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Sensitivity and qualitative changes in faba bean accessions influenced by black bean aphid

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Abstract

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The identification of low-sensitive accessions to aphid species has become an important subject of research and their use may increase plant production and reduce environmental pollution and control costs. Therefore, the present study aimed to present faba bean accessions that were less preferred by *Aphis fabae* Scopoli (Hemiptera, Aphididae) and to assess the qualitative plant changes affected by aphids. The field study was carried out at the Institute of Forage Crops (Pleven) from 2016 to 2018 with 12 *Vicia faba* L. accessions, and aphid infestation was assessed by recording the aphid number per plant at the pod formation stage. It was found the faba bean accession Fb 3270, followed by BGE 029055 had the lowest density of black bean aphids and were defined as very low sensitivity. The trend was similar for medium-sensitive BGE 002106. The Fb 3270, BGE 002106 and BGE 029055 accessions had significantly lower nitrogen concentration and plastid pigments, while crude fibre content was considerably higher. *Aphis fabae* infestation significantly changed the chemical content of plants leading to a reduction of the nitrogen, crude fibre, and plastid pigments in response to aphid injury. The reduction in nitrogen (6.9 and 8.6%) and fibre content (8.0 and 8.5%), as well as plastid pigments (9.7 and 13.1%), was the least pronounced in very low sensitive accessions (Fb 3270 and BGE 029055, respectively). Accessions having a low aphid preference and minor quality changes can be included in future breeding programmes to improve resistance to *A. fabae*.

Keywords: Aphis fabae; *Vicia faba* accessions; sensitivity; chemical change

Introduction

Among insects, aphids are important pests in the agricultural products of the world. Because many of them have become resistant to synthetic insecticides, many other control methods have been used against them (Abramson et al., 2006).

The black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae), is one of the most important pests of different cultivated crops throughout the world. The black bean aphid has a very wide host range. It has been recorded on more than 200 host plant species in the world, and many plant species are sensitive to attack by this aphid (CAB International, 2000). Large colonies of *A. fabae* may be very damaging to legumes, and cause direct damage by phloem-feeding, resulting in significant impairment of plant growth and reducing the quantity and quality of the yield (Shannag & Ababneh, 2007; Cahon et al., 2018; Du et al., 2021; Fuentes et al., 2021), leaf deformation and arrested development. Aphids are a vector of about 30 plant viruses. Host plants are damaged either directly by aphid feeding or indirectly by the transmission of viruses and excretion of honeydew (Neeraj et al., 1999).

In general, an economical and persistent method to overcome pests' attacks is the use of resistant/tolerant varieties. This method is the most useful technique in integrated pest management (IPM). Using persistent accessions is an effective method to control insect pests in different crops. The term antixenosis is representative of the morphological and biochemistry of plants that have an unfavourable effect on insects' behaviour and that insect uses another host plant (Nouri-Ghanbalani et al., 1995). It also influences oviposition behaviour and the placement of insects. This behaviour is the consequence of the morphological, biophysical, biochemistry and structural quality of the host plant (Panda & Khush, 1995). Many authors reviewed varietal resistance as a substantive and indubitable method for aphid control. They studied the effects of chemical variation in plants on their sensitivity to aphids in terms of host selection, plant water relations, the importance of the quality of food available to the aphids, and particularly of the soluble organic nitrogen content in the sieve-tube sap (Esmaeili-Vardanjani et al., 2013; Josefina et al., 2017).

Therefore, the present study aimed to present faba bean accessions that were less preferred by *A. fabae* and to assess the qualitative plant changes affected by aphids.

Material and Methods

The field experiment was performed at the Institute of Forage Crops (Pleven) from 2016 to 2018. Twelve accessions of faba bean (*Vicia faba* L.), originating in 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 to assess their sensitivity to *Aphis fabae* and qualitative plant changes affected by aphids. The experiment was laid out in Randomized Block Design (RBD) with three replications and an experimental plot of 4 m^2 and a total area of 155 m^2. The accessions were planted with a sowing rate of 30 seeds m^2 and kept devoid of insecticide application throughout the experimentation. Aphid infestation occurred naturally. The reaction of different accessions to *A. fabae* was assessed by recording the aphid number per plant at the pod formation stage of the faba bean. Therefore, twenty plants were selected randomly from each replication of the cultivar. The average number of aphids was calculated based on recorded aphids every 2 to 3 days in pod formation. The plant reaction was assessed using the grades described by Teotia & Lal (1970) (Table 1).

