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Susceptibility response of varieties and local lupine *Lupinus albus* L. population to *Bruchus rufimanus* Boheman, 1833 (Coleoptera: Chrysomeloidae)

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ABSTRACT

This study aimed to evaluate the susceptibility response of varieties and local populations of lupine *Lupinus albus* to broadbean seed beetle *Bruchus rufimanus* in multi-environment field tests. Seed damage 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 correlated to precipitation amount and humidity, and inversely to minimal and maximal temperature values. The seed damage rate was positively correlated 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 damage 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 the species' tolerance to *B. rufimanus* 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 by defending itself from *B. rufimanus* attack, which makes them particularly interesting for breeding purposes.

Key words: *Bruchus rufimanus*, beetle, lupine, seed damage rate, susceptibility index

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INTRODUCTION

Broad bean beetle, *Bruchus rufimanus* Boheman, 1833 (Coleoptera: *Chrysomelidae)* is a common pest on faba bean (*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 & Fernández-Carrillo (2011) firstly reported that lupine plants were a new host of different species of the genus *Bruchidius,* subfamily Bruchinae (*Bruchidius rubiginosus* Desbrochers). Harris (1980) established that *B. chinensis* L was an important lupine seed pest, but in a later study, the author found that broadbean seed beetle it is one of the most destroyed seed pests in lupine (Hurej, Twardowski, & Kozak, 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 (Bogatsevska et al, 2006; Carrillo-Perdomo et al, 2019).

The development duration, reproduction, damage degree and generation viability were determined largely by temperature in many insect species (Zhou Guo, Chen, & Wan, 2010; Kutcherov, 2015; Hasan & Ansari, 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 (Dermody, O'Neill, Zangerl, Berenbaum, & DeLucia, 2008; Hullé, d'Acier, Bankhead-Dromet, & Harrington, 2010).

Control of *Bruchus rufimanus* is primarily conducted by use of insecticides against adults before oviposition, at the stage of the mid-flowering and early pod-formation. Pyrethroids are ones 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. Ones of the effective alternative measures to beetle management are the identification of tolerant genotypes, integrate these genotypes in breeding programs, and to identify the genes involved in the tolerance mechanisms. In this regard, Szafirowska (2012) found that cultivars and their phenological development affect the activity of *B. rufimanus* and the quantity of damage. Southgate (1979) suggested that the seed size and portion remaining following Bruchinae larval feeding among different cultivars were important traits of germination capacity and damage extent. Roubinet (2016) observed differences in susceptibility between several cultivars of *B. rufimanus* and the timing of flowering or pod formation, turned out to be important factors influencing on the bruchid attack.

The application of alternative cropping strategies, specifically the use of different cultivars, is an efficacious and ecologically friendly approach to plant protection against main insect pests.

This study aimed to evaluate the susceptibility response of varieties and local populations of lupine *Lupinus albus* L. to *Bruchus rufimanus* in multi-environment field tests.

MATERIAL AND METHODS

Field trial was conducted with 23 white lupine cultivars: Astra, Nahrquell, Ascar, BGR 6305, Shienfield Gard, WAT, Kijewskij Mutant, Hetman, Start, Amiga (originated from Poland), Garant (originated from Ukraine), Tel Keram, *Bezimenii* 1, *Bezimenii* 2, Pflugs Ultra, *Termis Mestnii*, Horizont, *Solnechnii*, Pink Mutant, *Manovitskii*, Barde, Dega, *Desnyanskii* (originated from Russia) during the period 2014-2016 at the Institute of Forage Crops (Pleven, Bulgaria). Sowing was made by hand, in optimum sowing time, according to the technology of cultivation. 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 nitrogen 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.

