Evaluation of damage caused by *Bruchus pisorum* L (*Coleoptera*: *Chrysomelidae*) on some parameters related to seed quality of pea forage cultivars *(Pisum sativum* L.)

Оценка на вредата, причинена от *Bruchus pisorum* L (*Coleoptera*: *Chrysomelidae*) върху някои показатели, свързани с качеството на семената при сортове фуражен грах *(Pisum sativum* L.)

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Abstract

It was evaluated the damage caused by *Bruchus pisorum* L (*Coleoptera*: *Chrysomelidae)* on the germination ability of pea farage varieties (*Pisum sativum* L.). Result of damage by *Bruchus pisorum* in seeds with parasitized larva was significant decrease of the germination by 16.4% percentage points, the length and weight of primary radicle by 16.8 and 24.5%, the length and weight of plumule by 12.3 and 14.1%, the vigor index of primary radicle and plumule by 32.5 and 32.8% as well as the germination index by 17.4%. The inhibitory effect was on average 17.8%. Essential significant changes in regard to the studied parameters were found for damaged seeds with bruchid emergence hole. In these seeds the gewrmination decrease by 58.3% percentage points, the length and weight of primary radicle by 34.1 and 36.2%, the length and weight of plumule by 31.8 and 34.3%, the vigor index of primary radicle and plumule by 81.1 and 82.1% as well as the germination index by 83.1%. The inhibitory effect was on average 58.3%. It was found that the damaged seeds with parasitoid emergence hole provided better possibility for growth and development of plants whereas the damaged seeds with bruchid emergence hole had significantly low germination, vigor and sowing characteristics. These seeds could not provide the establishment of well-garnished stand and stable yields. As tolerant to damage by *Bruchus pisorum* was distinguished Glyans variety for which the values of parameters related to germination and vigor of seeds were influenced in the lowest degree from the damage unlike the sensitive Pleven 4 variety. Dominant factor influencing germination ability of seeds for all analyzed parameters was the type of seeds compared to varietal appurtenance.

Keywords: *Bruchus pisorum*, pea, seed qualities

Резюме

През 2012г. опитното поле на Института по фуражни култури – Плевен е изведен опит с 5 сорта пролетен фуражен грах Глянс; Модус; Камертон и Світ (украински сортове) и Плевен 4 (български сорт, стандарт). Направена е оценка на вредата, причинена от *Bruchus pisorum* L (*Coleoptera*: *Chrysomelidae*) върху кълняемата способност на сортовете грах (*Pisum sativum* L.). Резултат от вредата на *B. pisorum* при семена с паразитирана ларва е доказнано намаляване на кълняемостта средно с 16.4%[тни] единици, дължината и теглото на първичния корен с 16.8 и 24.5%, дължината и теглото на кълна с 12.3 и 14.1%, индекса на жизненост на първичния корен и кълн с 32.5 и 32.8%, както и индексът на кълняемост със 17.4%. Инхибиращият ефект е средно 17.8%. Съществени доказани промени по отношение на проучваните показатели се установяват при повредените с прозорче от имагинирал зърнояд семена, при които кълняемостта намалява с 58.3% [тни] единици, дължината и теглото на първичния корен с 34.1 и 36.2%, дължината и теглото на кълна с 31.8 и 34.3%, индекса на жизненост на първичния корен и кълн с 81.1 и 82.1%, както и индексът на кълняемост със 83.1%. Инхибиращият ефект е средно 58.3%. Установено е, че повредените с прозорче от имагинирал паразитоид семена осигуряват по-добра възможност за растеж и развитие на растенията, докато повредените с прозорче от имагинирал зърнояд семена са с доказана ниска кълняемост, жизненост и посевни качества. Тези семена не могат да осигурят създаването на добре гарниран посев и стабилни добиви. Като толерантен към повредите от *B. pisorum* се очертава сорт Глянс.

