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Stability of *Vicia faba* L. cultivars and responsible traits for *Aphis fabae* Scopoli, 1763 preference

Ivelina NIKOLOVA [1, 2]

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Stability of *Vicia faba* L. cultivars and responsible traits for *Aphis fabae* Scopoli, 1763 preference

Abstract: The study aimed to evaluate the responsible traits of preference of *Aphis fabae* to *Vicia faba* cultivars and their stability in multi-environment field tests. The experiment was carried out at the Institute of Forage Crops (Pleven) during the period 2016 to 2018. Aphid infestation was assessed by recording the number per plant at the pod formation, while the chemical composition was determined by standard Weende system methods. Canonical correspondence analysis showed that the aphid density was negatively correlated with the amount of rainfall and humidity until aphids were positively correlated with the temperature. According to GGE biplot analysis cultivar Fb 3270, followed by BGE 029055 and BGE 002106 were stable with a low density of aphids and were defined as tolerant. A significant negative correlation was found between the density of aphids and plant height (r = -0.447). The protein content showed a significant positive correlation (r = 0,686), while phosphorus and cyanogenic glycoside concentration were significantly negatively correlated with the aphid incidence (r = -0.411, r = -0.685, respectively). The results lay the groundwork for further analyses to finely dissect *A. fabae* tolerance in *V. faba* and pave the way for the development of methods to predict potential resistant genotypes in breeding programs.

Key words: *Aphis fabae* preference; faba bean cultivars; stability; morphological and chemical traits

Stabilnost sort boba (*Vicia faba* L.) in odzivne lastnosti črne fižolove uši (*Aphis fabae* Scopoli, 1763)

Izvleček: Namen raziskave je bil ovrednotiti preferenčne odzivne lastnosti črne fižolove uši na sorte boba in njihovo stabilnost v poljskem poskusu v različnih okoljih. Poskus je bil izveden na Institute of Forage Crops (Pleven, Bolgarija) v obdobju 2016-2018. Napad uši je bil ocenjen s štetjem uši na rastlino v razvojni fazi tvorbe strokov, kemijska sestava rastlin je bila določena s standardno Weende metodo. Kanonična korespondenčna analiza je pokazala, da je bila gostota uši v negativni korelaciji s količino padavin in vlažnostjo in v pozitivni korelaciji s temperaturo. Glede na GGE biplot analizo so bile sorte Fb 3270, BGE 029055 in BGE 002106 stabilne z majhno gostoto uši in so bile opredeljene kot odporne. Ugotovljena je bila značilna negativna korelacija med gostoto uši in višino rastlin (r = -0,447). Vsebnost beljakovin v rastlinah je pokazala značilno pozitivno korelacijo s pojavom uši (r = 0,686), medtem, ko sta bili vsebnosti fosforja in cianogenih glikozidov v negativni korelaciji s pojavom uši (r = -0,411; r = -0,685). Rezultati te raziskave dajejo osnovo za nadaljne analize tolerance črne fižolove uši na sorte boba in tlakujejo osnovo za nadaljne analize v razvoju metod za prognoziranje potencialno odpornih genotipov boba v žlahtniteljskih programih.

Ključne besede: preferenca črne fižolove uši na bob; sorte boba; stabilnost; morfološke in kemijske lastnosti boba

1 Agricultural Academy, Institute of Forage Crops, Pleven, Bulgaria
2 Corresponding author, e-mail: imnikolova@abv.bg

1 INTRODUCTION

Faba bean (*Vicia faba* L.) is an important grain legume, protein-rich and widely used for human and animal consumption (Dhull et al., 2022). In addition, faba bean has also a valuable agronomic function considering its high capacity for N_2 fixation.