Table 1. Sensitivity grades to *Aphis fabae* Scopoli

Grade	No. of Aphids per Plant	Sensitivity
0	0	No aphid
1	20	Insignificant
2	> 20-100	Very low
3	>101-200	Low
4	>201-350	Medium
5	>351-500	High
6	>501	Very high

Accessions were cultivated in parallel under standard technological practices for managing the faba bean pests. Keeping plants without aphid infestation was carried out by triple treatment with alternating insecticides with active substances alfa-cypermethrin 150 g/l and deltamethrin 25 g/l.

The chemical changes of the aboveground mass in the aphid-infested and uninfested accessions were determined by standard methods of the Weende system (AOAC, 2001), including nitrogen and crude fibre by the Kjeldahl method. In addition, in fresh plant samples, plastid pigments content (chlorophyll a, chlorophyll b, carotenoids and total) (mg/100 g fresh matter) was determined according to Zelenskii & Mogileva (1980).

The data was presented on average for the period 2016-2018. The statistical processing of experimental data was conducted using the Statgraphics Plus software program and was processed by analysis of variance (ANOVA) from a one-factor field trial.

Results and Discussion

The most indicative stage of the black bean aphid preference was pod formation. Bursts of asexual reproduction and live births on faba bean allowed large populations to build up quickly on plants which resulted in an average of 781.0 winged and wingless individuals/plant. The lowest density was found in Fb 3270, followed by BGE 029055 ($F_{11.5}$ = 44.900; p < 0.007) (Figure 1). According to sensitivity grades, the aphid number did not exceed 100 and accessions were defined as very low sensitive, ie. sustainable. Less preferred and numbers not exceeding 350 aphids were found in BGE 002106 and BGE041470 with negligible differences between them, followed by BGE 032012. That defined them as medium sensitive. Numerous colonies and an abundance of *A. fabae* were observed in BGE 046721, which had significantly the highest aphid density, followed by BGE 043776. Accessions were the most preferred and the aphid number was many times higher than the value of 500, which categorically defined them as very highly sensitive. Despite significant differences between them and Fb 2486 and Fb 1929, the last ones also belonged to the group of highly sensitive accessions. Other accessions were highly sensitive.

The results of the chemical composition and qualitative changes in the accessions, which occurred as a result of the aphid infestation and their nutritional activity, are shown in Figures 2–5.

The nitrogen content varied in a relatively wide range in the treated plants, uninfested by aphids(controls), from 29.8 to 37.6 g kg^{-1} dry matter (Figure 2). It was found that a significantly lower nitrogen concentration had the low-sensitive

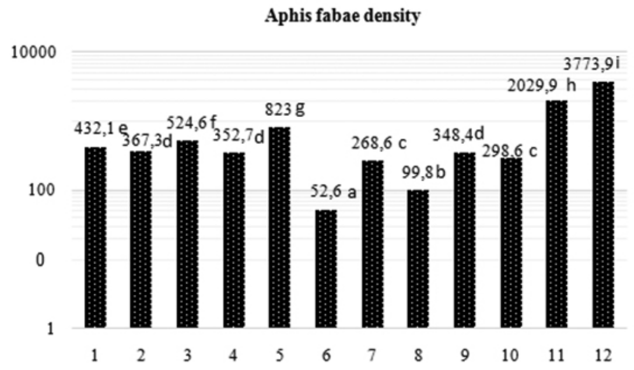
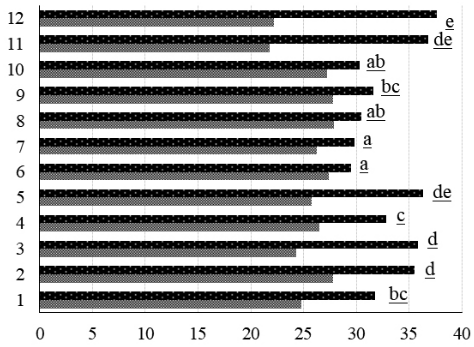


Fig. 1. ***Aphis fabae*** **Scopoli density in** ***Vicia faba*** **varieties**
Legend: The varieties are designated with the number from 1 to 12, as follows 1- Fb 1896, 2- Fb 1903, 3- Fb 1929, 4- Fb 2481, 5- Fb 2486, 6- Fb 3270, 7- BGE 002106, 8- BGE 029055, 9- BGE 032012, 10- BGE 041470, 11- BGE 043776, 12- BGE 046721

accessions Fb 3270 and BGE 002106, as differences compared to BGE 029055 and BGE 041470 were statistically insignificant ($F_{11.5} = 1.401$; $p < 0.034$). The main part of the studied cultivars was characterized by a considerably higher nitrogen content, as the sensitive cultivars Fb 1903, Fb 1929, Fb 2486, BGE 043776 and BGE 046721 were distinguished significantly by the highest nitrogen values than the others.