Ключови думи: *Bruchus pisorum,* грах, качество на семената

Подробно резюме

През 2012г. опитното поле на Института по фуражни култури – Плевен е изведен опит с 5 сорта пролетен фуражен грах Глянс; Модус; Камертон и Світ (украински сортове) и Плевен 4 (български сорт, стандарт). Използван е блоков метод с големина на реколтната парцелка – 4 m^2, в три повторения и сеитбена норма – 120 бр.к.с. m^{-2}. През вегетационния период не е извеждана химична борба срещу неприятелите. Лабораторната кълняемост на семената, както и дължината и теглото на първичния корен и кълн на узрелите семена е определена четири месеца след прибиране на реколтата. Направена е оценка на вредата, причинена от *Bruchus pisorum* L (*Coleoptera*: *Bruchidae*) върху кълняемата способност на сортове грах (*Pisum sativum* L.). Резултат от вредата на *B. pisorum* при семена с паразитирана ларва е доказнано намаляване на кълняемостта средно с 16.4%[тни] единици, дължината и теглото на първичния корен с 16.8 и 24.5%, дължината и теглото на кълна с 12.3 и 14.1%, индекса на жизненост на първичния корен и кълн с 32.5 и 32.8%, както и индексът на кълняемост със 17.4%. Инхибиращият ефект е средно 17.8%. Съществени доказани промени по отношение на проучваните показатели се установяват при повредените с прозорче от имагинирал зърнояд семена, при които кълняемостта намалява с 58.3% [тни] единици, дължината и теглото на първичния

корен с 34.1 и 36.2%, дължината и теглото на кълна с 31.8 и 34.3%, индекса на жизненост на първичния корен и кълн с 81.1 и 82.1%, както и индексът на кълняемост със 83.1%. Инхибиращият ефект е средно 58.3%. Установено е, че повредените с прозорче от имагинирал паразитоид семена осигуряват по-добра възможност за растеж и развитие на растенията, докато повредените с прозорче от имагинирал зърнояд семена са с доказана ниска кълняемост, жизненост и посевни качества. Тези семена не могат да осигурят създаването на добре гарниран посев и стабилни добиви. Като толерантен към повредите от *B. pisorum* се очертава сорт Глянс, при който стойностите на показателите, свързани с кълняемостта и жизнеността на семената се повлияват в най-ниска степен от вредата за разлика от чуствителния сорт Плевен 4. Установено е, че доминиращ фактор, влияещ върху кълняемата способност на семената при всички анализирани показатели е типът на семената в сравнение с сортовата принадлежност.

Introduction

The determination and emergence are important stages in crop cultivation. They have essential meaning on the following stages of plant growth and development (Kalisz, 1986) and for the realization of high productivity with good quality (Yari et al., 2010). On the other hand an important condition for implementation of this process is the use of qualitative seeds: with genetic purity, strong germination capacity, uniformity of seed size, freedom from seed borne diseases and absence of weed or other crop seeds (Hartmann and Kester, 1983). According to Thomson (1979) the seed quality is a multiple concept comprising several components. The seed vigour is an important component that can influence crop plant density and yield (Siddique and Wright, 2004). That vigour is related to the germination and seed ability to grow rapidly and jointly. It suggested that the speed and uniformity of emergence are important parameters of seed quality (Copeland and McDonald, 1995). Although many vigour tests are suggested only few are accepted by seed analysts and seed testing organization (Perry, 1981). Some of applied tests are germination test and seed vigour test which are used to determine seed viability (Hartmann and Kester, 1983; Fiala, 1987).

