ISSN 1314-6246 Nikolova *J. BioSci. Biotech.* **2021**, 10(1): 67-74

RESEARCH ARTICLE

Ivelina M. Nikolova

Susceptibility response of varieties and local populations of lupines to *Bruchus rufimanus* (Coleoptera: Chrysomelidae)

Authors' address:
Department "Forage production and livestock breeding", Institute of Forage Crops - Pleven, "General Vladimir Vazov" str. 89, Pleven 5800, Bulgaria.

Correspondence:
Ivelina M. Nikolova
Department "Forage production and livestock breeding", Institute of Forage Crops - Pleven, "General Vladimir Vazov" str. 89, Pleven 5800, Bulgaria.
Tel.: +359 64 805 882
e-mail: imnikolova@abv.bg

Article info:
Received: 11 August 2020
Accepted: 1 October 2020

ABSTRACT

This study aimed to evaluate the susceptibility response of varieties and local populations of lupines to *Bruchus rufimanus* in multi-environment field tests. Seed damaged rate and susceptibility index were assessed in each environment and subjected to a heritability-adjusted genotype and genotype x environment biplot analysis. It was found that the susceptibility index of damaged seeds was positively related to precipitation amounts and humidity, and inversely to min and max temperatures. The seed damaged rate was positively related to temperatures but negatively to rain and humidity. The local polish population WAT and cultivars Pink Mutant, Solnechnii, and Bezimenii 1 had the lowest seed damaged rate and stable position across environments. Meanwhile, these cultivars showed a low susceptibility index and low variability. The discrepancy between the early phenological development of Pink Mutant, Solnechnii, and Bezimenii 1 and the life cycle of *B. rufimanus* was one of the reasons for manifested tolerance. Correlations between damaged seed and susceptibility index as well as the mass of 1000 seeds and sensitivity index were strongly positive and negative, respectively. WAT, Pink Mutant, Solnechnii, and Bezimenii 1 had a clear advantage in defending itself from *B. rufimanus* attack, which makes them particularly interesting for breeding purposes.

Key words: *Bruchus rufimanus,* HA-GGE biplot analysis, Seed damaged rate, Susceptibility index

Introduction

Broad bean beetle, *Bruchus rufimanus* Boheman, 1833 (Coleoptera: Chrysomelidae) is a common pest on faba beans (*Vicia faba* L.) all over Europe and worldwide (Roubinet, 2016). Bean beetle hosts, in addition to *V. faba*, are various genera *Vicia*, *Pisum* and *Lathyrus* (Delobel & Delobel, 2006; Ward, 2018).

Ramos and Fernández-Carrillo (2011) first reported that lupin plants were a new host of different species from Bruchus genus (*Bruchidius rubiginosus* Desbrochers). Harris (1980) established that *B. chinensis* L was an important lupin seed pest, but in a later study, the author found that *B. rufimanus* it is one of the most destroyed seed pests in lupine (Hurej et al., 2013).

Bruchus rufimanus is univoltine insect. Adults emerge from overwintering sites and enter host crops to feed on pollen for several weeks, which females must do to terminate reproductive diapause. After that, females lay eggs on the pod surface. The larvae develop in the seeds and the adults emerge at harvest. Bruchids make a round output hole in seeds and go through it. Broad bean beetle moving to sheltered winter sites, or they remain in the seed until the following year doing no further damage during storage.

The development duration, reproduction, damage degree, and generation viability were determined largely by temperature in many insect species (Zhou et al., 2010; Kutcherov, 2015; Hasan & Ansary, 2016). For example, changes in development and damage rate by temperature were reported regarding *Acanthoscelides obtectus* Say (Stewart et al., 2015). However, climatic conditions have a considerable impact on the attack and pest damage.

Control of *B. rufimanus* is primarily conducted by the use of insecticides against adults before oviposition, at the stage of the mid-flowering and early pod-formation. Pyrethroids are one of the most use insecticides but managing adult pest attacks is difficult due to their mobility, and the lack of persistence of pyrethroids at high temperatures (Mansoor et al., 2015).

European restrictions and environmental concerns have increased the need for alternative measures. Site selection, crop rotation, cultivar and seed selection, sowing date, and plant density are potential means to pest control. The ones of the effective alternative measures to beetle management are the A field trial was conducted with 21 white lupine cultivars:

Astra, Nahrquell, Ascar, Shienfield Gard, Kijewskij Mutant, Hetman, Start, Amiga (originating from Poland), Garant (originating from Ukraine), Tel Keram, Bezimenii 1, Bezimenii 2, Pflugs Ultra, Termis Mestnii, Horizont, Solnechnii, Pink Mutant, Manovitskii, Barde, Dega, Desnyanskii (originating from Russia) and two local populations: BGR 6305 and WAT during the period 2014-2016 at the Institute of Forage Crops (Pleven, Bulgaria). Sowing was made by hand, in optimum sowing time, early in the spring (usually March) according to the technology of cultivation. It was used 5 kg/da active substance mineral nitrogen. The experiment was laid out using a randomized block design. The studied genotypes were grown in a density of 50 plants m^{-2}. Each plot unit (5,50 m broad × 2 m length) included twelve rows spaced 50 cm apart.

