Bulletin of Entomological Research (2016) **106**, 268–277 doi:10.1017/S0007485315001133

Pea weevil damage and chemical characteristics of pea cultivars determining their resistance to *Bruchus pisorum* L.

I. Nikolova*

Department of technology and ecology of forage crops, Institute of Forage
Crops General Vladimir Vazov 89, 5800 Pleven, Bulgaria

Abstract

Bruchus pisorum (L.) is one of the most intractable pest problems of cultivated pea in Europe. Development of resistant cultivars is very important to environmental protection and would solve this problem to a great extent. Therefore, the resistance of five spring pea cultivars was studied to *B. pisorum*: Glyans, Modus; Kamerton and Svit and Pleven 4 based on the weevil damage and chemical composition of seeds. The seeds were classified as three types: healthy seeds (type one), damaged seeds with parasitoid emergence holes (type two) and damaged seeds with bruchid emergence holes (type three). From visibly damaged pea seeds by pea weevil *B. pisorum* was isolated the parasitoid *Triaspis thoracica* Curtis (Hymenoptera, Braconidae). Modus, followed by Glyans was outlined as resistant cultivars against the pea weevil. They had the lowest total damaged seed degree, loss in weight of damaged seeds (type two and type three) and values of susceptibility coefficients. A strong negative relationship ($r = -0.838$) between the weight of type one seeds and the proportion of type three seeds was found. Cultivars with lower protein and phosphorus (P) content had a lower level of damage. The crude protein, crude fiber and P content in damaged seeds significantly or no significantly were increased as compared with the healthy seeds due to weevil damage. The P content had the highest significant influence on pea weevil infestation. Use of chemical markers for resistance to the creation of new pea cultivars can be effective method for defense and control against *B. pisorum.*

Keywords: *Bruchus pisorum*, pea cultivars, damaged seeds, chemical seed composition, resistance

(Accepted 21 December 2015; First published online 3 February 2016)

Introduction

The pea weevil, *Bruchus pisorum* (L.) (Coleoptera: Chrysomelidae), is one of the most intractable pest problems of cultivated pea, *Pisum sativum* L., in Europe (Burov, 1980; Marzo *et al.*, 1997; Girsch *et al.*, 1999). It causes considerable damage to the pea plants. Many authors (Ali *et al.*, 1994; Damte & Dawd, 2003) found that losses as high as 50% may often be encountered in pea. Even with only a small amount of actual biological losses by seed yield per plant, economic losses can reach up to 100% (Boeke *et al.*, 2004; Somta *et al.*, 2006).

Pea weevils attack peas that are grown in fields. Infestation results in seeds that may not germinate or produce weak plants. Weevils cannot persist in storage as they cannot re-infest stored seed. Females lay eggs on the outside of the pod. Larvae develop in growing seeds within the pods. After pupation within the seed, the adult chews an exit hole through the seed coat. Damage is distinctive. Both adult and larvae feed on the inside of seeds. The final effect of seeds with a beetle infestation on the germination of host legumes can be unforeseeable (Fox *et al.*, 2012). In some cases, the larva feeding effectively kills the embryo or removes so much endosperm that the seed cannot germinate (Fox *et al.*, 2012).

The pea weevil control is difficult and mainly conducted through chemical means. Development of resistant cultivars

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*Author for correspondence
Phone: +359 884684575
Fax: +359 64805882
E-mail: imnikolova@abv.bg

would solve this problem to a great extent. In addition, in terms of the modern organic farming, the use of resistant cultivars against phytophagous insects is very important to environmental protection.

Entomologists have investigated morphological and biochemical bases of resistance to storage insect pests (Morton *et al.*, 2000; Shaheen *et al.*, 2006; Srinivasan & Durairaj, 2007; Acosta-Gallegos *et al.*, 2008). Literature surveys indicate that cultivars of chickpea grains often differ in resistance to bruchid incidence due to variable traits, and it is now the generally agreed-upon fact that a broad genetic base, formed upon physical or chemical characteristics of grains, is essential for crop improvement (Sarwar, 2012). The author reported that the variation in seed parameters was primarily due to variation in percent infestation level, adult emergence, reduction in seed weight and inherent capacity of each genotype to be attacked by bruchids.

According to some authors, chemical composition is the main responsible for the resistance against bruchids, no tegument thickness (Moss & Credland, 1994; Maldonado *et al.*, 1996, Ghizdavu *et al.*, 1997). Regnault-Roger (1999) concluded that even though a clear mechanism of leguminous resistance against bruchids is not well known yet, isolation and characterization of the chemical components and the quantification of their role in resistance determination being necessary.

Haruta *et al.* (2001) found that plant–insect interaction is a dynamic system, subjected to continual variation and change. In order to reduce insect attack, plants developed different protective mechanisms, including chemical and physical barriers, such as the induction of defensive proteins. Similar results reported Leite de Lima *et al.* (2001) and according to them a reduction in the bruchids infestation probably due to chemical substances present in the grains that affected the survival of the insects. Yankova *et al.* (2007) suggested that varieties with low trypsin inhibitor activity had a relatively low percentage of damage seeds.