We used the coefficient of early-ripeness (Kuzmova, 2002) for quantitative assessment in the period from germination to early flowering:

Cr=1 + [{Nc-Nmin}/{Nmax-Nmin}],

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

During the growing season, insect pest control was not applied. The degree of *Bruchus rufimanus* seed damage was determined after lupine harvesting. Bulk samples, containing 1500 seeds, were taken for each accession. Susceptibility index (SI, %) was calculated by the following formula:

SI = (a-b) / a x 100, where:

a - weight of 1000 healthy seeds;

b - weight of 1000 seeds damaged by the broad bean beetle

In order to eliminate interactions between variables and to include genotype and genotype x environment (GGE) interactions as well, HA-GGE biplot analysis was carried out (Yan & Holland, 2010). Biplot graphs are suitable for simultaneous visualization of interacting factors and based mathematically on SVD (singular-value decomposition) models. Biplots 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 environments, and low G × E interactions.

To evaluate the influence of environmental factors on DR and SI, different climatic variables were subjected to 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 (National Institute of Meteorology and Hydrology, Pleven Branch). In order to focus on the occurrence of bruchids in the field, the climatic parameters used in the analysis ranged from March to June of 2014, 2015 and 2016 years. To determine a relative impact of the selected climatic variables on the performance of DR and SI, canonical correspondence analysis (CCA) (Ter Braak, 1986) was carried out. The analysis was performed using the Paleontological Statistics Software Package (PAST) (Hammer, Harper, & Ryanh, 2001). Pearson correlation was calculated to study the possible relationship between the parameters evaluated (DR and SI with genotype) at 5% probability ($p \leq 0.05$). Analyses were performed using CCA. Relationships between damaged seeds and certain plant traits were tested using multiple regression analysis of Statgraphics (1995) for Windows Ver. 2.1 Software program. The data were subjected to one-way ANOVA, and the averages were compared by Tukey’s test at 5% probability ($p \leq 0.05$).

RESULTS AND DISCUSSION

During the studied period the meteorological conditions varied (Fig. 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 (up to 1.0 and 0.7^{0}C in 2014 and 2016, respectively) as well as a lower rainfall and air humidity (up to 107.1 and 25.5 mm, and 9.7 and 6.7% in 2014 and 2016, respectively). Such conditions led to an earlier appearance of *B. rufimanus* and their stronger attack compared to 2014 and 2016 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 2015. 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 *B. rufimanus* attack. During 2014 the meteorological conditions were characterized by the highest rainfall amount, and relative humidity combined with low temperatures during the growing season suppressing infestation and damage rate of *B rufimanus*.

A wide range of the values obtained for DR and SI were noted for the 23 lupine cultivars studied in the three environments. ANOVA (Table 1) revealed a significant effect of genotype (G), environment (E) and G × E in both variables, being the highest average of a square for E, followed by G and the lowest for G × E.

A canonical correspondence analysis (CCA) helped to visualize the distinct relations of DR and SI components to climatic variables (Fig. 2). Whereas SI was positively correlated with bulk precipitation and humidity, and inversely to T_{min} and T_{max}, the seed damage rate was positively correlated with T_{min} and T_{max} but negatively to rain and humidity. Moreover, T_{min} and T_{max} were associated with the environmental 2 droughts (2015), and opposed to rain and humidity during the environmental 1 wet period (2014). Because of a negative effect of rainfall on DR, the seed damage decreasing at rainy seasons as could be seen in the driest environments. This might be due to the fact that rainfall could disturb bruchid oviposition and reduce egg viability (Roubinet, 2016). In the opposite, rainfall and humidity had a positive effect, with SI increasing at higher values.