The soil type is leached chernozem with pH $_{(KCl)}$ – 5.49 and content of total N – 34.30 mg/1000 g soil, P_2O_5 – 3.72 mg/100 g soil, and K_2O – 37.50 mg/100 g soil.

The period from germination to early flowering was determined for quantitative assessment we used the coefficient of early-ripeness (Kuzmova, 2002):

$$\mathrm{Cr} = 1 + \frac{\mathrm{Nc} - \mathrm{Nmin}}{\mathrm{Nmax} - \mathrm{Nmin}}$$

where: Nc is the duration of the period sowing - beginning of flowering for the particular cultivar; Nmax and Nmin are the maximum and minimum duration (in days) of the period sowing-beginning of flowering for all tested cultivars.

The values of the coefficient were as followed: for ultra-early ripening cultivars – from 1.00 to 1.17; for early-ripening cultivars – 1.17 to 1.33; for medium-early ripening cultivars – 1.34 to 1.66 and for late-ripening ones > 1.66.

No chemical control of insect pests was conducted during the growing season. The degree of Bruchus rufimanus damaged seeds was determined after lupin harvesting. Bulk samples containing 1500 seeds were taken for each accession, Susceptibility index (I, %) was calculated by the following formula:

$$\mathrm{SI} = \frac{a - b}{a} \times 100$$

where: a is weight of 1000 healthy seeds; b is weight of 1000 seeds damaged by the broad bean beetle

To eliminates interactions between variables and to include genotype and genotype x environment (GGE) interactions, an HA-GGE biplot analysis was carried out (Yan and Holland, 2010). Biplot graphs are suitable for simultaneous visualization of interacting factors and based mathematically on SVD (singular-value decomposition) models. They are used frequently, in a comparison of multiple genotypes in different environments (Rubiales et al. 2014; Sánchez-Martín et al., 2014). In this way, the best genotype will have the lowest values for the evaluated trait and stability through all the environments, and low G × E interactions.

To evaluate the influence of environmental factors on DR and SI, different climate variables were subjected to the Non-Metric Multidimensional Scaling (NMDS) ordination (Anderson, 2001). Data on the meteorological variables: rainfall, average air temperature, as well as average relative humidity were obtained from Pleven meteorological station for each environment. In order to focus on the occurrence of bruchids in the field, the climatic parameters used in the analysis ranged from March to June. To determine the relative impact of the selected climatic variables on the performance of DR and SI, canonical correspondence analysis (CCA) was carried out. The analysis was performed using the Paleontological Statistics Software Package (PAST) (Hammer et al., 2001). Relationships between damaged seeds and certain plant traits were tested using multiple regression analysis. The statistical processing of experimental data was conducted using the Statgraphics Plus software program.

Results and Discussion

The meteorological conditions during the studied period were different (Figure 1) and had an impact on *Bruchus rufimanus* development, reproduction, and damage rate. April, May, and June months in 2015 were characterized by a higher average daily temperature (by 1,0 and 0,7^{0}C to 2014 and 2016) as well as a lower rainfall and air humidity (by 107,1 and 25,5 mm, and 9,7 and 6,7% humidity to 2014 and 2016). Those conditions led to an earlier appearance of bean beetle and their stronger attack compared to other years. The plants were in the sensitive stage of flowering and pod formation to bruchid infestation in May and the first ten days of June. At the same time, the plants suffered from a lack of moisture. During 2016, after sowing, the subsequent dry weather delayed seed germination. In April-June the higher temperatures accelerated the plant development and favored the broad bean beetle attack. The meteorological conditions during 2014 characterized by the highest amount of rainfall and relative humidity combined with low temperatures during the growing season. That suppressed infestation and damage rate of *B. rufimanus* (Figure 1).

A wide range of values for DR and SI were noted for the 23 lupin 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 mean of a square for E, followed by G and the lowest for G × E (Table 1).