The most important common bean pest worldwide is the black bean aphid, *Aphis fabae* Scopoli, 1763 (Hemiptera, Homoptera: Aphididae), which causes considerable damage to plants and yield loss reaches 37 % (Munyasa, 2013). Aphids frequently grow and develop rapidly, allowing aphid populations to fastly exceed economic threshold levels (10–15 % attacked plants with single larval colonies, wingless, winged). Numerous colonies of *A. fabae* are very damaging to *V. faba* because of the direct negative impact on plant growth and the quantity and quality of the yield (Shannag & Ababneh, 2007). Injury caused by many aphid species is well documented to change the rate of photosynthesis, plant growth and physiological processes (Cahon et al., 2018; Du et al., 2021; Fuentes et al., 2021). Recurrent chemical applications are inappropriate because they represent a serious threat to pollinators and natural enemies in general. And, biological control approaches that have been attempted so far showed limited effectiveness, which makes studies of methods to control aphid populations extremely important. Insecticides' frequent treatments led to resistance evolution in many insect pests and synthetics are often more toxic to natural enemies than to the pests themselves. In addition, when insecticides negatively impact useful insects, secondary pest outbreaks can result (Straub et al., 2020). Breeding-resistant cultivars are an adequate approach to achieving efficient levels of pest resistance and promoting sustainable agriculture. Many authors reported that the application of resistant cultivars is a substantive and indubitable method for aphid control (Esmaeili-Vardanjani et al., 2013; Josefina et al., 2017). Béji et al. (2015) studied faba bean resistance to *A. fabae* and found that the best parameters describing resistance were pod mass and grain number. Meradsi & Laamari (2016) evaluated the resistance response of *V. faba* to black bean aphid by the relationship between the resistance level and plant morphological characteristics. Golizadeh et al. (2016) reported the relative impact of cultivars' resistance to black bean aphids, based on antibiosis and antixenosis.

The application of alternative cropping strategies, specifically the use of different cultivars, is an effective, environmentally friendly alternative to suppress crop pests.

This study aimed to evaluate the responsible traits of preference of *A. fabae* to *V. faba* cultivars and their stability in multi-environment field tests.

2 MATERIAL AND METHODS

The field study was carried out at the Institute of Forage Crops (Pleven; Bulgaria) during the period from 2016 to 2018. Twelve cultivars of faba bean (*Vicia faba* L.), originating from Portugal (Fb 1896, Fb 1903, Fb 1929, Fb 2481, Fb 2486, Fb 3270) and Spain (BGE 002106, BGE 029055, BGE 032012, BGE041470, BGE 043776, BGE 046721) were used. The experiment was laid out in Randomized Block Design (RBD) with three replications and an experimental plot of 4 m^2. The cultivars were planted with a sowing rate of 30 seeds m^2 and kept devoid of insecticide application throughout the experimentation to assess the susceptibility or resistance response to *Aphis fabae*. Aphid infestation occurred naturally. The reaction of different cultivars to *A. fabae* was assessed by recording the aphid number per plant at the 50 % pod formation stage of the faba bean. Therefore, twenty plants were selected randomly from each replication of the cultivar. The average aphid number was calculated based on three-time counts in each stage within 2-3 days. The height of the plants was measured in parallel.

To determine the chemical changes of the aboveground mass of cultivars in the aphid infestation, the plant samples taken of each cultivar were fixed for 15 minutes at 100 °C and dried to a constant mass at 60 °C in a thermostat. The chemical composition was determined by standard methods of the Weende system (AOAC, 2001) and includes crude protein (CP) by the Kjeldahl method (crude protein is calculated on the formulae CP = total N x 6.25) and phosphorus – colourimetrically by hydroquinone (Sandev, 1989). In addition, in fresh plant samples, cyanogenic glycosides contents (mg/100 g dry matter)) was determined according to Ermakov et al. (1987). Chemical compounds in cultivars were determined in the bedding of the pod formation.

To include genotype and genotype x environment (GGE) interplays and to remove interactions between variables, HA-GGE biplot analysis was used (Yan & Holland, 2010). Biplots are often applied to compare multiplex genotypes in different environments (Rubiales et al, 2014; Sánchez-Martín et al, 2014).

To determine the relative effect of the meteorological variables such as rainfall, air temperature and relative humidity on aphid density, canonical correspondence analysis (CCA) was carried out. The Paleontological Statistics Software Package (PAST) (Hammer et al., 2001) was used to perform the analysis.

The data were subjected to one-way ANOVA, and the means were compared by Tukey's test at 5 % probability ($p \leq 0.05$). The Multiple Regression Analysis of Statgraphics Plus (1995) for Windows Ver. 2.1 Software program was used.