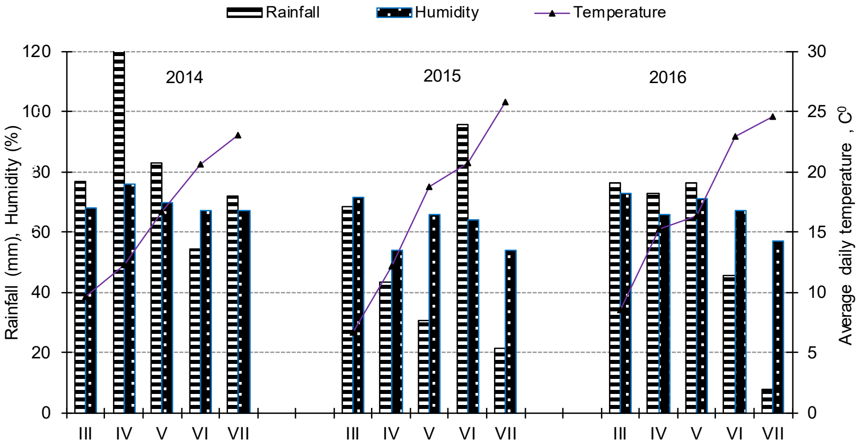

Fig. 1. Meteorological conditions of the period 2014-2016.

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

A HA-GGE biplot is the preferred GGE biplot for test environment and genotype evaluation (Yan & Holland, 2010). AGGE biplot presents the average 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 (Yan, Hunt, Sheng, & Szlavnics, 2000).

Although there are no exact 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 environments 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 (right angle). Additionally, within the environmental group, E1 was apparently less associated with E3, while strongly positively correlated were E2 and E3.

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

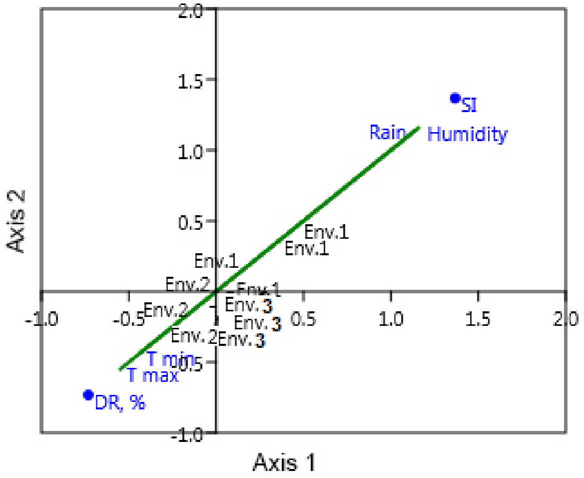

Fig. 2. CCA graph based on the correlation of DR and I of *Bruchus rufimanus* for 23 lupine cultivars according to several climatic parameters. The period analyzed was from April to June, T_{max} = maximum temperature; T_{min} = minimum temperature; DR = seed damage rate (%); SI, %= susceptibility index.