Table 1. *Analysis of variance for Bruchus rufimanus seed damage rate (DR) and susceptibility index (SI) of the 23 lupim genotypes.*

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
DR					
ENV	2	17878.48	8939.239*	3213.711	8.11E-10
REP(ENV)	6	16.690	2.782	58.494	8.03E-35
GEN	22	14129.08	642.231*	11.232	1.2E-11
ENV * GEN	44	2515.781	57.177 *	1202.361	9.9E-153
PC1	23	2511.448	109.193	2296.210	
PC2	21	4.333	0.206	4.340	
Residuals	132	6.277	0.048		
SI					
ENV	2	2755.412	1377.706*	381.713	4.74E-07
REP(ENV)	6	21.656	3.609	33.620	2.21E-24
GEN	22	4587.940	208.543*	11.733	5.64E-12
ENV * GEN	44	782.079	17.775*	165.566	1.74E-96
PC1	23	678.050	29.480	274.600	-
PC2	21	104.029	4.954	46.140	-
Residuals	132	14.171	0.107	-	-

Legend: DF- degrees of freedom; G * E- term of genotype * environment interaction);
* Significant at 0.0001 level probability

A canonical correlation analysis helped to visualize the distinct relations of DR and SI components to climate variables (Figure 2). Whereas SI was positively related to bulk precipitation and humidity and inversely to Tmin and Tmax, the seed damaged rate was positively related to Tmin and Tmax but negatively to rain and humidity. Moreover, Tmin and Tmax were associated with the environmental 2 droughts (2015) and opposed to rain and humidity during the environmental 1 wet period (2014). Because of the negative effect of rainfall on DR, the seed damage decreases in rainy seasons as could be seen in the driest environments. This might be due to the fact that rainfall might disturb bruchid oviposition and reduce egg viability (Roubinet, 2016). The opposite, rainfall, and humidity had a positive effect, with SI increasing at higher values (Figure 2).

The HA-GGE biplot is the preferred GGE biplot for test environment and genotype evaluation (Yan & Holland, 2010). The GGE biplot presents the mean characteristic and stability, which gives us an essential visualization of the data (Yan, 2001; Yan & Rajcan, 2002). A GGE biplot is a biplot based on environment-centered data (Gabriel, 1971), which removes the environment's main effect and integrates the genotypic main effect with the genotype-by-environment interaction effect of a genotype-by-environment dataset (Yanunt *et al*., 2000).

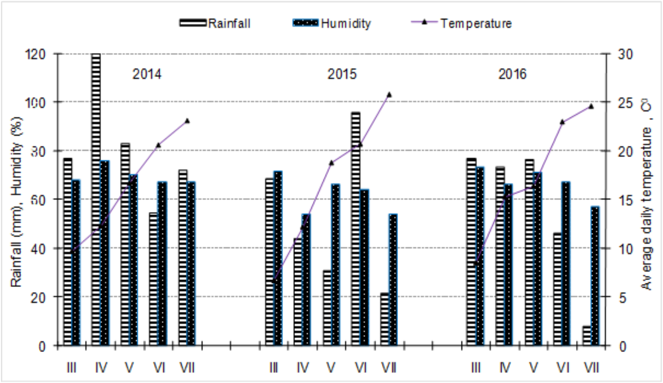

Figure 1. *Meteorological characteristic of the period 2014-2016.*

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

Although there are no strict relations, the goodness of approximation for the correlation coefficients by the angles is related to the goodness of fit of the biplot. Depending on the angle between two environment vector correlation is different. In that aspect, the environments were more or less positively correlated (acute angles). An exception was found between E1 and E2 environments which were not correlated (a right angle). In addition, within the environmental group, E 1 was apparently less associated with E3, while strongly positively correlated were E2 and E3 (Figure 3).

In order to determine which of the 23 lupin genotypes studied were the least affected by bean beetle attack 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. Thus, in the case of damaged seeds, the genotype with the lowest DR was G13 (6,3%) despite exhibiting environmental interactions, followed by the genotypes G18 (10,9%), G6 (11,8%), G19 (14,0%) and G17 (15,5%), whose responses were more stable, as indicated by their location close to the axis 1 (Figure 3).

The results showed that genotypes G19, G17, and G6 were considered as the most stable being the ones closest to the midpoint of the boxplot and less preferred from *B. rufimanus*.

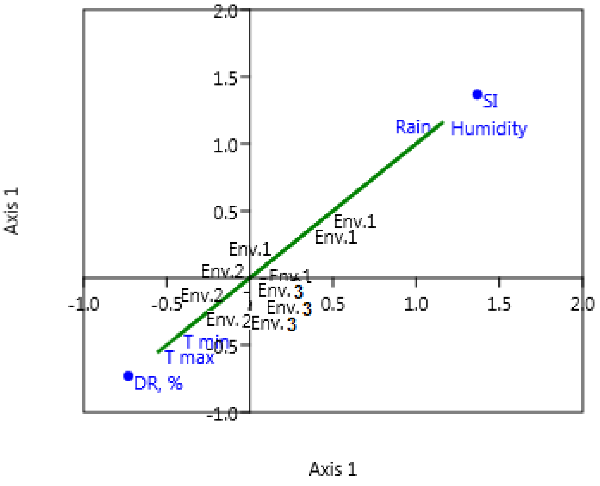

Figure 2. *CCA graph based on the correlation of DR and SI of Bruchus rufimanus for 23 lupin cultivars according to several climatic parameters. The period analyzed was from April to June, Tmax = maximum temperature; Tmin = minimum temperature; DR = Seed damaged rate (%); SI, %= Susceptibility index.*