In the earlier, our study was found that in the pea weevil damaged seeds, crude protein (CP), total phenols, water-soluble sugars and phosphorus (P) contents were increased, while the calcium (Ca) content and trypsin inhibitory activity were decreased (Nikolova *et al.*, 2009). Similar results reported War *et al.* (2012). According to the authors, the protein content was increased in insect-damaged groundnut genotypes as compared with uninfected control plants.

Use of different markers for resistance as a genetic material in the creation of new pea cultivars is one of the most effective methods for defense and control against *B. pisorum*. The introduction of resistant pea cultivars would help farmers reduce losses due to pea weevil and provide an environmentally safer option to weevil control.

The aim of this study was to determine the resistance in five pea cultivars to *B. pisorum* based on the weevil damage and chemical composition of seeds.

Material and methods

During the 2012–2014 period in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria (43°23.312′N; 24° 34.856′E; altitude 230 m), a study was conducted on the resistance of five spring pea cultivars to *B. pisorum* L. (Coleoptera: Chrysomelidae): Glyans, Modus; Kamerton and Svit (Ukrainian cultivars) and Pleven 4 (Bulgarian cultivar). The field trial was conducted using a long-plot design with a sowing rate of 120 germinating seeds m^{-2} in three replications, plot size of 4 m^2 and a natural background of soil supply with the major nutrients. In the long-plot design, the replications are arranged in an elongate strip, i.e. the replications are arranged one after the other. The method was applied because the soil fertility was equalized. The soil type was a leached chernozem with $pH_{(KCl)}$ – 5.49 and content of total N – 34.30 mg 1000^{-1} g soil, P_20_5 – 3.72 mg 100^{-1} g soil as well as K_20 – 37.50 mg 100^{-1} g soil. No pesticides were applied.

After pea harvesting bulk samples, containing 1500–2000 seeds, were taken for each cultivar. The seeds of each cultivar every year were classified as three types: healthy seeds (type one), damaged seeds with parasitoid emergence holes (type two) and damaged seeds with bruchid emergence holes (type three). For every cultivar and type of seeds, the seed damage ratings were evaluated by taking 200 seeds in each replication. Seed damage ratings were determined based on comparing the recorded number of seeds without damage with the number of damaged seeds.

Susceptibility coefficient (q, %) was calculated by the following formula: $q = (a–b)/a\ x\ 100$ (a, weight of 1000 healthy seeds; b, weight of 1000 seeds damaged by *B. pisorum*).

From visibly damaged pea seeds by pea weevil, *B. pisorum* was isolated the parasitoid *Triaspis thoracica* Curtis (Hymenoptera, Braconidae).

The chemical composition of the grain was determined in the chemical laboratory of the Institute as follows: CP by Keldahl method (Kjeldahl, 1883), crude fiber (CF) by Weende method (AOAC Official Method) and P colorimetrically by the hydroquinone method and Ca – complexometrically.

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

Results

Tested cultivars had a different proportion of seed damage from the three types (table 1). Modus was distinguished with the lowest seed damage with bruchid emergence holes (type three) over the years (ranged from 17.6 to 23.5%) and the average for the period (17.1%) ($P < 0.05$) – table 2. Differences between Modus and other cultivars were statistically significant. The opposite trend was observed in Pleven 4, which had the highest values (average 44.2%) and differences to others were statistically significant ($P < 0.05$). At the other cultivars, the seeds from the type three had similar values as in Glyans was found significantly lower seed damage in 2012 and the average for the period.

Of importance was that there were differences between cultivars concerning the damaged seeds with parasitoid emergence holes (type two). These seeds looked like a prick. The highest value of this type of damage over the years and average for the period was found in Modus by 19.4% (excluding 2013 when differences between Modus and Kamerton were not significant) and differences to others were significant. Prevailing trend over the years was a lower proportion of damaged seeds from type two in Pleven 4 and Kamerton an average of 10.8 and 12.3%, respectively as differences between them and other cultivars were significant ($P < 0.05$).

In terms of the total seed damage ratings regardless of seed types average for 2012–2014, Modus had the lowest values (36.5%) and Pleven 4 – the highest (55.0%) as differences between them and other cultivars were significant ($P < 0.05$). In the other cultivars, the parameter varied in similar levels as significant difference was observed between Glyans and Svit.

Table 1. Seed damage ratings by Bruchus pisorum in pea cultivars.

Cultivars	Number examined seeds	% damaged seeds with bruchid emergence holes	% damaged seeds with parasitoid emergence holes	% damaged seeds, total
		2012		
Glyans	1558	25.6 b[1]	15.0 b	40.7 a
Cvit	1770	31.7 c	17.1 d	48.9 b
Kamerton	1668	30.9 c	12.2 a	43.1 a
Modus	1401	17.6 a	22.3 e	40.0 a
Pleven 4	1720	47.6 d	16.1 c	63.7 c
		2013		
Glyans	2148	32.9 b	21.5 b	54.4 ab
Cvit	2249	36.7 c	20.9 b	57.6 b
Kamerton	2075	33.9 b	22.0 bc	55.9 b
Modus	1512	23.5 a	26.8 c	50.3 a
Pleven 4	2719	56.1 d	14.1 a	70.2 c
		2014		
Glyans	1267	19.2 b	3.3 a	22.6 ab
Cvit	1943	21.0 b	2.1 a	23.1 ab
Kamerton	1392	21.5 b	2.6 a	24.0b
Modus	2179	10.1 a	9.1 b	19.2a
Pleven 4	2443	28.8 c	2.2 a	31.1c
		Average for 2012–2014		
Glyans	1658	25.9 b	13.3 b	39.2 b
Cvit	1987	29.8 c	13.4 b	43.2 c
Kamerton	1712	28.8 c	12.3 ab	41.0 bc
Modus	1697	17.1 a	19.4 c	36.5 a
Pleven 4	2294	44.2 d	10.8 a	55.0 d

[1] Means in each row followed by the same letters are not significantly different ($P > 0.05$).