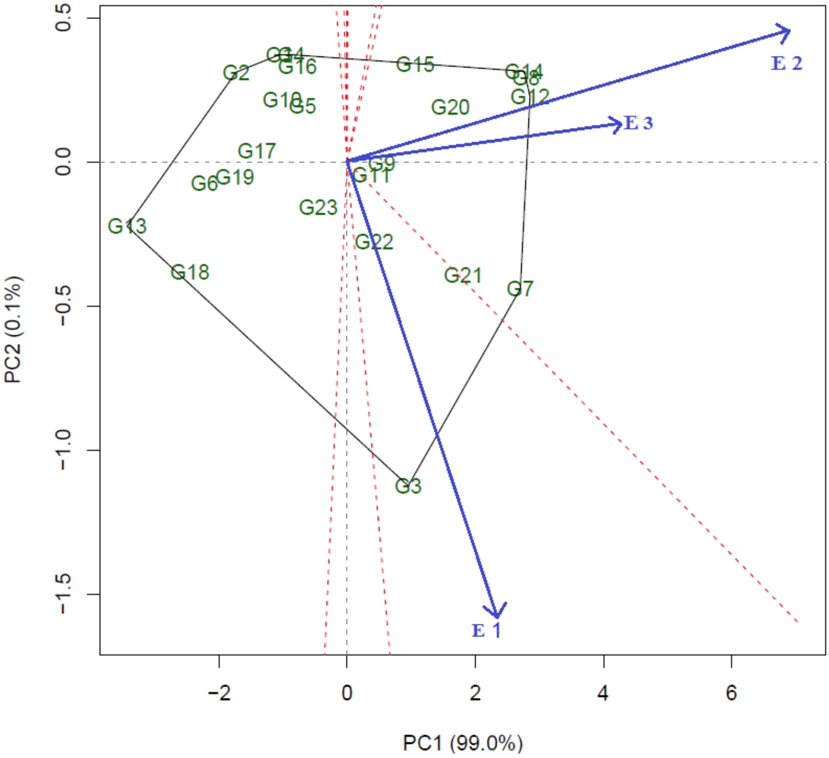

Fig. 3. The GGE biplot based on seed damage rate (2014-2016 period). The genotypes are designated with the symbol “G”, and the respective number from 1 to 23, as followed: G1-Astra, G2-Nahrquell, G3-Ascar, G4-BGR 6305, G5-Shienfield Gard, G6-WAT, G7-Kijewskij Mutant, G8-Hetman, G9-Start, G10-Amiga, G11-Garant, 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 strongly overlapped, as well as G1 and G4; G5 and G10 genotypes.

In order to determine which of the 23 lupine genotypes studied were the least affected by *B. rufimanus* 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 exposed 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 position close to the axis 1. 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 by *B. rufimanus*. Relatively stable and damage tolerant with somewhat difference among each other, exhibited G1, G4 and G16. Genotype G2 had lower values for that trait, but it 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, the values of G12, G8, and G14 to PC2 are equally distantly situated at zero pointing to higher variability (poorer stability). The same level of poor stability and damage sensitivity also showed G7 and G21 genotypes. The variables of the five genotypes above mentioned were highly expressed in E3 and E2 environments.

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

The first two principal components (PC1 and PC2) determined 99.1% of the dispersion.

The GGE biplot based on SI analysis (Fig. 4) represented 96.2% of the total trait variation between the first two principal components (PC1 and PC2). The environment with the shortest vector was E1, and the longest E2, respectively. The most discriminative environment was E2 in which less rainfall was registered. The genotype 6 (G6) was the most responsive to that trait (the lowest value of SI - 5.6%) followed by G19, G18, G13 (7.4; 7.9 and 9.0%, respectively) (see Table 2). A similar level of sensitivity showed G2 and G1, too. According to the ordinate, G6 was highly stable, followed by G19 within the group of the low susceptibility index. Lower variability had G18 and G13 genotypes. The sensitivity index at genotype 4 (G4) was close to the average for the biplot.

The genotype presented the highest value of SI and identified as highly sensitive was G14, followed by G12 and G7, respectively. Furthermore, the genotype 14 (G14) was considerably variable (poor stability) together with G22. Also, G14 had the highest value in E2, which was the most favourable for its susceptibility.

