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SENSITIVITY OF FABA BEAN (*Vicia faba* L.) CULTIVARS TO *Aphis fabae* Scopoli INFESTATION AND PLANT PARAMETERS RESPONSIBLE FOR LOW SUSCEPTIBILITY TO THE PEST

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Abstract

The most economically significant pest of V*icia faba* L. beans is the bean aphid *Aphis fabae* Scopoli (*Hemiptera*, *Homoptera*: *Aphididae*). The use of varieties resistant to various aphid species can increase the production of this crop, reduce environmental pollution and the cost of monitoring the condition of crops. It is known about the relationship between the degree of damage by aphids and the morphological features of the plant, however, information on chemical changes during damage by aphids and the role of chemical factors in sensitivity to A. fabae is ambiguous. In the present work, for 12 varieties of *V. faba* from the collection of the Institute of Forage Crops (Pleven), it was shown for the first time that the *A. fabae* infestation led to a decrease in plant height, crude protein, phosphorus, and chlorophyll a + b while the amount of cyanogenic glycosides increased significantly. Therefore, the aim of the present study was to assess the sensitivity of faba bean cultivars to *Aphis fabae* and to define the morphological and chemical parameters responsible for low aphid susceptibility. The field study was carried out at the Institute of Forage Crops (Pleven, 2016-2018) in the experiment laid out in Randomized Block Design (RBD). The infestation was assessed by recording the number of aphids per plant at the stages of budding, flowering and bean formation (n = 20, N = 3), resistance or susceptibility of plants was classified using a 0-12-point scale. The chemical composition (the content of crude protein, phosphorus, chlorophyll a, chlorophyll b, cyanogenic glycosides) was determined by standard methods (Weende system analysis). It was found that aphids reached the highest abundance at the stage of pod formation. Cultivars Fb 3270 and BGE 029055 were defined as very low susceptible to aphids, while BGE 002106, BGE 032012 and BGE 041470 were medium susceptible. Aphid infestation significantly affected the morphological and chemical traits of cultivars and led to a reduction of the plant height, crude protein, phosphorus and chlorophyll a + b content, while cyanogenic glycosides significantly increased in response to aphid injury. The extent of the decrease in chemical parameters and plant height depends on the aphid abundance, being significantly higher in very high susceptible and high susceptible cultivars. On the contrary, cyanogenic glycosides increase with an increase in the aphid abundance. Thereof, the levels of crude protein, phosphorus and cyanogenic glycosides can serve as key factors indicative of the aphid preference. Cultivars Fb 3270 and BGE 029055 with higher phosphorus and cyanogenic glycosides, lower crude protein, and shorter plants had significantly lower aphid infestations. Therefore, these varieties are significantly less susceptible to *A. fabae* than other varieties and can be involved in breeding programs to improve plant resistance to *A. fabae*.

Keywords: *Aphis fabae*, feed preferences, faba bean cultivars, susceptibility, morphological traits, chemical traits

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

The most important common bean pest worldwide is the black bean aphid, *Aphis fabae* Scopoli (*Hemiptera*, *Homoptera*: *Aphididae*), which causes considerable damage to plants and yield loss reaches 37% [1]. Aphids frequently grow and develop rapidly, allowing aphid populations to fastly exceed economical threshold levels. Numerous colonies of *A. fabae* is very damaging to *V. fabae* because of the direct negative impact on the plant growth and the quantity and quality of the yield [2]. Injury caused by many aphid species is well documented to change the rate of photosynthesis, plant growth and physiological and chemical processes [3-5]. For example, H.K. Shannag [6] found the negative effects of aphid feeding by reduction of crude protein levels of damaged leaves in 14-day-infested plants before signs appeared. On the other hand, P. Mawaand and A.B. Tambe [7] studied qualitative losses caused by aphids in lucerne and found a drastic reduction in chlorophyll, dry matter, crude protein and fibre, phosphorous, potassium, ash and calcium.

The control of aphids currently depends primarily on chemical insecticides. However, insecticides have a negative effect on useful insects and the environment. Thus, there is a need for effective, environmentally friendly alternatives to suppress crop pests. Identification of cultivars resistant to different aphid species has become an important subject of research [8-10]. The use of these cultivars may increase faba bean production affected by aphids and reduce environmental pollution and control costs.

Many authors reported that the application of resistant cultivars is a substantive and indubitable method to control aphids [11, 12]. B. Béji et al. [2] studied faba bean resistance to *A. fabae* and found that the best parameters describing resistance were pod weight and grain number. F. Meradsi and M. Laamari [13] evaluated the resistance response of *V. faba* to black bean aphid by the relationship between the resistance level and plant morphological characteristics. It was reported [8, 14} that there is the relative impact of cultivars' resistance to black bean aphids, based on antibiosis and antixenosis. However, there was less information on the chemical changes that occur after plant damage by aphids and on the role of various chemical factors in the susceptibility of different species to *A. fabae* colonization [15-17].

An integrated approach seems to be the most informative to reveal traits associated with the resistance to aphids. However, there was less information about the possible morphological and chemical parameters in *V. faba* cultivars responsible for resistance to black bean aphid and changes occurring after damage.