Seed yield and viability can be reduced by different types of environmental stress, one of which is damage of seed weevils. The final effect of seeds with beetle infestation on the germination of host legumes can be unpredictable (Southgate, 1979). In some cases the larva feeding effectively kills the embryo or removes so much endosperm that the seed cannot germinate (Camargo-Ricalde et al., 2004; El Atta, 1993; Tomaz et al., 2007). Larval feeding may also create openings for pathogenic bacteria and fungi (Chang et al., 2011; Cipollini and Stiles, 1991; Mucunguzi, 1995). Even if germination occurs prior seed infestation after that the infestation may distort the development of cotyledons or prevent the formation of true leaves (Hegazy and Eesa, 1991). Depletion of cotyledon reserves may slow plant growth and hence reduce the probability for its establishment. Despite these detrimental effects of seed-beetle damage, some proportion of infested seeds may germinate successfully (Halevy, 1974; Hoffman et al., 1989; Mack 1998; Miller, 1994a,b). The pea weevil is one of the most destructive pests in grain legumes (Clement et al., 2000) and cultivar improvement for bruchid resistance is among the most important strategies for mitigate the action of biotic factors.

The subject of present study was to evaluation of damage caused by *Bruchus pisorum* L. on seed germination, growth and vigor in pea forage varieties.

Material and Methods

In 2012 a field trial was carried out in the experimental field of the Institute of Forage Crops (Pleven) with 5 pea forage varieties: Glyans (Ukraine), Cvit (Ukraine), Kamerton (Ukraine), Modus (Ukraine), Pleven 4 (Bulgaria). It was laid out by the design of long plot method with three replications. Pesticides were not used during the vegetation period. The laboratory germination of seeds as well as the length and weight of primary radicle and plumule was determined four months after harvesting.

The seeds of each variety were classified into three types: healthy seeds (type 1), damaged seeds with parasitoid emergence hole (type 2) and damaged seeds with bruchid emergence hole (type 3).

For every cultivar and type of seeds, the germination percentage was evaluated by taking 200 seeds (50 seeds in each replication). The seeds were placed in Petri dishes on double-layered Filtrak 383 filter paper with 150 mm diameter, and kept in an incubator at a temperature of 20 ±1 °C for 8 days according to the ISTA (2010) rules. The germinated seeds were counted daily (Seed vigor, 1983). The seedlings with stunted primary roots were considered as abnormally germinated (ISTA, 2011). A seed was considered to have germinated when the radicle reached a length of 10 mm (Goertz and Coons, 1989).

The energy of emergence (EG) was recorded on the 4th day after sowing. It was the percentage of germinating seeds 4 days after planting relative to the total number of tested seeds (Ruan et al*.*, 2002).

The germination percentage was calculated 8 days after sowing using the formula below for each replication of the variant:

$GP \% = (n / N) *100$,

where GP is the germination percentage, n is the number of germinated seeds, and N is the total number of seeds (Belcher and Miller 1974).

The inhibitory effect (IE, %) from damage by *B. pisorum* on germination was calculated by the following formula:

$IE = [(a-b)/a)]x100$ (Ahn and Chung, 2000), where:

a – number of germinated seeds in the control variant (healthy seeds);
b – number of germinated seeds in the studied variant (damaged seeds).

After the final count in the standard germination test, seedling growth was assessed by measuring seedling length (plumule and radicle length) and seedling weight (fresh weight). The root length was measured from tip of the tap root to the joining point of the cotyledon.

The vigor indexes (VI) of primary radicle and plumule were calculated, using formula from Abdul-Baki and Anderson (1973), as follows:

Vigor Index = Standard Germination (%) × length of primary radicle (plumule) (cm).

The germination index (GI) was calculated as described in the Association of Official Seed Analysts (Seed Vigor, 1983) by following formula:

GI = (Number of germinated seed / Days of first count) +……+ (Number of germinated seed / Days of final count).

Mathematical statistical processing of experimental data was conducted after preliminary transformation of the percentage of damaged seeds relative to the control variant, by the formula: $Y = arcsin\sqrt{(x_{\%} / 100)}$ (Hinkelmann and Kempthorne, 1994).

All data analyses were conducted using the Statgraphics Plus software program and ANOVA for statistical analysis.