3 RESULTS AND DISCUSSION

The meteorological conditions from 2016 to 2018 were different (Figure 1) and affected *Aphis fabae* development and reproduction. The higher average daily temperature in April, May and June months in 2016 (by 2.0 and 0.4 ^{0}C compared to 2017 and 2018), as well as a lower rainfall (by 41.9 and 105.6 mm, respectively), led to an earlier appearance of aphids and a stronger infestation compared to other years. At the same time, the weather conditions were favourable for plant development and did not suffer from a lack of moisture. In 2018, aphids were suppressed by the high amount of rainfall and relative humidity combined with lower temperatures whereat the population density was considerably lower. A similar trend was observed in 2017.

A wide range of the values obtained for aphid density was noted for the 12 *Vicia faba* cultivars studied in the three environments. ANOVA (Table 1) revealed a significant effect of genotype (G), environment (E) and G × E in both variables, being the highest average of a square for G, followed by E and the lowest for G × E.

A canonical correspondence analysis (CCA) was used to illustrate the clear relations of the aphid number to climatic variables (Fig. 2). It was found that the aphid number was negatively correlated with the amount

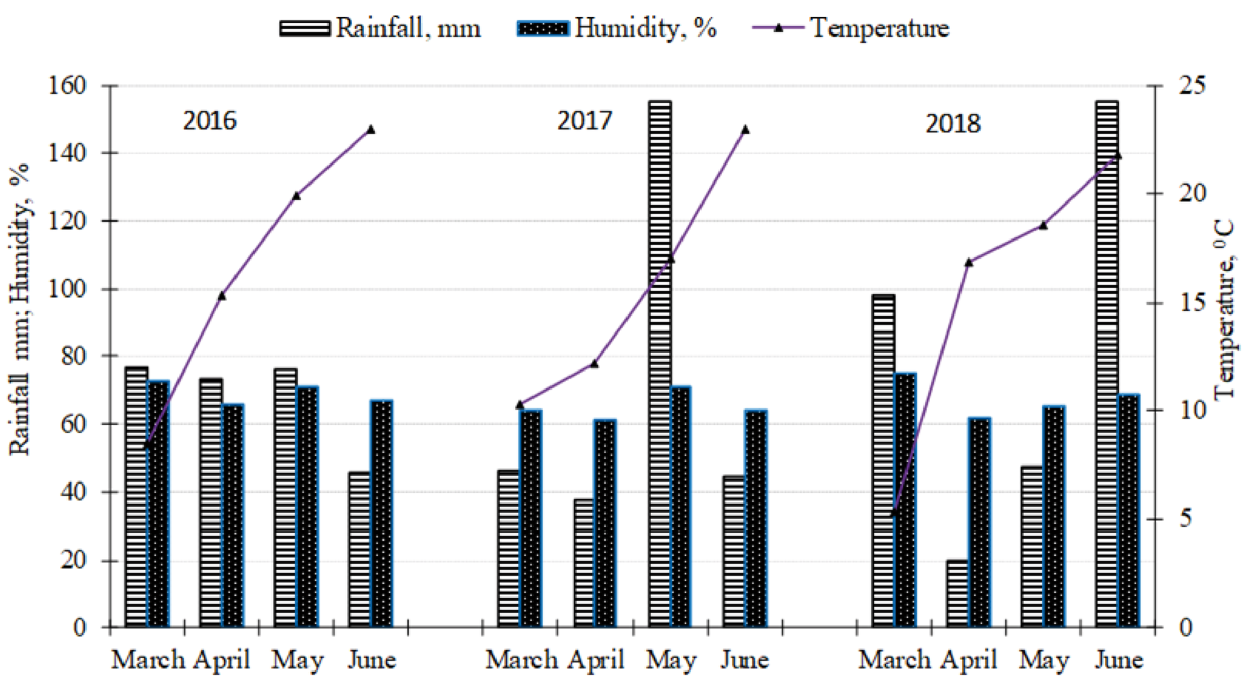

Figure 1: Meteorological characteristics of the period 2016-2018

Table 1: Analysis of variance for *Aphis fabae* number of the 12 faba bean genotypes

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Environment (E)	2	10925763	5462882	367,0335	5,33E-07
Replication/R	6	89303,26	14883,88	0,702751	0,648376
Genotype (G)	11	62417552	5674323	4,554665	0,001222
G^{x}E	22	27408186	1245827	58,82247	5,13E-35
PC1	12	26553829	2212819	104,48	0
PC2	10	854357	85435,7	4,03	0,0002
Residuals	66	1397843	21179,44	#N/A	#N/A