Relatively stable and damage tolerant with little difference in each other, exhibited G1, G4, and G16, Genotype G2 had lower values for that trait but was more affected by the environment. The most susceptible genotypes (high DR, represented on the opposite side of the biplot) were G12 (35,8%), G8 (34,7%), and G14 (34,6%). According to the GGE biplot analysis, values of G12, G8, and G14 to PC2 are distantly situated to zero. It pointed to greater variability (lower stability). The same level of poorer stability and damage sensitivity also showed G7 and G21. The variables of the above five genotypes were best expressed in E3 and E2 environments. The two principal components determined 99,1% of the dispersion.

The GGE biplot based on SI (Figure 4), analysis represented 96,2%% of the total trait variation between two principal components. The environment with the shortest vector was E1, and the longest - E2. The most discriminative environment was E2 in which less rainfall was registered. Genotype 6 was the most responsive to that trait (the lowest value of SI, 5,6%), and it was followed by G19, G18, G13 (7,4; 7,9 and 9,0%, respectively) (see Table 2). A similar level of sensitivity was observed in G2 and G1 too. According to the ordinate, G6 was highly stable, followed by G19 within the group of the low susceptibility index.

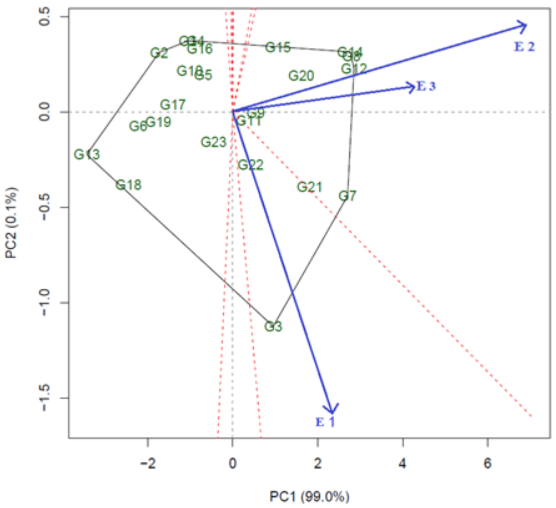

Figure 3. *The GGE biplot based on seed damaged rate (2014-2016). The genotypes are designated with the symbol "G" and the respective number from 1 to 23, as follow G1-Astra, G2-Nahrquell, G3-Ascar, G4-BGR 6305, G5-Shienfield Gard, G6-WAT, G7-Kijewskij Mutant, G8-Hetman, G9-Start, G10-Amiga, G11-Garant, G12-Tel Keram, G13-Bezimenii 1, G14-Bezimenii 2, G15-Pflugs Ultra, G16- Termis Mestnii, G17-Horizont, G18-Solnechnii, G19-Pink Mutant, G20-Manovitskii, G21-Barde, G22-Dega, G23-Desnyanskii. The years are designated with the letter E and number 1; 2; and 3 for 2014, 2015 and 2016, respectively, Note: G14 and G8 are heavily overlapped, as well as G1 and G4; G5 and G10.*

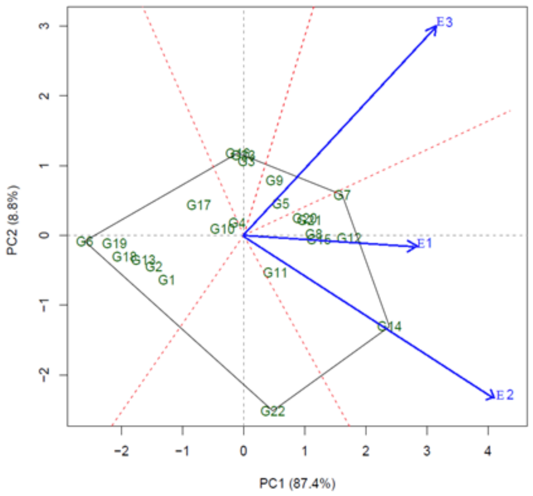

Figure 4. *The GGE biplot based on susceptibility index (2014-2016). The genotypes are designated with the symbol "G" and the respective number from 1 to 23, as follow G1-Astra, G2-Nahrquell, G3-Ascar, G4-BGR 6305, G5-Shienfield Gard, G6-WAT, G7-Kijewskij Mutant, G8-Hetman, G9-Start, G10-Amiga, G11-Garant, G12-Tel Keram, G13-Bezimenii 1, G14-Bezimenii 2, G15-Pflugs Ultra, G16- Termis Mestnii, G17-Horizont, G18-Solnechnii, G19-Pink Mutant, G20-Manovitskii, G21-Barde, G22-Dega, G23-Desnyanskii. The years are designated with the letter E and number 1; 2; and 3 for 2014, 2015 and 2016, respectively, Note: G23, G16 and G3 are heavily overlapped, as well as G21 and G20.*