Table 2. Analysis of variance.

Source	Sum of squares	df	Mean square	*F*-ratio	*P*-value	Source	Sum of squares	df	Mean square	*F*-ratio	*P*-value
2012						2014					
% damaged seeds with bruchid emergence holes											
Variety	1452.71	4	363.178	76.82	0.0000	Variety	537.895	4	134.474	18.9	0.0004
Replications	30.3849	2	15.1924	3.21	0.0945	Replications	4.53509	2	2.26755	0.32	0.7359
Residual	37.8216	8	4.7277			Residual	56.9223	8	7.11529		
Total (corr.)	1520.92	14				Total (corr.)	599.353	14			
% damaged seeds with parasitoid emergence holes											
Variety	166.037	4	41.5093	616.48	0.0000	Variety	105.947	4	26.4867	14.4	0.0010
Replications	2.66133	2	1.33067	19.76	0.0008	Replications	0.25996	2	0.12998	0.07	0.9324
Residual	0.538667	8	0.067333			Residual	14.7187	8	1.83984		
Total (corr.)	169.237	14				Total (corr.)	120.926	14			
% damaged seeds, total											
Variety	1161.15	4	290.287	58.03	0.0000	Variety	226.852	4	56.713	8.22	0.0062
Replications	50.5619	2	25.281	5.05	0.0381	Replications	2.63489	2	1.31745	0.19	0.8298
Residual	40.0205	8	5.00257			Residual	55.1673	8	6.89591		
Total (corr.)	1251.73	14				Total (corr.)	284.654	14			
2013						Average 2012–2014					
% damaged seeds with bruchid emergence holes											
Variety	1716.59	4	429.148	396.86	0.0000	Variety	1147.59	4	286.897	147.09	0.0000
Replications	0.600573	2	0.300287	0.28	0.7645	Replications	2.98812	2	1.49406	0.77	0.4962
Residual	8.65083	8	1.08135			Residual	15.6038	8	1.95048		
Total (corr.)	1725.84	14				Total (corr.)	1166.18	14			
% damaged seeds with parasitoid emergence											
Variety	248.13	4	62.0326	9.88	0.0035	Variety	130.035	4	32.5088	25.17	0.0001
Replications	23.8522	2	11.9261	1.9	0.2113	Replications	2.20937	2	1.10469	0.86	0.4606
Residual	50.2227	8	6.27784			Residual	10.3311	8	1.29139		
Total (corr.)	322.205	14				Total (corr.)	142.576	14			
% damaged seeds, total											
Variety	672.567	4	168.142	33.48	0.0000	Variety	612.446	4	153.111	97.77	0.0000
Replications	27.4757	2	13.7378	2.74	0.1244	Replications	8.86576	2	4.43288	2.83	0.1176
Residual	40.1825	8	5.02281			Residual	12.5282	8	1.56603		
Total (corr.)	740.225	14				Total (corr.)	633.84	14			

Total (corr.), – Total (corrected).

Table 3. Weight of 1000 seeds (g) and susceptibility coefficient (q, %) in pea cultivars.

Cultivars	Type one	Type two	Type three Weight	Total q_1	 q_2	 q_3
			2012			
Glyans	236.60 d[1]/c[2]	230.35 d/b	214.34 d/a	2.65 ab	9.42 ab	12.07 ab
Cvit	197.55 b/b	192.70 b/b	175.37 b/a	2.44 ab	11.23 bc	13.67 bc
Kamerton	195.89 b/b	188.55 b/b	169.84 b/a	3.76 b	13.23 c	17.00 c
Modus	207.76 c/c	204.76 c/b	194.09 c/a	1.44 a	6.57 a	8.01 a
Pleven 4	123.33 a/c	115.14 c/b	98.77 a/a	6.65 c	19.94 d	26.58 d
			2013			
Glyans	225.00 c/c	189.95 c/b	174.32 d/a	15.59 ab	22.5 b	38.09 b
Cvit	231.33 c/c	193.37 c/b	179.61 d/a	16.41 ab	22.36 b	38.77 b
Kamerton	192.00 b/c	155.47 b/b	141.50 b/a	18.95 b	26.28 c	45.23 c
Modus	186.03 b/b	158.75 b/a	153.02 c/a	14.65 a	17.76 a	32.41 a
Pleven 4	154.00 a/c	117.86 a/b	103.35 a/a	23.45 c	32.89 d	56.34 d
			2014			
Glyans	224.82 c/b	220.83 d/b	205.21 c/a	1.76 ab	8.73 c	10.49 bc
Cvit	220.27 c/b	215.82 cd/ab	207.44 c/a	2.03 ab	5.84 b	7.87 b
Kamerton	200.56 b/c	192.3 b/b	182.30 b/a	4.08 bc	9.09 c	13.17 c
Modus	208.00 b/a	206.10 c/a	203.00 c/a	0.89 a	2.38 a	3.27 a
Pleven 4	151.00 a/c	143.02 a/b	125.68 a/a	5.29 c	16.77 d	22.06 d
			Average 2012–2014			
Glyans	228.80 d/c	213.68 e/b	197.96 d/a	6.66 a	13.55 b	20.21 b
Cvit	216.38 c/c	200.63 d/b	187.47 c/a	6.96 a	13.14 b	20.10 b
Kamerton	196.15 b/c	178.77 b/b	164.55 b/a	8.93 b	16.20 c	25.13 c
Modus	200.60 b/b	189.87 c/a	183.37 c/a	5.66 a	8.90 a	14.56 a
Pleven 4	142.78 a/c	125.34 a/b	109.27 a/a	11.80 c	23.20 d	35.00 d