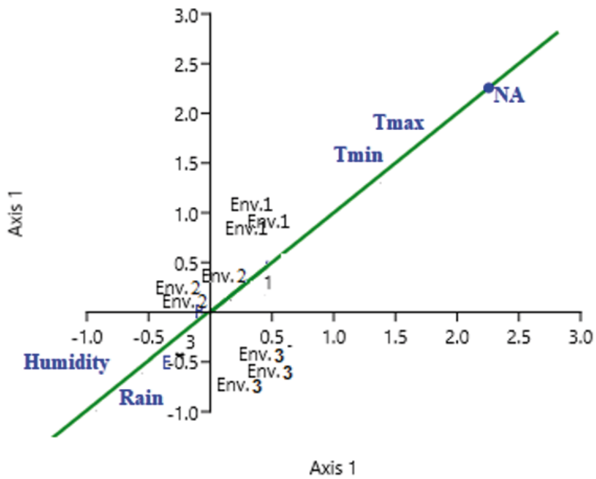

Figure 2: CCA graph based on the correlation of the aphid number for 12 faba bean cultivars according to several climatic parameters. The period analyzed was from March to June, Tmax = maximum temperature; Tmin = minimum temperature; NA – number of *Aphis fabae* Scopoli, 1763

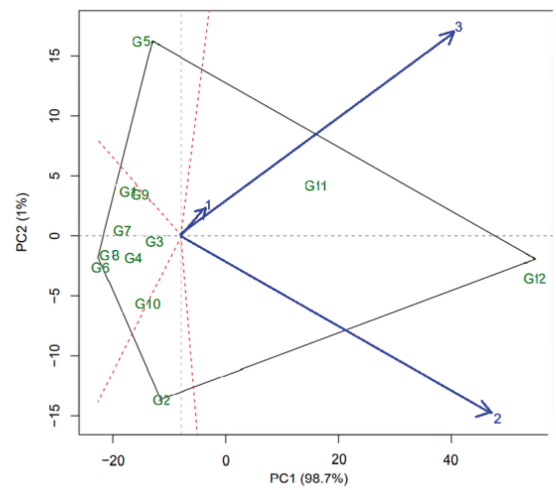

Figure 3: The GGE biplot based on seed damage rate (2016-2018). The cultivars are designated with the symbol "G" and the respective number from 1 to 12, as follows G1- Fb 1896, G2- Fb 1903, G3- Fb 1929, G4- Fb 2481, G5- Fb 2486, G6- Fb 3270, G7- BGE 002106, G8- BGE 029055, G9- BGE 032012, G10- BGE 041470, G11- BGE 043776, G12- BGE 046721. The years are designated with the letter E and numbers 1; 2; and 3 for 2016, 2017 and 2018, respectively, Note: G1 and G9 are heavily overlapped

of rainfall and humidity until *A. fabae* was positively correlated with T min and T max. Furthermore, temperatures were associated with the Environmental 1 drought (2016) and opposed to precipitation and humidity during the Environmental 3 wet season (2018).

Because of the negative effect of rainfall on aphids, the density decreased during rainy seasons, whereas in the driest environments, the aphid number increased. Rainfall and lower temperatures hinder the development of *A. fabae* or fully wash away species from the plants. On the opposite, the temperature had a positive effect, with the number increasing at higher values.

According to the results of the GGE biplot analysis (Fig. 3), the difference in vector length among environments showed phenotypic variances within different environments. Based on the discrimination power (vector length) E2, followed by E3 were the most discriminating, GGE biplot manifested clearly long vectors for E2 and E3, and a shorter vector for E1, respectively.

According to Yan & Holland (2010), an HA-GGE biplot is the preferred GGE biplot as regards test environment and genotype evaluation. A GGE biplot is based on environment-centred data (Gabriel, 1971), which integrates the genotypic main effect with the genotype-by-environment interaction effect of a genotype-by-environment dataset (Yan et al., 2000).

The best part of approximation for the correlation coefficients by the angles is linked to the fit goodness of the biplot although there are no exact relations. The vector correlation is varied depending on the angle between the two environments. Then, the environments were more or less positively correlated since the angles were acute angles. Furthermore, within the environmental group, E2 was apparently less associated with E3, while E1 and E3 were strongly positively correlated.