	1	2	3	4	5	6	7	8	9	10	11	12
■ Uninfested by A. fabae	31,8	35,5	35,8	32,8	36,3	29,5	29,8	30,5	31,6	30,3	36,8	37,6
■ Infested by A. fabae	24,8	27,8	24,3	26,5	25,8	27,4	26,2	27,8	27,8	27,2	21,8	22,2

Fig. 2. Nitrogen content (dry mass, g kg^{-1} DM) in dry mass ***Vicia faba*** **varieties**
Legend: The varieties are designated with the number from 1 to 12, as follows 1- Fb 1896, 2- Fb 1903, 3- Fb 1929, 4- Fb 2481, 5- Fb 2486, 6- Fb 3270, 7- BGE 002106, 8- BGE 029055, 9- BGE 032012, 10- BGE 041470, 11- BGE 043776, 12- BGE 046721

Nitrogen is necessary for many physiological processes of the plant and usually is considered the most important for aphid survival. As *A. fabae* is ingesting only soluble nitrogen sources from plant phloem, the aphid effect resulted in considerable nitrogen reduction up to 41.0% with significant differences compared to control plants (according to the figure numbers: 1: $F_{1,2} = 4.132$; $p < 0.008$; 2: $F_{1,2} = 0.608$; $p < 0.001$; 3: $F_{1,2} = 1.076$; $p < 0.012$; 4: $F_{1,2} = 0.608$; $p < 0.001$; 5: $F_{1,2} = 0.776$; $p < 0.022$; 9: $F_{1,2} = 2.376$; $p < 0.022$; 10: $F_{1,2} = 1.254$; $p < 0.038$; 11: $F_{1,2} = 0.962$; $p < 0.012$; 12: $F_{1,2} = 0.776$; $p < 0.008$). That decrease was usually proportional to the infestation levels of faba bean accessions and was the most pronounced in high and very high sensitive BGE 046721, BGE 043776 and Fb 1929 (40.1; 40.7 and 32.0% respectively). The nitrogen reduction disrupted not only the physiological mechanisms of the plant but also reduced the amount and nutritional value of the leaves.

A minimal change was found only in very low sensitive accessions Fb 3270, BGE 002106 and BGE 029055 where the nitrogen content decreased in the low range from 6.9 to 12.0% (6: $F_{1,2} = 3.290$; $p < 0.016$; 7: $F_{1,2} = 4.138$; $p < 0.001$; 8: $F_{1,2} = 2.886$; $p < 0.052$).

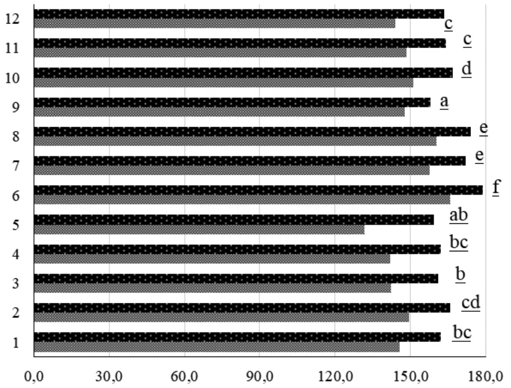
Obversely, as a result of the colonization and active nutritional activity of aphids in the sensitive accessions, nitrogen levels significantly decreased and losses were high varying from 21.6 to 41.2%. Results showed that black bean aphids preferred to settle and colonize on nitrogen-rich plants, while lower nitrogen levels were associated with weak preference and considerably fewer aphids.

A strong positive correlation was found between the aphid number and the nitrogen concentration ($r = 0.707$).

Plant–nitrogen–sucking insect interactions are the subject of much investigation, especially concerning aphid nutrition. The nitrogen preference of aphids observed in that study was consistent with those reported in several previous experiments. Mohamed & Siman (2001) studied different cultivars/accessions of broad bean for their resistance against *Aphis craccivora* and suggested that high sensitive/heavy infestation of the plant was possibly based on its higher nitrogen and protein content in plant leaves and stems. Comadira et al. (2015) studied the complex relationship between plant nitrogen and aphid infestation 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 nitrogen in the colonization choice of aphids on faba bean plants.