Here, for the first time, it was shown that *V. faba* plant height, the content of crude protein, phosphorus and chlorophylls a + b decreased while the amount of cyanogenic glycosides increased significantly in response to damage by aphids *A. fabae*. Among 12 varieties of *V. faba* from the collection of the Institute of Forage Crops (Pleven), the varieties have been identified that are promising in breeding for resistance of faba beans to *A. fabae*. The amount of protein, phosphorus, and cyanogenic glycosides in *V. faba* plants has been proposed as key indicators of plant attractiveness and aphid preference for such plants.

Therefore, the aim of the present study was to assess the sensitivity of faba bean cultivars to *Aphis fabae* and define the parameters responsible for low aphid susceptibility.

Materials and methods. The field study was carried out at the Institute of Forage Crops (Pleven) during the period 2016-2018. Twelve cultivars 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. The experiment was laid out in Randomized Block Design (RBD) with three replications and a 4 m^2 experimental plots. 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. Keeping plants without aphid infestation was carried out by triple treatment with alternating insecticides with active substances alfa-cypermethrin 150 g/l and deltamethrin 25g/l (20 ml/ha or 0.02 ml/m^2 for both preparations during budding, flowering and pod formation). The reaction of different cultivars to *A. fabae* was assessed by recording the aphid number per plant at 50% budding, 50% flowering and 50% pod formation stages 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.. Plant response was assessed on a point scale [18], where 0 is resistance (0 aphids per plant), 1 is slight sensitivity (20 aphids per plant), 2 is very low sensitivity (> 20-100 aphids per plant), 3 is low sensitivity (> 101-200 aphids per plant), 4 is medium sensitivity (> 201-350 aphids per plant), 5 is high sensitivity (> 351-500 aphids per plant), 6 is very high sensitivity (> 501 aphids per plant).

In order to determine the chemical changes of the aboveground mass of cultivars in the aphid infestation, 15 plant samples taken of each cultivar (n = 5, N = 3) were fixed for 15 min at 100 °C and dried to a constant weight at a 60 °C in a thermostat. The chemical composition was determined by standard methods of the Weende system [19] and includes crude protein (CP) by Kjeldahl method (CP = total N × 6.25), crude fibre (CF), phosphorus colourimetrically by hydroquinone method, calcium complexometrically [20]. In addition, in fresh plant samples, plastid pigments content (chlorophyll a, chlorophyll b, carotenoids, and total) (mg/100 g dry matter)) was determined according to M.I. Zelenskii and G.A. Mogileva [21] as well as the cyanogenic glycosides contents - according to A.I. Ermakov et al. [22]. The content of phosphorus, chlorophyll a, chlorophyll b was measured spectrophotometrically (a Spekol 11 spectrophotometer, Carl-Zeiss, Germany), the content of crude protein was measured using a KELDAL apparatus (model UDK-127, VELP Scientifica Srl, Italy). Chemical compounds in infested and uninfested cultivars were determined in the bedding of the pod formation.

The data were subjected to one-way ANOVA, the means (M) with standard radiation (±SD) were calculated, the means were compared by Tukey's test ($p \leq 0.05$). The Correlation Analysis was performed using Microsoft Office Excel 2007. Multiple Regression Analysis of Statgraphics Plus (1995) for Windows Ver. 2.1 Software program was used.

Results. Aphids appeared in the formation of the first buds, and at the plant development during the budding stage, their number increased proportionally. Despite the low density in that stage, aphids preferred certain cultivars. BGE 046721 had significantly the highest aphid number, followed by BGE 043776 and Fb 1903 ($F_{11.5}$ = 12.224; $p < 0.001$) (Table 1). The differences in the infestation in the other cultivars were mostly inconsiderable, but Fb 3270, Fb 1896, BGE 002106 and BGE 032012 were less preferred. The last ones stood out by a small aphid number, varying in the narrow range of 14.6-16.6 number of winged and wingless individuals/plant. The average population density of the species during budding was low (50 aphids per plant). According to the susceptibility grades, there was a stronger aphid preference for BGE 046721 and BGE 043776 as early as the early stage.

At the beginning of the flowering stage, there was a considerable increase in the number, which reached an average of 197.2 aphid number in the 50% flowering. The number was three times higher than in the earlier stage of the plant development. Fb 3270 had the lowest value, followed by BGE 029055 and the difference was significant ($F_{11.5}$ = 21.922; $p < 0.001$). A lower number was also found in BGE 002106 and Fb 2486 with minimal differences between them, followed by Fb 1903 and BGE 032012. The differences between the last three cultivars were insignificant.

1. *Aphis fabae* Scopoli number (number of winged and wingless individuals per plant) **in faba bean (*Vicia faba* L.) cultivars of different origine by plant development stages** (n = 20, N = 3, M±SD, Randomized Block Design, a 4 m^2 experimental plot, the Institute of Forage Crops, Pleven, Bulgaria, 2016-2018)