Table 2. *Ranking of the twenty-three lupin genotypes with the lowest levels of Bruchus rufimanus seed damaged rate (DR) and susceptibility index (SI) (ascending order).*

DR						**SI**					
1	G13	11	G5	21	G14	1	G6	11	G23	21	G7
2	G18	12	G23	22	G8	2	G19	12	G3	22	G12
3	G6	13	G11	23	G12	3	G18	13	G22	23	G14
4	G2	14	G22			4	G13	14	G11		
5	G19	15	G9			5	G2	15	G9		
6	G17	16	G3			6	G1	16	G5		
7	G1	17	G15			7	G17	17	G20		
8	G10	18	G20			8	G10	18	G21		
9	G4	19	G21			9	G4	19	G8		
10	G16	20	G7			10	G16	20	G15		

Stability throughout the environments has been taken into account by considering each genotype position in the biplots.

Lower variability had G18 and G13, G4 had a mean susceptibility index to the grand mean.

The genotype presenting the highest value in that trait and identified as strongly sensitive was G14, followed by G12 and G7. Furthermore, the genotype G14 was a considerable variable (low stability) together with G22. Also, G14 had the highest value in E2, which provoked its high susceptibility.

Pearson correlations between DR and SI with genotype as a weighting variable (r = + 0.812, *p*= 0.0001) revealed a significantly high coefficient value, which suggests a strong association between both parameters.

The reduced DR and SI for G6, G19, G18, and G13 might be the result of the combination of different resistance mechanisms. The antixenosis mechanisms might be involved in the resistance of these genotypes by reducing the oviposition over their pods as the result of morphological, phenological, or (and) chemical plant factors that adversely affect insect behaviour. Such morphological traits hindering the penetration of the larvae could be related to a pod or seed coat thickness, seed weight, chemical compounds that hamper the penetration of pods or seeds (alkaloids in lupins) (Keneni et al., 2011). The discrepancy between the phenological development of the host plant and the life cycle of bean beetle could be a marker for tolerance too. In our case, several differences among the phenological development of the genotypes, affecting *B. rufimanus* damage, were observed (Figure 5). After passing the budding stage, there were found differences in the growing period length. Astra, Termis Mestnii, and Barde were characterized with the shortest average duration of the period germination-beginning of flowering (37 days). Pink Mutant (G19), Solnechnii (G18), and Bezimenii 1 (G13) had a shorter average duration of the period (38 days) and occupied an intermediate position. In the remaining stages of the growing season, the trend remained. The early cultivars (with early flowering) reached technical maturity on average after about 129-134 days and the late ones – for 140-148 days. Cultivars Ascar (G3), Termis Mestnii (G16), Barde (G21), as well as Pink Mutant (G19), Solnechnii (G18), and Bezimenii 1 (G13), could be included in the group of ultra-early ripening cultivars (the coefficient of early-ripeness of 1.00-1.14). Medium-early ripening cultivars were Astra (G1), Kijewskij Mutant (G7), Start (G9), BGR 6305 (G4), WAT (G6), Garant (G11), Tel Keram (G12), Bezimenii 2 (G14), Pflugs Ultra (G15) (coefficient of early-ripeness >1.34) and the late-ripening ones - Hetman (G8), Shienfield Gard (G5) and Nahrquell (G2) (coefficient > 1.66).

Several cultivars of the ultra-early ripening group stood out with considerably lower values of damage traits (DR and SI).

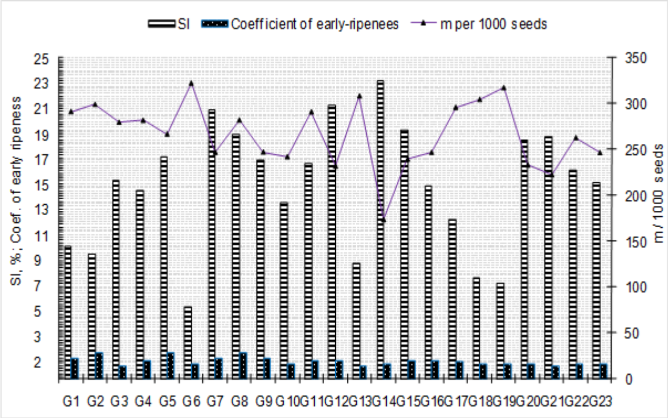

Figure 5. *Characteristics of lupine genotypes. Legend: SI-susceptibility index; G1-Astra, G2-Nahrquell, G3-Ascar, G4-BGR 6305, G5-Shienfield Gard, G6-WAT, G7-Kijewskij Mutant, G8-Hetman, G9-Start, G10-Amiga, G11-Garant, G12-Tel Keram, G13-Bezimenii 1, G14-Bezimenii 2, G15-Pflugs Ultra, G16- Termis Mestnii, G17-Horizont, G18-Solnechnii, G19-Pink Mutant, G20-Manovitskii, G21-Barde, G22-Dega, G23-Desnyanskii.*