Type one-healthy seeds, Type two-damaged seeds with parasitoid emergence holes, Type three-damaged seeds with bruchid emergence holes; q_1 – susceptibility coefficient of Type two seeds; q_2- susceptibility coefficient of Type three seeds; q_3- susceptibility coefficient of Type two and Type three seeds.

[1] Means in each column followed by the same letters are not significantly different ($P > 0.05$).

[2] Means in each row followed by the same letters are not significantly different ($P > 0.05$).

In a comparative analysis of influence of weight seeds on the degree of damage was observed following trend – the cultivars with higher weight seeds had lower rates of damage (table 3). Pleven 4 had the lowest weight of 1000 healthy seeds (142.8 g) as simultaneously, it had the highest total rate of damage (55.0%). It was found that between the weight of healthy seeds and the proportion of damaged seeds with bruchid emergence holes was a strong negative relationship ($r = -0.838$).

As a result of the harmful effect to *B. pisorum* was the loss in weight of damaged seeds (types two and three) as compared to healthy seeds differences were always statistically significant ($P < 0.05$) – table 4(C) and (D). It should be noted that Modus had the lowest reduction of the weight in types two and three damaged seeds. The cultivar was characterized by the lowest values of susceptibility coefficients q_1, q_2 and q_3 (8.9; 5.7 and 14.6%, respectively, average for the period) as compared with other cultivars, statistically significant differences not found only between the values of q_1 average for the period – table 4(A) and (B).

Pleven 4 had the highest values of q_1 and q_2 – 23.2 and 11.8%, respectively. The reduction in the seed weight, expressed through relevant susceptibility coefficients in Glyans and Cvit had similar values. In Kamerton were observed higher susceptibility coefficients, as differences between the cultivar and Glyans and Svit were significant ($P < 0.05$).

The chemical composition of healthy seeds (CP and *CF* contents, mineral composition) was different depending test cultivars (table 5). Glyans and Modus had the lowest CP content in the healthy seeds, while Pleven 4 – the highest ($P < 0.05$) as differences between them and the others were statistically significant ($P < 0.05$). The opposite trend was observed concerning CF content as Pleven 4 had the lowest value. The variety were characterized with the lowest Ca and the highest P content as differences between it and the others were statistically significant ($P < 0.05$). Ca and P concentrations were similar in the other cultivars.

The result of the harmful effect by pea weevil was an increase quantity of CP, CF and P in the type two and type three seeds. The increase of the CP, CF and P content was expressed to a greater extent in damaged seeds from type three than in the damaged seeds from type two. Differences between the health and damaged seeds from type three were statistically significant in all tested cultivars (except Cvit about CF). It simultaneously was observed a significant increase in the content of those components in the type three damaged seeds to type two (except Glyans regarding to CP and Glyans, Kamerton and Pleven 4 regarding to CF).

Trend of decrease of Ca content in damaged seeds predominated (in Cvit, Kamerton and Modus).

The results of carrying out analysis showed that the linear component in the regression of insect density with respect of the investigated chemical traits was significant (table 6). From the complex study of the traits was obtained model (1) which demonstrated the complicated character of the change of density depending on the variation of investigating plant traits.

The common type of the obtained equation of regression was:

$$Y = 11.3632 + 0.0340285^{*}X_1 - 0.147315^{*}X_2 - 3.79988^{*}X_3 + 4.11145^{*}X_4;$$

Table 4. Analysis of variance, (A) 2012 and 2013; (B) 2014 and average, 2012–2014; (C) 2012 and 2013; (D) 2014 and average 2012–2014.