To determine which of the twelve faba bean cultivars studied were the lowest density of *A. fabae* based on their representation in the biplots, the ranking of the genotypes (considering stability across the environments studied) for both variables assessed is shown in Table 2.

Stability throughout the environments has been taken into account by considering each cultivar (genotype) position in the biplots. Thus, the cultivar with the lowest density of *A. fabae* was G6/Fb 3270 (63.9 winged and wingless individuals/plant) despite exposed environmental interactions, followed by the G8/BGE 029055 (120.9 individuals/plant) and G7/BGE 002106 (214.9 individuals/plant), whose responses were more steady, as shown by their position close to the axis 1. The three cultivars were defined as tolerant.

The results indicated that the cultivar G3 was considered stable similar to G7 given the closeness to the midpoint of the boxplot but presented with a considerably higher value of the density and identified as a sensitive (446,8 individuals/plant). Relatively stable but sensitive with somewhat differences from each other showed G9, G4 and G1 (297.8; 338.9 and 421.4 individuals/plant,

Table 2: Ranking of the twelve faba bean cultivars with the lowest density of *Aphis fabae* (ascending order)

Rank	G	Cultivars
1	G 6	Fb 3270
2	G 8	BGE 029055
3	G 7	BGE 002106
4	G 9	BGE 032012
5	G 10	BGE 041470
6	G 4	Fb 2481
7	G 2	Fb 1903
8	G 1	Fb 1896
9	G 3	Fb 1929
10	G 5	Fb 2486
11	G 11	BGE 043776
12	G 12	BGE 046721

respectively) too. Poor stability and aphid sensitivity showed G2, Q5 and G10 genotypes but G2 and G5 were more affected by the environment.

Genotype G12 (2868,1 individuals/plant) had the highest values for that trait, followed by G11 (1514,9 individuals/plant) which determined them as the most susceptible cultivars. According to the GGE biplot analysis, G11 had the highest value in E3 while G12 - in E2, which were the most favourable for their susceptibility. Genotypes G11 and G12 were less affected by the environment compared to G2 and G5.

The first two principal components (PC1 and PC2) determined 98.7 % of the dispersion.

The tolerance of cultivars Fb 3270, BGE 029055, and BGE 002106 (G6, G8 and G7) might be the result of the combination of different resistance mechanisms. Morphological characteristics such as plant height influenced the abundance of aphids as tolerant cultivars were considerably higher than susceptible ones. The correlation between the aphid density and the height of the cultivar plants was calculated. It was found that *A. fabae* preferred significantly lower plants and a middle negative correlation was found, r = -0.447, p = 0.014 (Fig. 4).

There have been different hypotheses regarding the effects of plant height on the preference of aphids. For example, Meradsi & Laamari (2016) found that morphological characteristics such as plant height did not affect *A. fabae* infestation but resistant cultivars had a longer leaflet than highly susceptible cultivars. Chaudhari et al. (2013) found the degree of association between the aphid population with morphological traits was not significant except for plant height which showed a highly significant negative correlation. On the other hand, Lebbal (2010) mentioned that aphid-resistant and highly susceptible bean cultivars had the same morphological characteristics.

The effect of some chemical traits on aphid preference was examined by regressing models too (Fig. 4).

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 considerably lower concentration of protein in the aphid-tolerant cultivars was detected. Probably high levels were a key factor supporting high *A. fabae* density and fecundity. A positive significant interaction between aphid density and protein content was detected, r = 0.686, p = 0.022.

The protein preference of aphids observed in that study was consistent with those reported in several previous experiments. Meradsi & Laamari (2016) studied different cultivars of *V. faba* for their resistance against the black bean aphid and found that the low susceptibility of the plant was possibly based on its lower nitrogen and protein and high phosphor content in plant leaves. Chaudhari et al. (2013) reported that resistant lucerne varieties against *Therioaphis maculata* (Buckton, 1899) had a lower total chlorophyll, crude protein, sugar and magnesium contents. The authors found also a highly significant positive correlation between the aphid population and chemical component levels in plants. Comadira et al. (2015) studied the complex relationship between plant N and aphid infestation (*Myzus persicae* (Sulzer, 1776)) and found that in N-deficient barley leaves, the progenitor aphids failed to survive until maturity despite the observed large increase in free amino acids.