For example, Pink Mutant, Solnechnii, and Bezimenii 1 had early flowering and slightly preference by bean beetle, while late-ripening Hetman and Shienfield Gard was considerably preferred by bruchids. The discrepancy between the early phenological development of those cultivars and the life cycle of *B. rufimanus* was one of the reasons for manifested tolerance.

There was published evidence for the influence of cultivar on damage caused to *Vicia faba* grain by *B. rufimanus* (Ebedah et al., 2006; Szafirowska, 2012). In those studies was suggested that plant architecture, flowering period and abundance, and the timing of pod formation were the key factors that influence the activity of *B. rufimanus*. According to Bruce et al. (2011), Ceballos et al. (2015), several plant characteristics could adversely affect insect behaviour. Authors found that some susceptible genotypes flowered later than the average, which could have contributed in some way to the escape of these pea plants from bruchid infestation. More recent research identified phenological tolerance in cultivars with an early flowering stage becoming unavailable to the weevils during the period when the attack is likely to be most severe (Bell & Crane, 2016).

On the other hand, results showed the mass of 1000 seeds strongly negatively correlated with the sensitivity index, r= −0.842. It was noticed that genotypes exceeding 300 g per 1000 seeds, such as G6 (322.2g), G19 (317.1g), G13 (308.2g), and G18 (304.3g) were distinguished by low susceptibility indexes (from 5.6 to 7.9%). In contrast, genotypes with much smaller seeds like G14, G21, and G20 (173.2, 222.2, and 232.9, respectively) were characterized by higher SI values (from 19 to 23%). Larger seeds are considerably richer in nutrients than small seeds, where larvae destroyed a large amount of them. For example, Mateus et al. (2011) reported that the attack by bruchids caused a significant reduction in seed weight, between 0.03 (large seeds) and 0.08 g (smaller seeds), depending on the genotypes/cultivars, corresponding to a decrease in nutrients available to the embryo development. In that aspect, the genotype G14, G21, and G20 were one of the cultivars with the highest susceptibility indexes as the larva destroyed most of the grain content for its feeding.

Also, antixenosis mechanisms might be involved in the tolerance of these genotypes by reducing the preference of bean beetle adults for feeding as the result of chemical plant factors that adversely affect insect behaviour. Probably, studied lupin cultivars may differ chemically to a great extent (in alkaloid content), and in that context, some species of them may even be toxic to some animals. The negative role of different alkaloids in cultivated lupins was indicated by Ströcker et al. (2013). The presence of such antinutrient substances in the genotype-host probably explains the preferences of bruchids.

About the effect of some botanical oils, including lupin seeds on the granary weevil, *Sitophilus granarius* reported Makarem et al. (2017). According to authors, lupine oil protected the grain against weevils up to the 6th-week post-treatment achieving mortalities between 60.0 and 100%. Meanwhile, the highest degree of inhibited oviposition and adult emergence was detected with a lupine oil treatment compared with other oils.

On the other hand, proteinase inhibitors are potential candidates for biocontrol of insect pests since insect digestive proteinases are promising targets towards the control of various insects (Sharma et al., 2012). Proteases have been found to be effective against many Coleopteran (Elden, 2000). Scarafoni et al. (2008) reported for the inhibitory properties of a trypsin inhibitor from *Lupinus albus* L, a leguminous plant believed to be devoid of any protease inhibitor. Several protease inhibitors have been reported to exhibit inhibitory activity against insect proteases.

It is necessary to examine not only the individual effect of plant traits but also their mutual impact on the beetle damage. The applied regression analysis (ANOVA) in Table 3 showed that the interaction of plant traits had a significant effect on the damaged seed rate. The susceptibility index had the highest regression coefficient (r=1.915) (Table 3, below). It had a significant positive effect. The coefficient of early-ripeness had a significantly strong effect on the *B. rufimanus* choice (r= −1.687) but correlated negatively. The mass of 1000 seeds had a low positive effect (r=0.048) on the damaged seeds in the complex interaction between plant traits and seed damage rate.

According to the results above, G6, G19, G18, and G13 seem to have a clear advantage in defending itself from *B. rufimanus* attack. The low DR and SI make genotypes particularly interesting for breeding purposes because it probably presents a combination of different mechanisms like seed weight and phenological development adversely affect *B. rufimanus* behaviour. The possibility of combining these two types of resistance mechanisms have great importance because of the durability of tolerance. If one of these levels is broken the other resistance mechanism will successfully overcome weevil attacks.