The trend was opposite completely in the case of crude fibre, where control plants with a high content were characterized by a low number of black bean aphids. The low sensitive accession Fb 3270 had significantly the highest fibre content, followed by BGE 002106 and BGE 029055 ($F_{11.5} = 2.872$; $p < 0.029$) (Figure 3). Some differences were observed between the other variants, but the values were considerably lower and did not exceed 167 g kg^{-1} fibre content. The losses of crude fibre in medium and highly sensitive accessions as a result of the feeding attack of *A. fabae* were statistically significant in compared to control plants (1: $F_{1,2}$ = 8.205; p < 0.030; 2: $F_{1,2}$ = 8.143; p < 0.012; 3: $F_{1,2}$ = 10.056; p < 0.001; 4: $F_{1,2}$ = 11.830; p < 0.025; 5: $F_{1,2}$ = 19.407; p < 0.043; 7: $F_{1,2}$ = 10.228; p < 0.047; 9: $F_{1,2}$ = 1.900; p < 0.001; 10: $F_{1,2}$ = 7.306; p < 0.015; 11: $F_{1,2}$ = 7.066; p < 0.033; 12: $F_{1,2}$ = 11.118; p < 0.017).



	1	2	3	4	5	6	7	8	9	10	11	12
Uninfes.by A. fabae	162,0	166,0	161,0	162,0	159,4	179,0	172,0	174,0	157,9	166,9	164,0	163,4
Infested by A. fabae	145,6	149,5	142,2	141,8	131,6	165,7	157,5	160,4	147,6	151,2	148,6	143,9

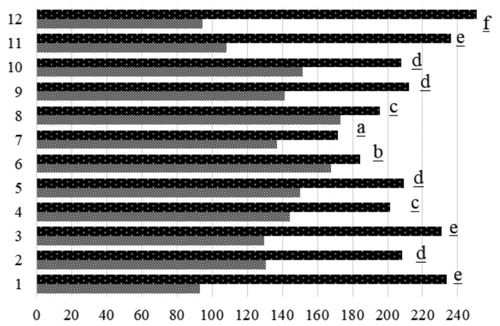
Fig. 3. Crude fibre content (dry mass, g kg^{-1} DM) in dry mass *Vicia faba* varieties

Legend: The varieties are designated with the number from 1 to 12, as follows 1- Fb 1896, 2- Fb 1903, 3- Fb 1929, 4- Fb 2481, 5- Fb 2486, 6- Fb 3270, 7- BGE 002106, 8- BGE 029055, 9- BGE 032012, 10- BGE 041470, 11- BGE 043776, 12- BGE 046721

The decrease varied in a relatively narrow range from 9.2 to 13.6%, except Fb 2486, where it reached 21.1%. A higher reduction was found in Fb 1929, Fb 2481, Fb 2486 and BGE 046721, while for very weakly sensitive Fb 3270 (8.0%) and BGE 029055 (8.5%) it was insignificant (6 $F_{1,2}$ = 14.902; p < 0.011; 8 $F_{1,2}$ = 15.412; p < 0.027). The reduction in fibre content was considerably less pronounced than that of nitrogen and did not lead to high essential qualitative changes in the chemical composition of damaged plants. In addition, a low negative correlation was found between aphid numbers and fibre content (r = -0.280).

The effect of aphids on the photosynthetic process was traced by determining the content of plastid pigments in the faba bean accession leaves. It is known that aphid feeding causes considerable losses in the plastid pigment content, an important nitrogen source for these insect pests (Anjali et al., 2017; Mawar & Tambe, 2018). The chlorophyll content and carotenoids are one of the most important parameters in the relationships between plants and pests. Changes in their content in the leaf tissue are an important indicator of chloroplast developmental disturbances and photosynthetic disturbance in plants (Sytykiewicz et al., 2013).

The analysis results of the plastid pigments in the leaves (Figure 4) showed that significant differences were found in the control plants. Statistically, BGE 046721 stood out with the highest value of "chlorophyll a + b + carotenoids", followed by BGE 043776, Fb 1896 and Fb 1929 ($F_{11,5}$ = 6.304; *p* < 0.011). Accessions were highly sensitive, characterized by a high number of *A. fabae*. The low-sensitive Fb 3270, BGE 002106 and BGE 029055 contained significantly lower plastid levels.