Source	Sum of squares	df	Mean quare	*F*-ratio	*P*-value	Source	Sum of squares	df	Mean quare	*F*-ratio	*P*-value
(A)											
2012						2013					
Weight of healthy seeds											
Variety	20996.7	4	5249.18	303.84	0.0000	Variety	11858.9	4	2964.72	207.5	0.0000
Replications	49.5841	2	24.792	1.44	0.2934	Replications	13.8915	2	6.94573	0.49	0.632
Residual	138.208	8	17.276			Residual	114.301	8	14.2876		
Total (corr.)	21184.5	14				Total (corr.)	11987.1	14			
Weight of damaged seeds with parasitoid emergence holes											
Variety	22174.1	4	5543.53	306.89	0.0000	Variety	11264.9	4	2816.22	562.36	0.0000
Replications	89.5732	2	44.7866	2.48	0.1452	Replications	11.7879	2	5.89393	1.18	0.3564
Residual	144.509	8	18.0637			Residual	40.063	8	5.00788		
Total (corr.)	22408.2	14				Total (corr.)	11316.7	14			
Weight of damaged seeds with bruchid emergence holes											
Variety	22942.2	4	5735.54	371.59	0.0000	Variety	11176.3	4	2794.06	292.89	0.0000
Replications	40.7797	2	20.3899	1.32	0.3193	Replications	16.2631	2	8.13153	0.85	0.4618
Residual	123.481	8	15.4351			Residual	76.3174	8	9.53968		
Total (corr.)	23106.4	14				Total (corr.)	11268.8	14			
q parasitoid emergence holes											
Variety	48.0432	4	12.0108	12.01	0.0018	Variety	150.027	4	37.5068	6.83	0.0108
Replications	2.22937	2	1.11469	1.11	0.374	Replications	1.88437	2	0.942187	0.17	0.8455
Residual	7.99876	8	0.999845			Residual	43.9638	8	5.49548		
Total (corr.)	58.2713	14				Total (corr.)	195.875	14			
q bruchid emergence holes											
Variety	303.537	4	75.8841	28.61	0.0001	Variety	382.666	4	95.6666	53.82	0.0000
Replications	4.23921	2	2.11961	0.8	0.4826	Replications	0.429373	2	0.214687	0.12	0.8878
Residual	21.2186	8	2.65232			Residual	14.2206	8	1.77758		
Total (corr.)	328.994	14				Total (corr.)	397.316	14			
q total											
Variety	588.897	4	147.224	25.24	0.0001	Variety	1001.43	4	250.358	22.31	0.0002
Replications	6.83764	2	3.41882	0.59	0.5787	Replications	2.53492	2	1.26746	0.11	0.8946
Residual	46.663	8	5.83288			Residual	89.7565	8	11.2196		
Total (corr.)	642.398	14				Total (corr.)	1093.72	14			
(B)											
2014						Average, 2012–2014					
Weight of healthy seeds											
Variety	10462.4	4	2615.6	68.75	0.0000	Variety	13020.6	4	3255.15	262.74	0.0000
Replications	80.7269	2	40.3634	1.06	0.3902	Replications	41.6844	2	20.8422	1.68	0.2456
Residual	304.358	8	38.0448			Residual	99.1149	8	12.3894		
Total (corr.)	10847.5	14				Total (corr.)	13161.4	14			
Weight of damaged seeds with parasitoid emergence holes											
Variety	11792	4	2948.01	78.4	0.0000	Variety	13897.1	4	3474.28	984.74	0.0000
Replications	47.5764	2	23.7882	0.63	0.5558	Replications	19.1189	2	9.55946	2.71	0.1263
Residual	300.805	8	37.6007			Residual	28.225	8	3.52813		
Total (corr.)	12140.4	14				Total (corr.)	13944.5	14			
Weight of damaged seeds with bruchid emergence holes											
Variety	14284.1	4	3571.04	198.22	0.0000	Variety	14918.1	4	3729.52	680.06	0.0000
Replications	55.1985	2	27.5992	1.53	0.2734	Replications	31.3484	2	15.6742	2.86	0.1157
Residual	144.125	8	18.0157			Residual	43.8727	8	5.48408		
Total (corr.)	14483.5	14				Total (corr.)	14993.3	14			
q parasitoid emergence holes											
Variety	39.5016	4	9.87539	5.04	0.0251	Variety	70.7944	4	17.6986	15.32	0.0008
Replications	5.15685	2	2.57843	1.32	0.3203	Replications	0.784893	2	0.392447	0.34	0.7218
Residual	15.6605	8	1.95756			Residual	9.24504	8	1.15563		
Total (corr.)	60.3189	14				Total (corr.)	80.8244	14			
q bruchid emergence holes											
Variety	339.9	4	84.975	70.24	0.0000	Variety	334.337	4	83.5843	102.26	0.0000
Replications	3.11225	2	1.55613	1.29	0.3278	Replications	1.09297	2	0.546487	0.67	0.5389
Residual	9.67848	8	1.20981			Residual	6.53909	8	0.817387		
Total (corr.)	352.691	14				Total (corr.)	341.969	14			
q total											
Variety	588.365	4	147.091	26.6	0.0001	Variety	707.313	4	176.828	56.97	0.0000
Replications	0.74788	2	0.37394	0.07	0.9351	Replications	2.38692	2	1.19346	0.38	0.6927
Residual	44.2342	8	5.52927			Residual	24.8305	8	3.10382		
Total (corr.)	633.347	14				Total (corr.)	734.531	14			

Table 4. (*Cont.*)