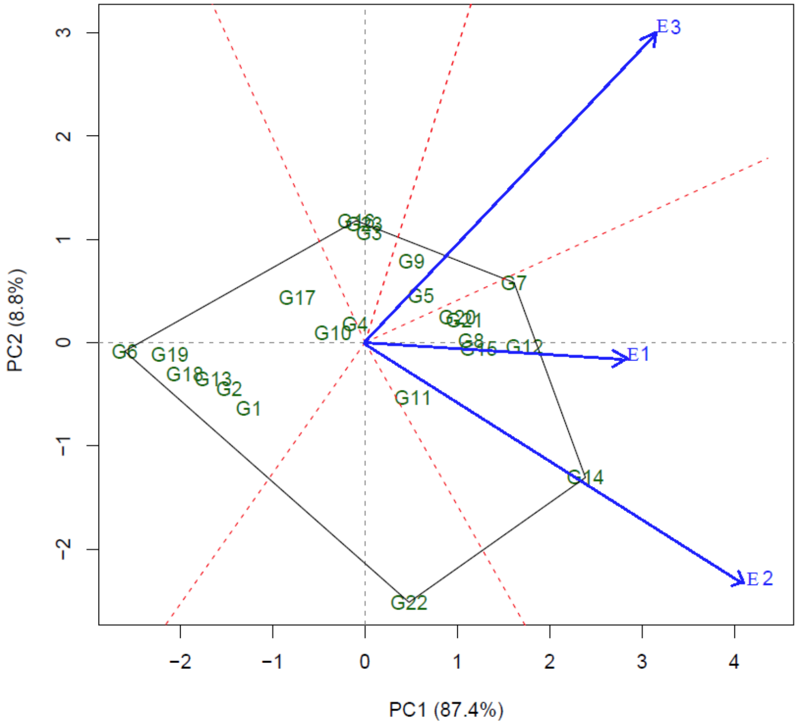

Fig. 4. The GGE biplot based on susceptibility index (2014-2016 period). The genotypes are designated with the symbol "G" and the respective number from 1 to 23, as follow G1-Astra, G2-Nahrquell, G3-Ascar, G4-BGR 6305, G5-Shienfield Gard, G6-WAT, G7-Kijewskij Mutant, G8-Hetman, G9-Start, G10-Amiga, G11-Garant, 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 strongly overlapped, as well as G21 and G20 genotypes.

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 decreased DR and SI values 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 the 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 lupines) (Keneni et al, 2011). The discrepancy between the phenological development of the host plant and the life cycle of *B. rufimanus* 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 (Fig. 5). After passing of the budding stage were found differences in the growing period length. *Astra*, *Termis Mestnii* and *Barde* were characterized by the lowest duration of the germination-beginning of flowering period (37 days). *Pink Mutan*t (G19), *Solnechnii* (G18), and *Bezimenii* 1 (G13) had a lower duration of that period (38 days). The early cultivars (with early flowering) reached technical maturity on average after 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 1.00-1.14, N_{min} and N_{max}: 37-38 days). 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, N_{min} and N_{max}: 39-40 days), and the late-ripening ones - *Hetman* (G8), *Shienfield Gard* (G5) and *Nahrquell* (G2) (coefficient of early-ripeness > 1.66, N_{min} and N_{max}: 41 days).

Several cultivars of the ultra-early ripening group stood out with considerably lower values of damage traits (DR and SI). For example, *Pink Mutant*, *Solnechnii*, and *Bezimenii* 1 had early flowering and slightly preference by *B. rufimanus*, 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 the species' tolerance to *B. rufimanus*.

In the previous studies, the influence of cultivar on damage caused to *Vicia faba* L. grain by *B. rufimanus* was evidented (Ebedah, Mahmoud, & Moawad, 2006; Szafirowska, 2012). In these studies was suggested that plant architecture, flowering period and abundance, and the timing of pod formation were the key factors affect the activity of *B. rufimanus*. According to Bruce, Martin, Smart, & Pickett (2011), Ceballos, Fernbndez, Zyciga, & Zapata (2015), several plant characteristics could adversely affect insect behaviour: the 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 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, the data obtained from the present study 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 index values (from 5.6 to 7.9%). As contrary, 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, Mexia, Duarte, Pereira & Tavares de Sousa (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 embryonic 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 *B. rufimanus* adults for feeding as the result of chemical plant factors that adversely affect insect behaviour. Probably, studied lupine 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 lupines was indicated by Ströcker, Wendt, Kirchner & Struck (2013). The presence of such antinutrient substances in the genotype-host probably repelled *B. rufimanus* and explain the weak preferences of bruchids.

Regarding effect of some botanical oils, including lupine seeds on the granary weevil, *Sitophilus granarius* L. (Curculionidae) reported Makarem, Kholy, Abdel-Latif & Seif (2017). According to the 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 to other oils.

