strongly declined.

The dependence of the higher aphid number on plants with longer duration of the sensitive stages and vegetation period in the third experimental year was confirmed. Line 13, characterized by the longest vegetation of 232 days and a long period of flowering and pod formation until the beginning of technical seed maturity (59 days), favored the mass reproduction of *A. pisum* and resulted in the highest number of individuals. Conversely, a shorter duration of flowering (5 days) and flowering-maturity period (41 and 39 days, respectively) had lines 6 and 12A. Due to poor synchronicity between the aphid biological cycle and phenological plant development lines were slightly preferred. An exception to that relationship, wherein the expressed preference of the species did not correspond to the shorter generative period was observed in line 14. The reasons probably were others.

The effect of the plant phenology on *A. pisum* preference was examined by regressing models (Figure 1). Positive significant interaction was detected between aphid density and generative period ($F_{1,\ 17} = 7.065$; $P = 0.003$, $R^2 = 0.757$). Pea aphid density significantly increased with prolonging the period from the flower beginning to started of technological maturity in studying lines.

Due to a significantly lower density of the species and shorter generative period, lines 6 and 12A were much less preferred and defined as tolerant.

Another reason for the preferences of *A. pisum* was related to the nitrogen content in the aboveground mass (Table 5). Hybrid lines significantly differed in terms of the concentration in most cases over the years and trends were similar. Line 6, followed by 12A was distinguished with a significantly lower nitrogen content average for the period. Over the three years, the lines were poor in nitrogen and occupied stable positions. Conversely, significantly higher concentrations characterized sensitive to aphid infestation lines like 13 and PL. Insignificant were differences between line 14 and PL in 2016 and 2017, as well as between line 14 and 13 in 2018.

The concentration of nitrogen was positively correlated to aphid population variation in the present work, suggesting that they affected aphid biology ($r = 0.909$). A significant lower

Table 5. *Nitrogen content in hybrid lines of winter forage pea (g/kg dry matter).*

Lines	2016		2017		2018		Average	
№ 6	29.86	a	19.14	a	17.25	a	22.09	a
№ 14	38.32	d	20.58	cd	25.88	d	28.26	d
№ MR	36.14	c	20.16	bc	22.98	c	26.43	c
№ 13	40.66	e	22.03	e	26.81	d	29.83	e
№ PL	36.88	cd	21.51	de	31.41	e	29.94	e
№ 12A	32.04	b	18.71	a	20.70	b	23.82	b
$F_{5,17}$	1.844		1.340		1.497		1.147	
P =	0.001		0.041		0.025		0.010	

concentration of nitrogen on the aphid tolerant lines was also detected. Something more, a positive significant interaction was found between aphid density and total nitrogen ($F_{1, 17}$ = 8.742; P = 0.010; R^2 = 0.826) (Figure 2). Present data highlighted the relation between nitrogen and aphids on winter pea and the possibility to be considered in breeding programs.

Because aphids feed exclusively on the phloem, their diet is rich in sugar but relatively poor in nitrogen, necessitating the ingestion of large volumes so that the insects can acquire sufficient nitrogen (Douglas et al., 2006). A large number of authors concluded that the high nitrogen inputs were an important factor contributing to a high aphid population and aphid fecundity (Staley et al., 2011; Gash, 2012; Santiago et al., 2012). Meradsi & Laamari (2016) suggested that the bean resistant cultivars to *A. fabae* were poor in nitrogen and sugars and rich in phosphor. Furthermore, Bala et al. (2018) specified that increased nitrogen and phosphorus content in plant tissues had positive effects on population growth and other parameters of aphid performance. Similar results were reported in a previous study where the high content of nitrogen resulted in significantly greater *A. pisum* population density in winter vetch varieties (Nikolova, 2017).

Similarly, the present data showed a preference of pea aphid to lines characterized by significantly higher nitrogen content while tolerant lines (6 and 12 A) were poor in nitrogen.

Conclusions

The population dynamics of the pea aphid during the vegetation period had a characteristic course, but the factors such as temperature, rainfall, and relative humidity largely affected the *Acyrthosiphon pisum* density. The highest positive and significant effect on pea aphid infestation had the temperature (r = 6.748), followed by humidity (r = 1.992). The rainfall amount had a considerably weaker negative influence.

A peak in the aphid population density was reached in the sensitive stage of pod formation, as well as at flowering under a favorable weather condition.

Lines 6 and 12A stood out with a significantly lower aphid density, nitrogen concentration and shorter generative period. They were much less preferred by aphids and defined as tolerant.

The interaction between aphid density and generative period as well as total nitrogen was positive and significant.

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