The present data revealed the key role of plant protein on the quantity and colonization choice of aphids on faba bean plants.

The phosphorus and cyanogenic glycoside content in the aboveground mass were other reasons for the preferences of aphids. Phosphorus is important for the formation of nucleic acids and phospholipids and is needed for the energy metabolism of photosynthesis (Wang et al., 2020). The results of the regression model showed that the highly preferred cultivars by aphids had a considerably lower phosphorus content, while the high phosphorus level determined a lower *A. fabae* density. A significant negative correlation was found between aphid number and phosphorus levels, r = -0.411, p = 0.013. The same trend was found in cyanogenic glycosides. The low aphid-affected cultivars had a high concentration of cyanogenic glycosides while the preferred plants had low levels. Faba bean leaves contain cyanoglycoside and during their hydrolysis is released hydrogen cyanide which

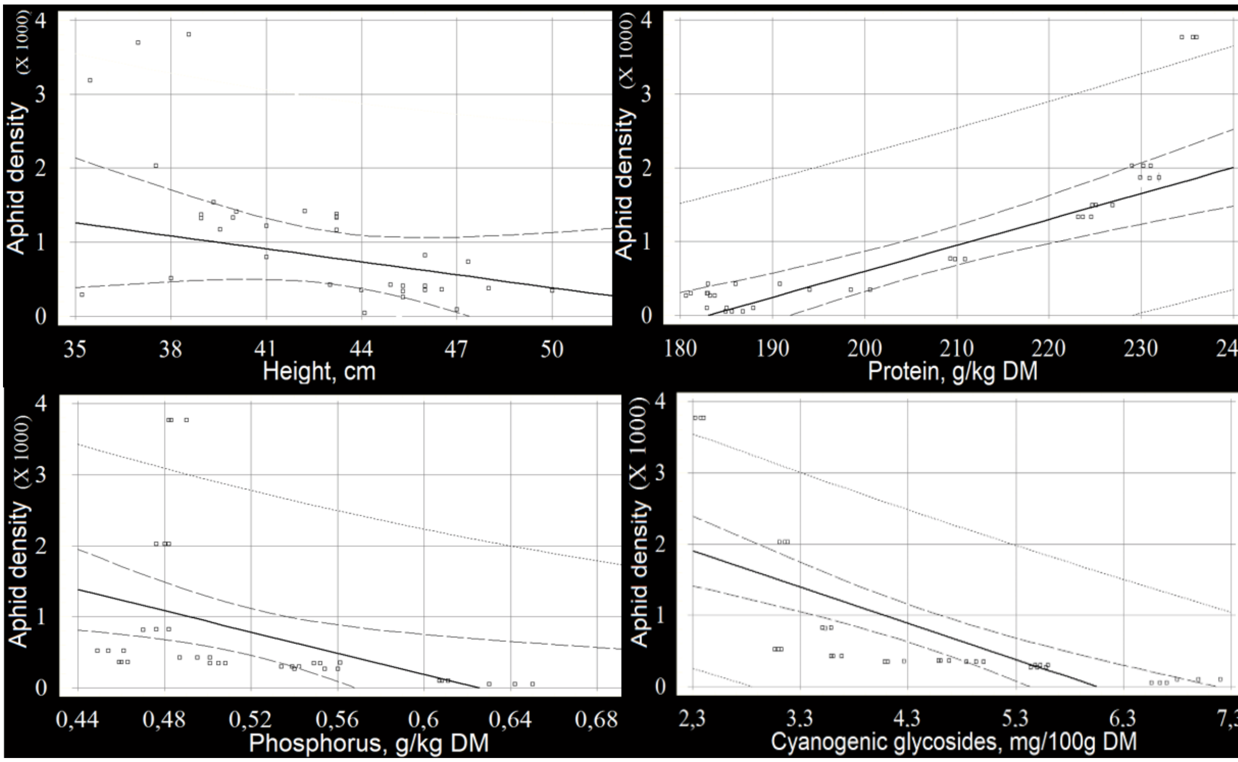

Figure 4: Effect of some traits on the *Aphis fabae* Scopoli density

has an insect-toxic effect in a concentration above 50-75 mg/100g of DM according to Naydenova et al. (2018). These compounds play an important role in plant defence producing bitter taste and toxic hydrogen cyanide which repel pests (Gleadow & Møller, 2014). Therefore, cyanogenic glycoside concentration was significantly negatively correlated with the incidence of black bean aphid (r = -0.685, p = 0.001).