Source	Sum of squares	df	Mean quare	*F*-ratio	*P*-value	Source	Sum of squares	df	Mean quare	*F*-ratio	*P*-value
(C)											
2012						2013					
Glyans											
Seed type	791.08	2	395.54	151.71	0.0002	Seed type	4039	2	2019.5	263.94	0.0001
Replications	34.1772	2	17.0886	6.55	0.0547	Replications	6.6744	2	3.3372	0.44	0.674
Residual	10.4287	4	2.60718			Residual	30.6053	4	7.65133		
Total (corr.)	835.686	8				Total (corr.)	4076.28	8			
Cvit											
Seed type	815.887	2	407.944	54.95	0.0012	Seed type	4306.45	2	2153.23	608.99	0.0000
Replications	29.5425	2	14.7712	1.99	0.2513	Replications	16.5692	2	8.28458	2.34	0.2121
Residual	29.6967	4	7.42418			Residual	14.1428	4	3.53571		
Total (corr.)	875.126	8				Total (corr.)	4337.17	8			
Kamerton											
Seed type	1082.54	2	541.271	119.32	0.0003	Seed type	4079.55	2	2039.78	109.52	0.0003
Replications	417.627	2	208.814	46.03	0.0017	Replications	36.1068	2	18.0534	0.97	0.4537
Residual	18.1449	4	4.53623			Residual	74.4994	4	18.6249		
Total (corr.)	1518.31	8				Total (corr.)	4190.16	8			
Modus											
Seed type	309.444	2	154.722	204.7	0.0001	Seed type	1867.06	2	933.532	488.22	0.0000
Replications	2.6502	2	1.3251	1.75	0.284	Replications	70.8065	2	35.4032	18.52	0.0095
Residual	3.02333	4	0.755833			Residual	7.64851	4	1.91213		
Total (corr.)	315.118	8				Total (corr.)	1945.52	8			
Pleven 4											
Seed type	937.919	2	468.96	311.78	0.0000	Seed type	4082.35	2	2041.18	816.11	0.0000
Replications	34.8278	2	17.4139	11.58	0.0217	Replications	5.56647	2	2.78323	1.11	0.4128
Residual	6.01653	4	1.50413			Residual	10.0045	4	2.50112		
Total (corr.)	978.764	8				Total (corr.)	4097.92	8			
(D)											
2014						Average 2012–2014					
Glyans											
Seed type	644.536	2	322.268	25.03	0.0055	Seed type	1426.52	2	713.262	130.67	0.0002
Replications	59.2523	2	29.6261	2.3	0.2162	Replications	28.0131	2	14.0065	2.57	0.1919
Residual	51.4955	4	12.8739			Residual	21.8335	4	5.45837		
Total (corr.)	755.284	8				Total (corr.)	1476.37	8			
Cvit											
Seed type	254.405	2	127.203	3.09	0.1546	Seed type	1257.33	2	628.667	210.85	0.0001
Replications	80.2384	2	40.1192	0.97	0.4524	Replications	10.0718	2	5.03588	1.69	0.2939
Residual	164.83	4	41.2076			Residual	11.9265	4	2.98163		
Total (corr.)	499.474	8				Total (corr.)	1279.33	8			
Kamerton											
Seed type	501.478	2	250.739	11.4	0.0223	Seed type	1502.77	2	751.385	115.69	0.0003
Replications	7.97736	2	3.98868	0.18	0.8406	Replications	69.9083	2	34.9541	5.38	0.0734
Residual	87.9639	4	21.991			Residual	25.9797	4	6.49493		
Total (corr.)	597.42	8				Total (corr.)	1598.66	8			
Modus											
Seed type	337.473	2	168.737	5.27	0.0756	Seed type	454.069	2	227.035	94.77	0.0004
Replications	38.22	2	19.11	0.6	0.5931	Replications	80.8833	2	40.4416	16.88	0.0112
Residual	128.031	4	32.0076			Residual	9.58298	4	2.39574		
Total (corr.)	503.724	8				Total (corr.)	544.536	8			
Pleven 4											
Seed type	1005.46	2	502.729	466.47	0.0000	Seed type	1685.32	2	842.659	1349.01	0.0000
Replications	11.2185	2	5.60923	5.2	0.0771	Replications	2.6666	2	1.3333	2.13	0.234
Residual	4.31093	4	1.07773			Residual	2.4986	4	0.62465		
Total (corr.)	1020.99	8				Total (corr.)	1690.48	8			

Total (corr.), Total (corrected).

where Y was the *B. pisorum* infestation; X_1 the crude protein; X_2 the crude fiber; X_3 the calcium; X_4 the phosphorus.

The applied analysis in table 7 showed that on pea weevil infestation, the highest significant influence had P content (4.111) followed by Ca (−3.800) which was with negative value. Considerably weaker influence had CP and CF.

Discussion

The number of emergence holes is a better indicator of seed resistance than the number of eggs present on the pods (Makanurn, 2010). It, therefore, was calculated the ratio of damaged seed from types two and three to healthy seeds (type one).

Table 5. Chemical composition of pea seed cultivars (g/kg dry matter) (on average for the period 2012–2014).