On the other hand, proteinase inhibitors are potential candidates for biocontrol of insect pests since insect digestive proteinases are promising targets towards control of various insects (Sharma, Nath, Kumari, & Bhardwaj, 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. Although the proteases were not investigated in the present study, seed genotypes slightly affected by *B. rufimanus* had presumably protease inhibitors strongly suppressing its activity.

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 in Table 3 showed that the interaction of plant traits had a significant effect on the damaged seed rate. The susceptibility index (SI) 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* selection (r= −1.687), but negatively correlated. 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 obtained , G6, G19, G18 and G13 genotypes seems to have a clear advantage in defending itself from *B. rufimanus* attack. The low DR and SI values make genotypes particularly interesting for breeding purposes due to 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 is of great importance due to the durability of the tolerance to *B. rufimanus*, and successfully overcome an attack if one of these levels is broken.

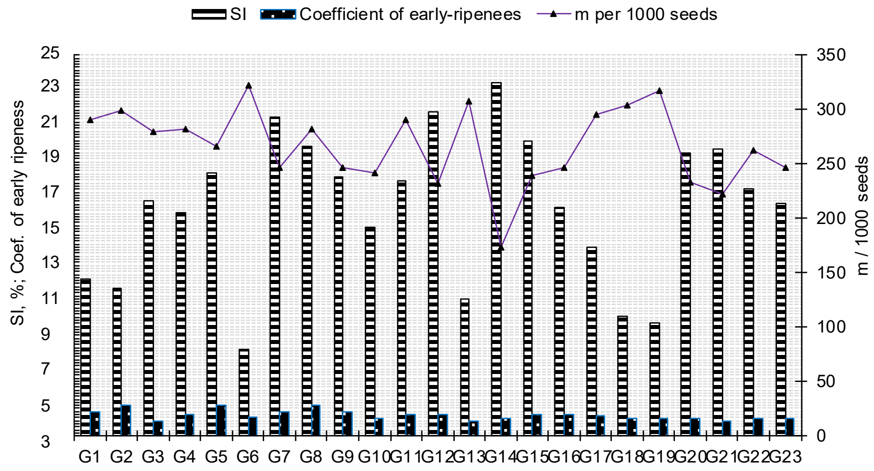

Fig. 5. Characteristics of lupine genotypes. Legend: SI- susceptibility index; G1-Astra, G2-Nahrquell, G3-Ascar, G4-BGR 6305, G5-Shienfield Gard, G6-WAT, G7-Kijewskij Mutant, G8-Hetman, G9-Start, G10-Amiga, G11-Garant, G12-Tel Keram, G13-Bezimenii 1, G14-Bezimenii 2, G15-Pflugs Ultra, G16-Termis Mestnii,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

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

CONCLUSIONS

Bruchus rufimanus damage was affected by climatic parameters. The susceptibility index (SI) of damaged seeds was positively correlated with precipitation amount and humidity, and inversely to minimal and maximal temperature values. The seed damage rate was positively correlated with temperature, but negatively to rain and humidity.

The local Polish population *WAT* and cultivars *Pink Mutant*, *Solnechnii*, and *Bezimenii* 1 (G6, G19, G18 and G13 genotypes, respectively) had the lowest seed damage rate and stable position across all environments. Meanwhile, these cultivars showed a low susceptibility index (SI) 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 tolerance to bruchids. Correlations between seed damage rate (DR) and susceptibility index (SI) as well as the mass of 1000 seeds and sensitivity index were strongly positive and negative, respectively.

Cultivars *Pink Mutant*, *Solnechnii*, *Bezimenii* 1 and local Polish population *WAT* had a clear advantage in defending itself from *B. rufimanus* attack, which makes them particularly interesting for breeding purposes.

The matching of early flowering with higher seed weight in cultivars could be used as a marker of tolerance to *B. rufimanus* broad bread weevil, and apparently an effective mechanism of plant defense.

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