Cultivars	Budding stage		Flowering stage		Pod formation		Susceptability estimates	Susceptability group
Fb 1896	14.8±1.93	a[1]/a[2]	125.3±27.75	f/b	432.1±17.07	e/c	$F_{2.5}$ = 47.840; p < 0.019	High
Fb 1903	49.0±3.46	d/a	99.2±22.40	de/b	367.3±19.81	d/c	$F_{2.5}$ = 18.706; p < 0.033	High
Fb 1929	33.8±4.83	c/a	148.9±23.70	g/b	524.6±15.89	f/c	$F_{2.5}$ = 9.798; p < 0.028	Very high
Fb 2481	26.2±2.64	abc/a	114.4±24.50	ef/b	352.7±16.57	d/c	$F_{2.5}$ = 9.117; p < 0.048	High
Fb 2486	20.4±3.40	ab/a	89.5±25.70	cd/b	823.0±20.64	g/c	$F_{2.5}$ = 24.531; p < 0.040	Very high
Fb 3270	14.6±2.16	a/a	34.9±17.54	a/b	52.6±15.12	a/c	$F_{2.5}$ = 6.578; p < 0.012	Very Low
BGE 002106	16.1±1.59	a/a	70.2±16.63	bc/b	268.6±18.19	c/c	$F_{2.5}$ = 12.152; p < 0.035	Medium
BGE 029055	25.0±3.79	abc/a	59.5±17.13	b/b	99.8±19.91	b/c	$F_{2.5}$ = 14.649; p < 0.044	Very Low
BGE 032012	16.6±2.52	a/a	93.0±23.90	de/b	348.4±24.13	d/c	$F_{2.5}$ = 39.732; p < 0.006	Medium
BGE041470	31.0±3.01	bc/a	135.5±18.41	fg/b	298.6±20.55	c/c	$F_{2.5}$ = 24.635; p < 0.005	Medium
BGE 043776	ax	e/a	514.6±23.30	h/b	2029.9±25.09	h/c	$F_{2.5}$ = 33.671; p < 0.029	Very high
BGE 046721	235.0±8.26	f/a	881.1±29.98	i/b	3773.9±22.23	i/c	$F_{2.5}$ = 87.942; p < 0.031	Very high
Average	50.0		197.2		781.0			

N o t e. In columns, values before the slash ([1]) and marked with the same letters have no statistically significant differences at p < 0.05. In a row, values after the slash ([2]) and marked with the same letters have no statistically significant differences at p < 0.05.

2. Height of plants colonized and not colonized by *Aphis fabae* Scopoli in faba bean (*Vicia faba* L.) cultivars of different origine by plant development stages (n = 20, N = 3, M±SD, Randomized Block Design, a 4 m^2 experimental plot, the Institute of Forage Crops, Pleven, Bulgaria, 2016-2018), 2016-2018)

Cultivars	Budding stage				Flowering stage				Pod formation			
	colonized		not colonized		colonized		not colonized		colonized		not colonized	
1	23.4±2.79	ab[1]/a[2]	31.1±2.88	abcd/b	41.7±9.04	ab/a	67.7±4.85	bcd/b	69.8±7.45	b/a	91.5±6.94	ef/b
2	31.2±4.18	cde/a	37.2±4.66	ef/b	73.2±10.45	f/a	86.2±10.17	g/b	79.3±10.16	cd/a	101.1±9.75	g/b
3	20.0±6.19	a/a	26.7±5.12	a/b	50.9±6.64	c/a	64.2±4.49	abc/b	58.4±6.45	a/a	77.9±6.15	ab/b
4	27.1±9.01	bc/a	33.7±4.69	cde/b	69.8±6.62	ef/a	77.9±6.15	ef/b	73.5±4.19	bc/a	83.8±9.42	bcd/b
5	19.6±8.44	a/a	28.1±10.73	ab/b	47.9±10.24	bc/a	60.8±9.69	ab/b	56.8±3.00	a/a	80.4±5.39	abc/b
6	32.8±6.99	de/a	39.2±6.74	f/a	65.8±6.33	de/a	72.1±6.06	de/a	84.9±3.11	de/a	86.4±5.60	cde/a
7	24.5±7.50	ab/a	29.7±7.73	abc/a	41.4±10.71	**a/a**	58.9±7.62	a/b	60.0±8.55	a/a	74.5±11.18	a/b
8	33.7±5.76	e/a	37.7±3.97	ef/a	74.0±8.55	fg/a	75.5±10.78	e/a	84.0±5.45	de/a	87.9±4.72	de/a
9	27.7±2.75	bcd/a	31.1±6.50	abcd/b	62.4±3.41	d/a	70.9±8.72	cde/b	75.3±3.20	bc/a	85.3±8.43	cde/b
10	28.9±7.48	bcde/a	32.5±4.08	bcde/a	75.3±3.20	fg/a	85.3±8.43	fg/b	77.3±7.76	c/a	87.8±6.95	de/b
11	28.7±5.81	bcde/a	32.8±6.49	bcde/b	66.4±5.30	de/a	77.1±4.79	e/b	70.0±3.82	b/a	95.8±5.20	fg/b
12	31.8±6.29	cde/a	36.0±4.50	def/b	80.4±5.41	g/a	95.4±11.11	h/b	86.4±5.96	e/a	123.8±7.87	h/b
Average	27.5		33.0		62.4		74.3		73.0		89.7	

Note. 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. In columns, values before the slash ([1]) and marked with the same letters have no statistically significant differences at $p < 0.05$. In a row, values after the slash ([2]) and marked with the same letters have no statistically significant differences at $p < 0.05$.

The unifying trait between all these variants was that the aphid number did not exceed the value of 100. At that stage, they were classified as very low susceptible. Conversely, the most preferred cultivar significantly with the highest *A. fabae* number was BGE 046721, followed by BGE 043776 and the trend of the budding stage was fully preserved. It should be noted that the aphid infestation in BGE 046721 and BGE 043776 exceeded 350 winged and wingless individuals/plant and plants were defined as highly susceptible as early as the flowering stage. Other cultivars were defined as low susceptible.