Seed type	Glyans	Cvit	Kamerton	Modus	Pleven 4	St.er.
CP						
One	203.7 a^1/a^2	227.85 a/c	236.00 a/d	222.45 a/b	242.65 a/d	1.432
Two	239.6 b/a	266.35 b/c	281.00 b/d	256.50 b/b	277.40 b/d	1.543
Three	244.4 b/a	283.77 c/c	292.53 c/d	267.27 c/b	307.90 c/e	1.339
St.er.	2.158	1.374	1.137	0.414	1.54	
CF						
One	71.55 a/bc	68.05 a/b	70.90 a/bc	72.15 a/c	62.70 a/a	1.08
Two	72.3 a/a	68.60 a/a	76.50 ab/b	72.60 a/ab	69.30 b/a	1.102
Three	75.3 a/ab	77.57 b/bc	80.37 b/c	84.63 b/d	71.80 b/a	0.987
St.er.	1.042	0.717	1.372	1.005	1.047	
Ca						
One	1.905 a/c	2.165 b/d	2.175 c/d	1.625 c/b	0.665 a/a	0.067
Two	2.14 a/c	1.745 a/b	1.520 b/b	0.965 b/a	1.575 b/b	0.088
Three	2.17 a/c	1.757 a/b	1.040 a/a	1.237 a/a	1.410 b/ab	0.104
St.er.	0.138	0.059	0.086	0.054	0.075	
P						
One	3.955 a/a	4.480 a/ab	4.795 a/b	4.910 a/b	5.495 a/c	0.15
Two	4.59 b/a	5.250 b/b	5.420 b/b	5.310 a/b	5.965 a/c	0.099
Three	5.28 c/a	5.880 c/b	6.293 c/c	6.407 b/c	7.677 b/d	0.092
St.er.	0.077	0.11	0.009	0.181	0.108	

Type one-healthy seeds, Type two-damaged seeds with parasitoid emergence holes, Type three-damaged seeds with bruchid emergence holes; St.er, Standard error.

[1] Means in each column followed by the same letters are not significantly different ($P > 0.05$).

[2] Means in each row followed by the same letters are not significantly different ($P > 0.05$).

Table 6. Regression analysis (ANOVA) of the insect density with regard to the chemical traits.

Dispersion	df	SS	MS	*F*-ratio	*P*-value
Model	4	244.578	61.145	17.262	0.000
Residual	10	35.422	3.542		
Total (Corr.)	14	280			

Modus was characterized with the lowest damaged seeds from the type three and the highest damaged seeds from type two ($P < 0.05$) as differences between Modus and others were significant. In the most sensitive cultivar, Pleven 4, was observed reverse trend. It was observed a negative correlation between the seed damage from type two and type three ($r = -0.863$). It was important for the developmental stage of the pea weevil at harvest time. When weevil larvae in the damaged seeds were in early instars, parasitoids reduced part of them. Modus had the shortest duration of flowering and pod development stages, and they occurred earlier than other cultivars (Nikolova, 2015). The development time of larvae to adult very likely was relatively insufficient and shortly, and damaged seeds from type two predominated at harvest time. In addition, the parasitoid probably was entered into the seeds in the moment, when bigger part of damaged seeds had younger host larvae. That resulted in the highest percentage of damaged seeds with the parasitoid emergence holes by 19.4% (on average over the period) in Modus. The difference between Modus and other cultivars was statistically significant. The biological control of the pea weevil by its natural enemy, *T. thoracica* can be quite successful in cultivars with earlier and shorter stages of flower and pod development.

Parasitoid control in pea cultivars, which had the larger duration of pod development stage, was ineffective. The beginning of stages of flowering and pod development in Pleven 4 compared with other cultivars occurred up to 7 days later, which affected the seasonal dynamics of *B. pisorum* (Nikolova, 2015). The parasitoid was entered into the seeds in the moment, when maybe a greater part of damaged seeds had older host larvae. Pleven 4 was distinguished with long flowering and pod development duration, and a bigger part of the larvae could complete their development to the adult stage at harvest time.

Similar results reported Schmale *et al.* (2005), according to which suppression of the *Acanthoscelides obtectus* population with a high level of initial infestation depended on the developmental stage of the weevil population at harvest time. When weevil larvae were present as early instars, parasitoids (*Dinarmus basalis*) reduced weevil populations by 88–97%, while development of populations of older weevil instars was delayed by the parasitoid, without reducing the build-up of the population (Schmale *et al.*, 2006). In a previous study, Schmale *et al.* (2001) concluded that feeding on the host' shaemolymph acted as a source of addition energy.

The development and use of cultivars with pod and seed resistance to *B. pisorum* would provide an environmentally safer option than contact insecticides for adult weevil control. Many authors studied the resistance of different cultivars against *B. pisorum*. Ahmed *et al.* (1989) evaluated chickpea genotypes for their susceptibility to pulse beetle, *Callosobruchus maculatus* F. (Bruchidae) taking into account the number of undamaged seeds (resistance to bruchids), number of eggs oviposited (ovipositional preference), and number of emergence holes (adult survival) per 50 seeds. The authors found that resistance to bruchids appeared to be a more heritable trait than the other two damage characters. According to Doss (2000), some *P. sativum* lines with the *Np* gene respond to the presence of pea weevil eggs on pods by forming callus (neoplastic pod trait) that reduces larval entry into the pod. In addition, the authors found that in a field trial, this pod-based resistance was responsible for a lower rate of weevil-infested seed (62.2%) in *Np* plants compared with that in a susceptible line

Table 7. Regression coefficients of the insect density with regard to the chemical composition.