In general, *B. rufimanus* damage was affected by climate parameters. The susceptibility index of damaged seeds was positively related to precipitation amounts and humidity, and inversely to min and max temperatures.

The local population WAT and cultivars Pink Mutant, Solnechnii, and Bezimenii 1 (G6, G19, G18, and G13, respectively) had the lowest seed damaged rate and stable position across environments. Meanwhile, these cultivars showed a low susceptibility index and low variability. Cultivars had a clear advantage in defending itself from *B. rufimanus* attack, which makes them particularly interesting

Table 3. *Regression coefficient of the damaged seed rate depending on some plant parameters for lupine genotypes*.

Source	**df**	**SS**	**MS**	**F**	**Significance F**
Regression	3	1319.330	439.780	33.140	0.051
Residual	19	252.143	12.270		
Total	22	1571.470			

Parameter	**Coefficients**	**Standard Error**	**t Stat**	**P-value**	**Lower 95%**	**Upper 95%**
Intercept	-17.145	15.206	-1.127	0.000	-48.970	14.681
SI	1.915	0.339	5.653	0.000	1.206	2.623
M of seeds	0.048	0.045	1.059	0.087	-0.047	0.142
CER	-1.687	2.843	-0.593	0.100	-7.639	4.264

Legend: SI- Susceptibility index, M of seeds- m per 1000 seeds, CER- Coefficient of early-ripeness

for breeding purposes. The matching of an early flowering with higher seed weighs in cultivars could be used as markers for tolerance against broad bread weevil, and like an effective method for plant defense.

References

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32–46.

Bell S, Crane E. 2016. Farming oilseed rape without neonicotinoids. Research Report Commissioned by Friends of the Earth. Retrieved from https://cdn.friendsoftheearth.uk/sites/default/files/downloads/Farming%20Oilseed%20Rape%20without%20Neonicotinoids.pdf

Bruce TJ, Martin JL, Smart LE, Pickett JA. 2011. Development of semiochemical attractants for monitoring bean seed beetle, Bruchus rufimanus. Pest Manag Sci 67: 1303–1308.

Ceballos R, Fernbndez N, Zyciga S, Zapata N. 2015. Electrophysiological and behavioral responses of pea weevil *Bruchus pisorum* L. (*Coleoptera: Bruchidae*) to volatiles collected from its host *Pisum sativum* L. Chilean JAR 75: 202–209.

Delobel B, Delobel A. 2006. Dietary specialisation in European species groups of seed beetles (Co*leoptera: Bruchidae: Bruchinae*). Oecologia 149: 428-443.

Ebedah IMA, Mahmoud YA, Moawad SS. 2006. Susceptibility of some faba bean cultivars to field infestations with some insect pests. Res G Agric Biol Sci 2: 537-540.

Elden TC. 2000. Effects of proteinase inhibitors and plant lectins on the adult alfalfa beetle (Coleoptera: Curculinoidae). J Entomol Sci 35: 62−69.

Gabriel KR. 1971. The biplot graphic display of matrices with application to principal component analysis. Biometrika 58: 453–467.

Hammer Ø, Harper DAT, Ryanh PD. 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron 4: 1–9.

Harris BM. 1980. Insects associated with the lupin (Lupinus angustifolius) cultivars fest and uniharvest. Ph.D. thesis, University of Canterbury, Lincoln College, New Zealand, pp. 248.

Hasan F, Ansari S. 2016. Temperature-dependent development and demography of *Zygogramma bicolorata* (*Coleoptera: Chrysomelidae*) on *Parthenium hysterophorus*. ANN APPL BIOL 168: 81-92.

Hurej M, Twardowski JP, Kozak M. 2013. Weevil (*Coleoptera*: *Curculionidae*) assemblages in the fields of narrow-leafed lupin sown as pure stand and intercropped with spring triticale. Zemdirbyste-Agriculture 100 (4): 393–400.

Keneni G, Bekele E, Getu E, Imtiaz M, Damte T, Mulatu B, Dagne K. 2011. Breeding Food Legumes for Resistance to Storage Insect Pests: Potential and Limitations. Sustainability 3(9): 1399-1415.

Kutcherov D. 2015. Temperature dependent development in *Chrysomela vigintipunctata* (*Coleoptera: Chrysomelidae*), a stenothermal early-season breeder. J Therm Biol 53: 9-14.

Kuzmova K. 2002. World agro-climatic analogues of Bulgaria on the conditions of cultivation of peas. Jubilee session "120 years of agricultural science in Sadovo", 113-118.