The most indicative of the black bean aphid preference was the pod formation stage, where the number reached maximum values in the studied cultivars and exceeded on average three times and fifteen times the infestation during the flowering and budding stages, respectively. 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. Stable position and with the lowest density stood out Fb 3270, followed by BGE 029055 ($F_{11.5} = 44.900$; $p < 0.007$). According to susceptibility grades, the aphid number did not exceed 100 and cultivars were defined as very low susceptible, 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 susceptible. Numerous colonies and abundance of *A. fabae* were observed in BGE 046721, which had significantly the highest aphid density, followed by BGE 043776. Cultivars were the most preferred and aphid number was many times higher than the value of 500, which categorically defined them as very high susceptible. Despite significant differences between them and Fb 2486 and Fb 1929, the last ones also belonged to the group of high susceptible cultivars. Other cultivars were high susceptible.

The number of *A. fabae* showed a significant difference between the three stages of growth and plant development. The comparative analysis about the aphid number unequivocally showed that cultivars were the most strongly attacked during the pod formation, followed by the flowering and budding stages infestation.

A.J. Biddle and N.D. Cattlin [23] reported a similar result. According to the authors, colonies of aphids developed rapidly on the upper parts of *Vicia* beans during flowering and pod formation. At first, individual stems were infested, later, aphids spread to surrounding plants, developing into localized patches of the infested plants. *Aphis fabae* population increased rapidly at the flowering stage and reached the highest density on the developing pods. M.R.Amin et al. [24] studied the population dynamic, infestation, and harmful effects of aphids on several bean plant species and found that aphid abundance was on the leaves, flowers and pods in the pod formation stage. In addition, he reported that species with the shortest duration of the growth stages had the highest infestation [24]. M.S.A. Mamun et al. [25] found that usually aphid infestation consistently increase from the early development stage and reached the highest values at the pod formation. Then followed a trend to decrease.

On the other hand, S.A. Dwivedi et al. [26] explored mustard varieties' resistance against aphids and found that the highest aphid infestation index on the based of aphid number was at the full pod formation followed by the flowering stage. In opposite to the present study, M. Esmaeili-Vardanjania et al. [11] found that the maximum number of *A. fabae* in bean cultivars was observed at a two-leaf stage in comparison to the flowering stage and the differences between the various stages of growth were significant. According to the authors during increasing plant age, resistance to black bean aphid was increased and the aphid population in all cultivars at the flowering stage was declined.

The height of the aphid-infested plants in the budding stage varied in a relatively narrow range and occupied similar values. Only Fb 1929 and Fb 2486 were up to 20 cm high (Table 2) and the differences compared to the other cultivars were significantly to be lower ($F_{11.9} = 5.666$; $p < 0.017$) (except for Fb 1896 and BGE 002106). Significantly higher value was observed for BGE 029055 compared to Fb 1896, Fb 1929, Fb 2481, BGE 002106 and BGE 032012. The trend was similar in the treated plants, as the height in Fb 3270 was significantly higher, followed by BGE 029055, Fb 1903 and BGE 046721 (differences between the last three were minimal) ($F_{11.9} = 5.341$; $p < 0.011$). A comparative analysis between infected and non-infected plants showed that *A. fabae* had a primarily depressant effect on the growth, reducing the values significantly at eight cultivars despite the low aphid number in the budding stage (according to the Table 2 numbers: 1 — $F_{1.9} = 5.341$; $p < 0.011$; 2 — $F_{1.9} = 4.161$; $p < 0.027$; 3 — $F_{1.9} = 5.301$; $p < 0.036$; 4 — $F_{1.9} = 5.750$; $p < 0.046$; 5 — $F_{1.9} = 7.070$; $p < 0.031$; 9 — $F_{1.9}=3.269$; $p < 0.021$; 11 — $F_{1.9}=3.775$; $p < 0.035$; 12 — $F_{1.9} = 4.135$; $p < 0.044$). Only Fb 3270, BGE 002106, BGE 029055 and BGE041470 did not show statistically reduced values under the aphid activity (6 — $F_{1.9} = 6.453$; $p < 0.064$; 7 — $F_{1.9} = 7.158$; $p < 0.017$; 8 — $F_{1.9} = 4.646$; $p < 0.284$; 10 — $F_{1.9} = 5.600$; $p < 0.087$).

The plant height is a genetic trait and the ratio between the cultivars remained relatively constant. In the flowering stage, treated BGE 046721 plants had a significantly higher value, followed by Fb 1903 with a minor difference to BGE041470 ($F_{11.9} = 7.628$; $p < 0.005$). The trend was similar for untreated variants as BGE 046721 had significantly the highest height except for BGE 029055 and BGE041470 ($F_{11.9} = 6.728$; $p <0.004$).