	1	2	3	4	5	6	7	8	9	10	11	12
Uninfested by A. fabae	233,8	208,2	230,8	201,4	209,1	184,3	171,4	195,7	212,1	207,8	236,0	251,7
Infested by A. fabae	92,9	130,5	129,6	144,5	150,3	168,0	136,8	173,0	141,3	151,6	108,2	94,4

Fig. 4. Plastid pigments content in fresh mass in *Vicia faba* varieties

Legend: The varieties are designated with the number from 1 to 12, as follows 1- Fb 1896, 2- Fb 1903, 3- Fb 1929, 4- Fb 2481, 5- Fb 2486, 6- Fb 3270, 7- BGE 002106, 8- BGE 029055, 9- BGE 032012, 10- BGE 041470, 11- BGE 043776, 12- BGE 046721

The correlation between the aphid density and the content of plastid pigments was calculated given the significant differences in the positions of the uninfected accessions. *Aphis fabae* preferred richer chlorophyll and carotenoid plants, and a significant strong positive correlation was found r = +0.722.

Similar results were reported by Chaudhari et al. (2013), according to which (2013) resistant alfalfa accessions against *Therioaphis maculata* (Buckton) had a lower total chlorophyll, crude protein, sugar and magnesium contents.

As a result of the attack and nutritional activity of aphids, a significant reduction in plastid pigment content was found in almost all cultivars (according to Figure 4 numbers: (1: $F_{1,2}$ = 26.894; p < 0.047; 2: $F_{1,2}$ = 31.204; p < 0.001; 3: $F_{1,2}$ = 26.181; p < 0.031; 4: $F_{1,2}$ = 27.208; p < 0.033; 5: $F_{1,2}$ = 21.391; p < 0.001; 7: $F_{1,2}$ = 30.280 ; p < 0.015; 9: $F_{1,2}$ = 29.284; p < 0.036; 10: $F_{1,2}$ = 33.248; p < 0.040; 11: $F_{1,2}$ = 37.177; p < 0.023; 12: $F_{1,2}$ = 32.886; p < 0.042). The reduction corresponded to the infestation grade and it was most pronounced in very high and highly sensitive cultivars. Only in Fb 3270 and BGE 029055 plastid pigments were not affected by black bean aphid as the decrease was insignificant by 9.7 and 13.1% ($F_{1,9} = 28.256$; $p < 0.028$; $F_{1,9} = 23.795$; $p < 0.036$, respectively). The pigment loss in BGE 002106 was 25.3%. The reduction of the total content of plastid pigments due to the bean aphid attack in the other accessions varied in a wide range from 37.1 to 166.6%, especially in Fb 1896, BGE 043776 and 12- BGE 046721 (118.2 – 166.6%).

Similar results were reported in previous studies. Mawar & Tambe (2018) studied qualitative losses caused by aphids in lucerne and found a drastic reduction in chlorophyll, dry matter, crude protein and fibre. Anjali et al. (2017) quantitatively measured the carotenoid content in sorghum and found significant losses ranging between 10 and 40% in aphid-infested plants. The authors reported a strong reduction in total chlorophyll content and moisture in the plant leaves.

The use of resistant cultivars to aphids has become a major research purpose. The application of accessions in the practice may increase plant productivity, reduce environmental pollution and control costs.

Conclusions

The faba bean accession Fb 3270, followed by BGE 029055 had the lowest density of black bean aphids. They were defined as very low sensitive, i.e. sustainable to aphid infestation. The trend was similar for medium-sensitive BGE 002106.

The Fb 3270, BGE 002106 and BGE 029055 accessions had significantly lower nitrogen concentration and plastid pigments, while crude fibre content was considerably higher.

Aphis fabae Scopoli infestation significantly changed the chemical content of *Vicia faba* L. accessions leading to a reduction of the nitrogen, crude fibre, and plastid pigments in response to aphid injury.

The reduction in nitrogen (6.9 and 8.6%) and fibre content (8.0 and 8.5%), as well as plastid pigments (9.7 and 13.1%), was the least pronounced in very low sensitive accessions (Fb 3270 and BGE 029055, respectively).

Accessions having a low aphid preference and minor quality changes can be included in future breeding programmes to improve resistance to *Aphis fabae* Scopoli.

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