Trait	Coefficients	S.E.	*t* Stat	*P*-value	Lower 95%	Upper 95%	Lower 95, 0%	Upper 95, 0%
CP	0.034	0.041	0.829	0.426	−0.125	0.057	0.125	0.057
CF	−0.147	0.124	−1.191	0.261	−0.423	0.128	−0.423	0.128
Ca	−3.800	1.218	−3.121	0.011	−6.513	−1.087	−6.513	−1.087
P	4.111	1.209	3.400	0.007	1.417	6.805	1.417	6.805

CP, crude protein; CF, crude fiber, Ca, calcium, P, phosphorus.

(85.4%). Clement *et al*. (2002) identified sources of natural weevil resistance in the *Pisum* genome (26 moderately resistant and resistant accessions of *P. fulvum*) to endow pea cultivars with pod and/or seed resistance to *B. pisorum*.

The loss in seed weight varied depending on the cultivars from 14.6 to 35.0% (total *q*) because of the harmful effect to *B. pisorum*. Mateus *et al*. (2011) reported that the attack by bruchids caused a significant reduction in seed weight, between 0.03 and 0.08 g, depending on the genotypes/cultivars, corresponding to a decrease in nutrients available to the embryo development. In addition, Zubareva (2006) found that the pea damage by *B. pisorum* was accompanied not only by a reduction of seed weight by 31%, but also the seed sowing quality: germination energy by 35% and germination by 54%.

In the present study, the seed weight was affected by the degree of damage seeds as the cultivars with a higher weight of 1000 seeds had lower rates of damage and lower values of the susceptibility coefficient. The small-seeded cultivar Pleven 4 had significantly the smallest seed weight and the highest susceptibility coefficients. It was characterized with the highest percentage of damaged seeds as the larva destroyed most of the grain content for its feeding. Nikolova & Pachev (2008) found that the small-seeded varieties were characterized by the highest percentage of *B. pisorum* damaged seeds (46.4 and 38.1%), and they had the longest period of flowering and pod formation. The degree of attack was associated with plant height and there was a positive correlation between that trait and percentage of damaged seeds. Similar results reported Poryazov (1990).

In a comparative analysis, concerning the contents of chemical components in pea cultivars was found that cultivars with lower protein and P content had lower levels of damaged seeds (for example, Glyans and Modus). The preference of the pea weevil concerning to CP and P content in seeds was related to a higher concentration. This resulted in a higher rate of damaged seeds. Pleven 4 had the highest protein and P content, which resulted in the highest damaged seed percent. Similar trend observed Marzo *et al*. (1997), which found a linear correlation between both protein and phytic acid content and *B. pisorum* infestation ($r^2 = 0.735$ and 0.732, respectively). However, the authors suggest that greater phytate and protein contents reduce the risk of Bruchus infestation in pea seeds. Opposite opinion had Odagiu & Porca (2002), according to which the chemical components had no direct influence on the tolerance against bruchids so that grains must be deeply studied in order to determine the influence of both pigments, and amino acids on tolerance of beans.

The results in table 7 indicated that the CP, CF and P content in damaged seeds of the cultivars of *P. sativum* significantly or no significantly was increased as compared with the healthy seeds due to weevil damage. In addition, the increase was expressed to greater extent in damaged seeds from the type three than in the damaged seeds from type two. The increase in the protein concentration may be due to the generation of defense-related proteins after insect infestation, which resulted in higher protein content in damaged seeds from the type three than type two. Similar results were reported in an earlier study (Nikolova *et al*., 2009). Lawrence & Koundal (2002) was found that plants defend themselves by producing these defense related proteins at high concentrations. Our results are similar to Rani & Pratyusha (2013) who found that infested cotton plant expressed higher levels of proteins than normal plant. The protein content was increased in insect damaged groundnut genotypes as compared to uninfected control plants according to War *et al*. (2012). Zubareva (2006) added that the pea weevil damage led to an increase of total protein content at the expense of albumin fraction and induced increase of trypsin inhibitor activity almost double.

The present data suggest that two pea cultivars may be tolerant cultivars and can be used through breeding programmes. In general, the eventual incorporation of yield traits and the biochemical markers for the selected pea cultivars are efficient tools, which are to be applied as marker-assisted selection closely linked to important traits, which greatly contribute to practical crop improvement programmes.

Conclusions

Modus, followed by Glyans was outlined as resistant cultivars against the pea weevil. They had the lowest total damaged seed degree, while Pleven 4 – the highest. A strong negative relationship ($r = -0.838$) between the weight of healthy seeds and the proportion of damaged seeds with bruchid emergence holes was found.

B. pisorum damage resulted in loss in weight of damaged seeds (type two and three) as Modus had the lowest reduction and the lowest values of susceptibility coefficients, followed by Glyans.

Cultivars with lower protein and P content had a lower level of damage. The CP, CF and P content in damaged seeds of the pea cultivars significantly or no significantly were increased as compared with the healthy seeds due to weevil damage. The P content had the highest significant influence on pea weevil infestation.

Use of chemical markers for resistance to the creation of new pea cultivars may be effective methods for defense and control against *B. pisorum*.

Acknowledgements

I am very grateful to Institute of Forage Crops for technical assistance and to the anonymous reviewers whose suggestions highly improved an earlier version of the manuscript.

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