At that stage, as a result of the higher aphid number and intensive nutritional activity, there were more pronounced differences in height and a significant decrease in ten of the studied cultivars (1 — $F_{1.9} = 6.819$; $p < 0.008$; 2 — $F_{1.9} = 9.691$; $p < 0.015$; 3 — $F_{1.9} = 5.326$; $p < 0.022$; 4 — $F_{1.9} = 6.010$; $p < 0.048$; 5 — $F_{1.9} = 9.371$; $p < 0.027$; 7 — $F_{1.9} = 8.738$; $p < 0.003$; 9 — $F_{1.9} = 6.222$; $p < 0.009$; 10 — $F_{1.9} = 5.992$; $p < 0.008$; 11 — $F_{1.9} = 4.744$; $p < 0.014$; 12 — $F_{1.9} = 10.660$; $p < 0.005$). The plant growth was decreased by an average of 21.7%. The low number of black bean aphids in Fb 3270 and BGE 029055 led to an insignificant reduction in the height of 9.6 and 2.0%, respectively ($F_{1.9} = 6.827$; $p < 0.019$; $F_{1.9} = 9.142$; $p < 0.005$).

In the pod formation, the plants reached their maximum height and the trend for the highest plants of BGE 046721 in the treated variants was definitely confirmed, followed by Fb 1903 and BGE 043776 with negligible differences between them ($F_{11.9} = 6.7065$; $p < 0.020$). The height trait was strongly influenced in aphid-infected plants as only the genetically highest cultivar (BGE 046721) retained a leading position but the second and third sites were occupied by resistant Fb 3270 and BGE 029055. The differences between the last three cultivars were minimal ($F_{11.9} = 6.345$; $p < 0.001$). Slightly preferred Fb 3270 and BGE 029055 by aphids during the reproductive stage developed under favourable conditions, ensuring normal growth and metabolic processes, which resulted in higher plants than others. On the based of the *A. fabae* abundance in the pod formation stage, the reduction in growth was the most pronounced as the height decreased by an average of 23.8%.

Particularly indicative change was the considerable reduction of height in Fb 1896, Fb 1929, BGE 043776, Fb 2486 and BGE 046721, reaching 31.1, 33.4, 36.9, 41.5 and 43.3%, respectively. The differences from the treated cultivars were significant (according to the table numbers 1 — $F_{1.9} = 8.339$; $p < 0.043$; 3 — $F_{1.9} = 8.059$; $p < 0.013$; 5 — $F_{1.9} = 3.827$; $p < 0.006$; 11 — $F_{1.9} = 3.934$; $p < 0.009$; 12 — $F_{1.9} = 6.562$; $p < 0.015$). Differences for other cultivars were also significant but the height decreased to a relatively lower degree (2 — $F_{1.9} = 10.361$; $p < 0.045$; 4 — $F_{1.9} = 6.854$; $p < 0.024$; 7 — $F_{1.9} = 9.355$; $p < 0.042$; 9 — $F_{1.9} = 5.992$; $p < 0.008$; 10 — $F_{1.9} = 6.924$; $p < 0.017$). Only the Fb 3270 and BGE 029055 heights were not affected by black bean aphid as the decrease was insignificant, by 1.8 and 4.6% ($F_{1.9} = 4.256$; $p < 0.028$; $F_{1.9} = 4.795$; $p < 0.036$, respectively).

Given the relative preservation of the positions of the uninfested cultivars in the measurement of height, the correlation between the aphid number and the height of the control plants was calculated. It was found that *A. fabae* prefered higher plants as in the budding stage the height had a weak positive effect on the number ($r = +0.244$, $p \leq 0.05$), while in the flowering and pod-forming stages a significant middle and strong positive correlation was found, $r = +0.606$ and $r = +0.803$; $p \leq 0.05$, respectively.

Black bean aphid had a highly significant depressant effect on plant growth during the three stages of development, preferring higher cultivars. An exception was found for very low susceptible plants at Fb 3270 and BGE 02905.

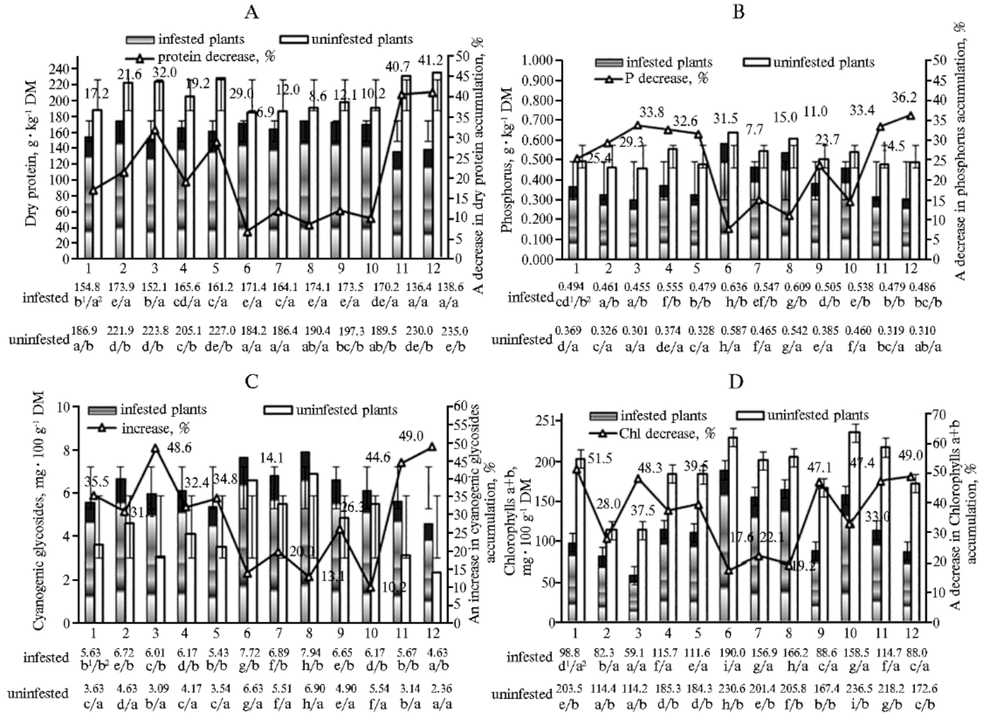
Similar results for a high reduction in plant height under aphid abundance were reported by other authors. According to M.R. Amin et al. [24], aphid infestation and damage had a negative effect not only on the height but also on the leave, flower, and pod number. Furthermore, A.S. Aldawood and A. Soffan [27] supplemented that a cultivar of *V. faba* with the lowest total numbers of aphids had the lowest plant height, while strongly infected cultivars had a higher height. I.A. Khan et al. [28] reported that aphid injury reduced the plant height as height losses were significantly higher (38.78%) in infested cultivars and lower in less infested ones (25.32%). Also, aphid damage delayed plant development and the correlation among aphids and plant height per cent loss was highly significant ($r = 0.75$) [28].