Makarem HAE, Kholy SEE, Abdel-Latif A, Seif AI. 2017. Effect of some botanical oils on the granary weevil, *Sitophilus granarius* (L.) (*Coleoptera: Curculionidae*). Egypt J Exp Biol 13(2): 273 – 282.

Mansoor MM, Afzal M, Raza ABM, Akram Z, Waqar A, Afzal MBS. 2015. Post-exposure temperature influence on the toxicity of conventional and new chemistry insecticides to green lacewing *Chrysoperla carnea* (Stephens) (*Neuroptera: Chrysopidae*). Saudi J Biol Sci 22: 317–321.

Mateus C, Mexia A, Duarte I, Pereira G, Tavares de Sousa M. 2011. Evaluation of damage caused by bruchids (*Coleoptera*: *Bruchidae*) on peas (*Pisum sativum* L.). Acta Hortic 917: 125-132.

Pearson’s Correlation Coefficient. 2008. In: Kirch W. (eds) Encyclopedia of Public Health. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-5614-7_2569

Ramos RY, Fernández-Carrillo E. 2011. Life cycle and behaviour of the lupine seed beetle, *Bruchidius rubiginosus* (Desbrochers, 1869) in the Iberian Peninsula (*Coleoptera: Bruchidae*). Boletín de la SEA 31 (12): 253–259.

Roubinet E. 2016. Management of the broad bean weevil (*Bruchus rufimanus* Boh.) in faba bean (*Vicia faba* L.). Department of Ecology, Swedish University of Agricultural Sciences (SLU). Technical report. Retrieved from https://pub.epsilon.slu.se/13631/1/roubinet_e_160704.pdf

Rubiales D, Flores F, Emeran AA, Kharrat M, Amri M, Rojas-Molina MM, Sillero JC. 2014. Identification and multi-environment validation of resistance against broomrapes (*Orobanche crenata* and *Orobanche foetida*) in faba bean (*Vicia faba*). Field Crops Res 166: 58–65.

Sánchez-Martín J, Rubiales D, Flores F, Emeran AA, Shtaya MJY, Sillero JC, Allagui MB, Prats E. 2014. Adaptation of oat (*Avena sativa)* cultivars to autumn sowings in Mediterranean environments. Field Crops Res 156: 111–122.

Scarafoni A, Consonni A, Galbusera V, Negri A, Tedeschi G, Rasmussen P, Magni C, Duranti M. 2008. Identificaion and characterization of a Bowman-Birk inhibitor active towards trypsin but not chymotrypsin in *Lupinus albus* seeds. Phytochemistry 69: 1820−1825.

Sharma P, Nath AK, Kumari R, Bhardwaj SV. 2012. Purification, characterization and evaluation of insecticidal activity of trypsin inhibitor from *Albizia lebbeck* seeds. J For Res 23(1): 131−137.

Southgate BJ. 1979. Biology of the Bruchidae. Annu Rev Entomol 24: 449-473.

Stewart AJA, Bantock TM, Beckmann BC, Botham MS, Hubble D, Roy DB. 2015. The role of ecological interactions in determining species ranges and range changes. Biol J Linn Soc Lond 115: 647-663.

Ströcker K, Wendt S, Kirchner WH, Struck Ch. 2013. Feeding preference of the weevils *Sitona gressorius* and *Sitona griseus* on different lupin genotypes and the role of alkaloids. Arthropod–Plant Interact 7: 579–589.

Szafirowska A. 2012. The role of cultivars and sowing date in control of broad bean weevil (*Bruchus rufimanus* Boh.) in organic cultivation. Veg Crop Res Bull 77: 29-36.

Ward RL. 2018. The biology and ecology of *Bruchus rufimanus* (bean seed beetle). Ph.D. tesses, Newcastle University England.

Yan W, Holland JB. 2010. A heritability-adjusted GGE biplot for test environment evaluation. Euphytica 171: 355–369.

Yan W, Hunt LA, Sheng Q, Szlavnics Z. 2000. Cultivar evaluation and mega-environment investigation based on GGE biplot. Crop Sci 40: 597–605.

Yan W. 2001. GGEbiplot—a Windows application for graphical analysis of multi-environment trial data and other types of two-way data. Agron J 93: 1111–1118.

Yan W, Rajcan I. 2002. Biplot analysis of test sites and trait relations of soybean in Ontario. Crop Sci 42(1): 11-20.

Zhou Z, Guo J, Chen H, Wan F. 2010. Effects of temperature on survival, development, longevity, and fecundity of *Ophraella communa* (*Coleoptera: Chrysomelidae*), a potential biological control agent against *Ambrosia artemisiifolia* (*Asterales*: *Asteraceae*). Environ Entomol 39: 1021-1027.

Zhang XP, Wang M. 1990. A genetic male-sterile (*ms*) watermelon from China. Cucurbit Genetics Coop. Rpt, 13: 45-46.