The present results showed that cyanogenic glycosides may play a key protective role against *A. fabae* preventing the colonization and abundance of the species. There was indisputable evidence for the role of cyanogenic glycosides as insect pest deterrents. According to Ballhorn et al. (2008; 2009), resistant bean genotypes had strong cyanogenesis and therefore were efficiently directly defended against insect pests. On the other hand, some authors reported that insect pest damage was responsible for catalyzing the synthesis of cyanogenic glycosides as a defence mechanism (Irmisch et al., 2014; Chunming et al., 2018).

Unlike the cyanogenic glycosides, there have been different hypotheses regarding the effects of concentrations of phosphorus in leaves on the preference of insect pests. For example, Vannette & Hunter (2009) reported that the greater concentrations of P in leaves affected the attractiveness of plants to sap-sucking pests. On the other hand, Azouz et al. (2014) studied how plant mineral status affected the aphid population under field conditions. Authors reported that the susceptible eggplant cultivars had lower potassium, sodium, calcium and phosphorus content and the phytochemical constituents were negatively correlated with the *A. gossypii*Glover, 1877 amount as well as with the level of infestation. Facknath & Lalljee (2005) explained that phosphorus decreases the host's suitability against various insect pests by changing secondary metabolites such as phenolics and terpenes and accumulation of phenolics which acts as a barrier having deterring (antifeedant) or directly toxic (insecticidal) effects on herbivores.

The above graphical representation of the relationships between the aphid density and studied traits allowed statistical results to be obtained (with sufficient approximation) and main dependencies to be established while a regression analysis allowed determining which factors matter most and how these factors influence each other.

The results of the regression analysis (Table 3) showed that the linear component in the regression of aphid numbers according to the chemical traits was significant.

Table 3: Regression analysis and regression coefficients (R) of *Aphis fabae* Scopoli number regarding main chemical traits in *Vicia faba* L. cultivars (Institute of Forage Crops, Pleven, Bulgaria, 2016-2018)

Regression analysis					
Dispersion	df	SS	MS	F-Ratio	P-value
Model	4	2.69668E7	6.74169E6	18.55	0.00001
Residual	31	1.12638E7	363349.0		
Total (Corr.)	35	3.82306E7			
Regression coefficients					
Factors	Coefficients	Standard Error	t Stat	P-value	
Intercept	10734.500	1662.140	6.4582	0.001	
Height	- 47.765*	12.181	-3.921	0.001	
Protein	2.560	31.574	0.081	0.935	
Phosphorus	- 2260.290	1903.530	-1.187	0.244	
Cyanogenic glycosides	- 516.057*	245.832	-2.099	0.004	

Based on the complex trait study was obtained regression equation (1) indicated the impact of each trait on the variation of chemical content:

$$Y = 10734.5 - 47.765*X1 + 2.560*X2 -2260.290*X3 - -516.057*X4 \quad (1)$$

where *Y* - was *Aphis fabae* number; *X1* - height; *X2* - protein; *X3* – phosphorus; *X4* - cyanogenic glycosides

Results showed that on black bean aphid infestation, the highest negative significant influence had cyanogenic glycosides (r = -516.1) followed by height (r = -47.8). Protein content had a positive influence (r = 2.6), while phosphorus - was negative but the effect on aphid attack was not significant.

4 CONCLUSIONS

Canonical correspondence analysis showed that the aphid number was negatively correlated with the amount of rainfall and humidity until *A. fabae* was positively correlated with Tmin and Tmax.

According to GGE biplot analysis cultivar Fb 3270, followed by BGE 029055 and BGE 002106 were stable with a low density of aphids and were defined as tolerant.

A significant negative correlation was found between aphid number and plant height (r = -0.447). Among biochemical constituents, crude protein content showed a significant positive correlation (r = 0,686) while phosphorus and cyanogenic glycosides concentration were significantly negatively correlated with the incidence of black bean aphid (r = -0.411, r = -0.685, respectively).

Cultivars with high phosphorus and cyanogenic glycosides concentration and low crude protein content can be included in future breeding programmes to improve resistance to *A. fabae*.

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