In contrast, F. Meradsi and M. Laamari [13] found that morphological characters as plant height did not affect *A. fabae* infestation, but resistant cultivars had a longer leaflet than highly susceptible cultivars. On the other hand, S. Lebbal [29] mentioned that bean resistant and highly susceptible cultivars had the same morphological characteristics.

The composition of available food in the host plant plays possibly the most important role in determining the relative resistance to aphids. 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, its effect on the crude protein resulted in considerable protein reduction up to 41.2% (Fig., A) with significant differences compared to uninfested plants (according to the figure numbers 1 — $F_{1.2} = 26.610$; $p < 0.008$; 2 — $F_{1.2} = 24.259$; $p < 0.001$; 3 — $F_{1.2} = 25.852$; $p < 0.012$; 4 — $F_{1.2} = 21.181$; $p < 0.001$; 5 — $F_{1.2} = 20.257$; $p < 0.022$; 9 — $F_{1.2} = 14.361$; $p < 0.022$; 10 — $F_{1.2} = 7.697$; $p < 0.038$; 11 — $F_{1.2} = 15.178$; $p < 0.012$; 12 — $F_{1.2} = 14.033$; $p < 0.008$). The decrease was usually proportional to the infestation levels of faba bean cultivars and crude protein and aphid numbers showed a medium significant positive correlation ($r = +0.696$, $p \leq 0.05$).

A minimal change was found only in very low susceptible cultivars Fb 3270, BGE 002106 and BGE 029055 where the protein content decreased in the low range from 6.9 to 12.0% (6 — $F_{1.2} = 20.252$; $p < 0.016$; 7 — $F_{1.2} = 25.254$;

$p < 0.001$; 8 — $F_{1.2} = 18.321$; $p < 0.052$).



Changes in the chemical composition of the colonized and not colonized plants in faba bean (*Vicia faba* L.) cultivars affected by *Aphis fabae* Scopoli: 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 — BGE041470, 11 — BGE 043776, 12 — BGE 046721 ($n = 5$, $N = 3$, $M \pm SD$, Randomized Block Design, a 4 m^2 experimental plot, pod formation stage; the Institute of Forage Crops, Pleven, Bulgaria, 2016-2018). In a row, values before the slash (^{1}) and marked with the same letters have no statistically significant differences at $p < 0.05$. In columns, values after the slash (^{2}) and marked with the same letters have no statistically significant differences at $p < 0.05$.

The sensitive varieties Fb 1903, Fb 1929, Fb 2486, BGE 043776 and BGE 046721 had statistically higher protein than the others ($F_{11,2} = 8{,}643$; $p < 0{,}031$). As a result of the colonization and active nutritional activity of aphids, protein levels significantly decreased and losses were high varying from 21.6 to 41.2% ($F_{11.2} = 5.104$; $p < 0.046$). Our results showed that black bean aphid preferred to settle and colonize on protein-rich plants, while lower protein levels were associated with weakly preference and considerably fewer aphids.

The protein preference of aphids observed in that study was consistent with those reported in several previous experiments. A.M. Mohamed and F.A.A. Siman [30] studied different cultivars/varieties of broad bean for their resistance against *Aphis craccivora* and suggested that high susceptible/heavy infestation of the plant was possibly based on its higher nitrogen and protein content in plant leaves and stems. C.J. Chaudhari et al. [31] reported that resistant lucerne varieties against *Therioaphis maculata* (Buckton) 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. G. Comadira et al. [32] studied the complex relationship between plant N 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 plant protein on the quantity and colonization choice of aphids on faba bean plants.

When determining the content of crude fiber, calcium, carotenoids and the total amount of pigments, no differences were found between plants infested and not infested with aphids. The content of these chemical components in plants infested and not infested with aphids was similar in value and did not affect the food preferences of the pest.

Phosphorus (P) is important for the formation of nucleic acids and phospholipids and is needed for the energy metabolism of photosynthesis [33]. In a comparative analysis concerning the P content in uninfested plants (see Fig., B) was found that Fb 3270 had significantly the highest content followed by BGE 029055 ($F_{11.2} = 0.015$; $p < 0.009$) and losses by aphids were low (7.7 and 11.0% respectively). The tendency for statistically higher P levels in the same plants after infection was reserved ($F_{11.2} = 0.013$; $p < 0.018$). In contrast, highly aphid preferred Fb 1929, Fb 2486, BGE 043776 and BGE 046721 had a significantly lower P content in both treated and untreated plants and losses were high varying from 31.5 to 36.2%. Despite the significant P reduction in all infested cultivars (1 — $F_{1.2} = 0.033$; $p < 0.005$; 2 — $F_{1.2} = 0.019$; $p < 0.012$; 3 — $F_{1.2} = 0.043$; $p < 0.001$; 4 — $F_{1.2} = 0.031$; $p < 0.020$; 5 — $F_{1.2} = 0.016$; $p < 0.001$; 6 — $F_{1.2} = 0.034$; $p < 0.007$; 7 — $F_{1.2} = 0.037$; $p < 0.011$; 8 — $F_{1.2} = 0.019$; $p < 0.017$; 9 — $F_{1.2} = 0.026$; $p < 0.026$; 10 — $F_{1.2} = 0.022$; $p < 0.001$; 11 — $F_{1.2} = 0.016$; $p < 0.031$; 12 — $F_{1.2} = 0.022$; $p < 0.015$), the high P content determined a markedly lower *A. fabae* number and low losses. It was found a negative significant correlation between the aphid abundance and the P content in plants ($r = -0.518$; $p \leq 0.05$).

There have been different hypotheses regarding the effects of concentrations of N and P in leaves on the preference of insect pests. For example, R.L. Vannette and M.D. Hunter [34] reported that the greater concentrations of N and P in leaves affected the attractiveness of plants to sap-sucking pests. On the other hand, H.A. Azouz et al. [35] 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) amount as well as with the level of infestation. S. Facknath and B. Lalljee [36] explained that phosphorus decreases the host suitability for 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.

Our results showed that *A. fabae* amount was considerably lower in cultivars with greater concentrations of P but lower content of crude protein in leaves which determined them as important indicators for the aphid preference.

Chemical traits such as cyanogenic glycosides determined the quality of food offered by the host plant and varied from one cultivar to another, with different effects on aphid population levels. Low aphid affected BGE 029055, followed by Fb 3270 had the highest concentration of cyanogenic glycosides in both uninfected ($F_{11.2} = 0.187$; $p < 0.024$) and infected ($F_{11.2} = 0.149$; $p < 0.008$) plants (see Fig., C). The trend was reversed for very high and high susceptible cultivars. Unlike the previous chemical components, where their content decreased after aphid damage, the cyanogenic potential significantly increased in response to injury (1 — $F_{1.2} = 0.443$; $p < 0.005$; 2 — $F_{1.2} = 0.336$; $p < 0.017$; 3 — $F_{1.2} = 0.428$; $p < 0.001$; 4 — $F_{1.2} = 0.424$; $p < 0.035$; 5 — $F_{1.2} = 0.215$; $p < 0.001$; 6 — $F_{1.2} = 0.320$; $p < 0.001$; 7 — $F_{1.2} = 0.308$; $p < 0.027$; 8 — $F_{1.2} = 0.456$; $p < 0.039$; 9 — $F_{1.2} = 0.259$; $p < 0.011$; 10 — $F_{1.2} = 0.272$; $p < 0.001$; 11 — $F_{1.2} = 0.215$; $p < 0.005$; 12 — $F_{1.2} = 0.177$; $p < 0.033$). The considerable higher aphid pressure resulted in a strongly expressed increase of concentrations from 31.1 to 49.0% in high and very high susceptible cultivars as opposed to the slightly preferred ones. Additionally, a strong significant negative correlation between the cyanogenic glycosides content and aphid number was observed ($r = -0.729$; $p \leq 0.05$).

The results showed that cyanogenic glycosides may play a central protective role against *A. fabae* preventing the colonization and abundance of the species. Bigger differences in the levels of that compound determined not only the different preferences of aphids to the cultivars but also the induction of different cyanogenic content after injury. Probably plants catalyzed their defence mechanisms with increasing aphid pressure, as the induction grade of cyanogenic glycosides was in response to the attack degree.

There was indisputable evidence for the role of cyanogenic glycosides as insect pest deterrents. According to R.M. Gleadow and B.L. Møller [37], these compounds play important role in plant defence producing bitter taste and toxic hydrogen cyanide which repel pests. On the other hand, some authors reported that insect pests damage were responsible for catalyzing the synthesis of cyanogenic glycosides as a defence mechanism [38, 39], while others found that insects were able to detoxify them and either used it as a carbon source or sequester hydrogen cyanide as a defence against predators [40].

In the present study, cultivars with significantly higher cyanogenic glycosides were efficiently directly defended from aphids and responded with a slight increase in compounds, while the preferred plants had a strong increase in cyanogenic glycosides but were not defended by *A. fabae* attack.

Chlorophyll content was used as an indicator of the level of photosynthesis and in turn, the level of carbohydrate produced per leaf. The protein content promoted aphid growth and development while carbohydrates contributed to their energy requirements [41].

Among the uninfected plants with statistically the highest content of chlorophyll a + b was BGE041470, followed by Fb 3270 and BGE 043776 ($F_{11.2} = 2.310$; $p < 0.018$), while the trend in infected cultivars was different and the leading position was occupied by Fb 3270, followed by BGE 029055 ($F_{11.2} = 1{,}861$; $p < 0.024$) (see Fig., D). No correlation was found between the aphid number and the chlorophyll content, which indicated that the chemical component was not related to the preferences of the species. However, as a result of the attack and nutritional activity of *A. fabae*, a significant reduction in chlorophyll a + b content was found in all cultivars (according to the figure numbers 1 — $F_{1.2} = 2.894$; $p < 0.047$; 2 — $F_{1.2} = 5.204$; $p < 0.001$ 3 — $F_{1.2} = 6.181$; $p < 0.031$; 4 — $F_{1.2} = 3.208$; $p < 0.033$; 5 — $F_{1.2} = 4.391$; $p < 0.001$; 6 — $F_{1.2} = 4.176$; $p < 0.017$; 7 — $F_{1.2} = 4.280$; $p < 0.015$; 8 — $F_{1.2} = 3.130$; $p < 0.001$; 9 — $F_{1.2} = 6.284$; $p < 0.036$; 10 — $F_{1.2} = 5.248$; $p < 0.040$; 11 — $F_{1.2} = 6.177$; $p < 0.023$; 12 — $F_{1.2} = 5.886$; $p < 0.042$). The reduction corresponded to the infestation grade and it was most pronounced in very high and high susceptible cultivars.

Similar results were reported in previous studies. T.I. Huang et al. [42] and M.S. Anjali et al. [43] reported that aphid infestation caused chlorophyll losses and the grade of decreased pigment contents was depended on the aphid density and plant growth stage. In addition, M.R. Amin et al. [24] reported that aphid abundance considerable affected the chemical traits of several bean species and led not only to a high reduction of chlorophyll but also the moisture content in the leaves. Different from our results were reported by D.C. Munthali and A.B. Tshegofatso [41], who studied aphid abundance affected by chlorophyll content in *Brassica oleracea* cultivars. They found that plants with high chlorophyll concentration had a significantly lower aphid infestation. Studies to find out the degree of association of the aphid population with biochemical characters such as chlorophylls are controversial and need further research.

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

Parameters of linear regression:

Dispersion	df	SS	MS	*F*-Ratio	p-value
Model	5	2.73226E7	5.46452E6	15.03	0.00001
Residual	30	1.09078E7	363594.0		
Total (Corr.)	35				

Regression coefficients:

Factors	Coefficient	Standard error	*t*-Stat	p-value
Intercept	–8653.04	2907.290	–2.976	0.005
Height	–26.133	30.273	–0.863	0.009
Protein	33.958	9.175	3.701	0.001
Phosphorus	8471.670	4172.730	2.030	0.051
Cyanogenic glycosides	–506.335	162.323	–3.119	0.004
Chlorophylls a+b	7.65142	3.804	2.011	–0.053

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

$$Y = -8653.04 - 26.1328X_1 + 33.9578X_2 + 8471.67X_3 - 506.335X_4 + 7.65142X_5,$$

where Y is *Aphis fabae* number; X_1 is hieght; X_2 is protein; X_3 is Phosphorus; X_4 is cyanogenic glycosides; X_5 is Chlorophylls a + b.

Results showed that on black bean aphid infestation, the highest negative significant influence had cyanogenic glycosides ($\beta = -506.3$; $p = 0.004$) followed by height ($\beta = -26.1$; $p = 0.009$). Protein content had a positive significant influence ($\beta = 34.0$; $p = 0.001$), while other traits had no significant influence on aphid attack.

Thus, *Aphis fabae* appeared in the formation of the first buds, and at the plant development during the budding and flowering stage, their number increased proportionally and reached a maximum in the pod formation. Stable position and with the lowest density stood out the cultivar Fb 3270, followed by BGE 029055 and according to susceptibility grades, they were defined as very low susceptible, i.e., sustainable to aphid infestation. Medium susceptible to aphids were BGE 002106, BGE 032012 and BGE041470. *Aphis fabae* infestation significant affected the morphological and chemical traits of *Vicia faba* cultivars and led to a reduction of the plant height (by 23.8%, $p \le 0.05$), crude protein (28.2%, $p \le 0.05$), phosphorus (by 31.0%, $p \le 0.05$) and chlorophylls a + b content (28.0%, $p \le 0.05$), while cyanogenic glycosides significantly increased (by 28.6%, $p \le 0.05$) in response to aphid injury. The grade of the decreased content of chemical traits and height was depended on the aphid number and losses were significantly higher in very high and high susceptible plants (BGE 046721, BGE 043776, Fb 2486, Fb 2481, Fb 1896, Fb 1903), while cyanogenic glycosides reciprocally increase with the aphid growth population. Protein and cyanogenic glycosides can be used as key indicators for aphid preference. Phosphorus may also be an important parameter for faba beans influencing *A. fabae* preferences given the negative significant correlation between aphid abundance and plant phosphorus content ($r = -0.518$; $p \le 0.05$). Cultivars Fb 3270 and BGE 029055 with high phosphorus (0.636 and 0.609 g/kg DM, $p \le 0.05$) and cyanogenic glycosides (7.72 and 7.94 mg/100 g DM, $p \le 0.05$) and low plant height and crude protein content (184.2 and 190.4 g/kg DM, $p \le 0.05$) had a significantly lower aphid infestation. Therefore, those cultivars having significantly less susceptibility to black bean aphid than other cultivars can be included in future breeding programmes to improve resistance to *A. fabae*.

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