Results

Varietal appurtenance of seeds was a factor influencing their germination and sowing characteristics (Gunn, 1972). At comparing the emergence energy and germination of healthy seeds among studied pea varieties was observed a high percentage of normally germinated seeds without significant difference among them (Table 1). The germination was in limits 77.08 – 84.76% which means that seed were suitable for sowing. It provided development of normal plants with possibilities for maximum yield according to the requirements for minimum germination in pea sowing (Guide to Seed Certification Procedures, 2012) – Table 1.

Table 1 Effect of the damage caused by *Bruchus pisorum* on seed germination in different varieties of spring forage pea

Таблица. 1 Влияние на повредите, причинени от *Bruchus pisorum* върху покълването на семената при различни сортове пролетен фуражен грах

Varieties	1	1	2	2	3	3				
	EG	GP	EG	GP	EG	GP	LSD 0.05%	IE $_1$, %	IE $_2$, %	LSD 0.05%
Glyans	54.23	84.76 c*/a**	50.77	70.78 b /b	19.97	27.74 a / c	8.797	10.11a/a	78.13 b/a	10.937
Cvit	48.35	80.90 c /a	45.30	65.91 b /b	23.23	26.57 a /bc	6.826	14.46 a/a	79.59 b/a	13.665
Kamerton	52.24	79.48 c /a	47.87	70.03 b /b	20.70	21.42 a / b	7.747	11.78 a/a	86.44b/ab	7.720
Modus	53.23	80.90 c /a	40.69	64.04 b /b	19.22	22.79 a/bc	9.149	17.08 a/a	84.64 b/a	11.525
Pleven 4	47.39	77.08 c /a	40.69	50.28 b /a	10.51	12.92 a / a	8.096	37.32 a/б	94.56 b/b	12.879
Average	51.09	80.63	45.06	64.21	18.73	22.29		18.15	84.67	
LSD 0.05%		5.980		9.103		7.674		10.383	9.692	

Legend: Type 1 – healthy seeds; Type 2 – damaged seeds with parasitoid emergence hole; Type 3 – damaged seeds with bruchid emergence hole; EG - Energy of emergence, %; GP - Germination percentage, %; IE 1, % - - Inhibitory effect of damaged seeds with parasitoid emergence hole; IE 2, % - - Inhibitory effect of damaged seeds with bruchid emergence hole, %;

* – significance among the type of seeds for a variety

** – significance among the varieties

As a result of damage from *Bruchus pisorum* and related to that deterioration of seed quality was changed the germination of seeds. The germination was in limits from 50.28 to 70.78% in damaged seeds with parasitoid emergence hole (type 2). It significantly decreased from 9.45 to 26.80 percentage points to the germination of the relevant healthy seeds (type 1). Among varieties with statistically significant lower germination was distinguished Pleven 4 (50.28%) with decrease by 26.80 percentage points. This variety was distinguished with significantly the highest inhibitory effect (IE_1) – 37.32%. The damaged seeds with bruchid emergence hole (type 3) were characterized with lowest energy of emergence and germination. Compared to the healthy seeds the germination significantly reduced from 54.34 to 64.16 percentage points as among the varieties the lowest significant germination and high inhibitory effect (IE_2) was found in Pleven 4 (12.92 and 94.56% respectively). At this type of seeds (type 3) Glyans variety was with significantly lower germination to Kamerton and with lowest inhibitory effect (IE_2) – 78.13%.

As expression of the sowing characteristics of seeds was the growth strength of primary radicle and plumule within certain variety. Data from biometric measurements in regard to the length of primary radicle and plumule (cm) and their weight (g) permitted objectively to evaluate the reaction of studied varieties in the initial stages of their development as a result of damage by *B. pisorum* (Table 2). It was observed some difference with respect to the up-growth length of primary radicle and plumule in the healthy seeds as significantly the highest values were found for Pleven 4 variety (9.015 and 3.773 cm respectively). Exception for significant difference was observed only relative to Glyans variety in regard to the radicle length. At the other varieties the values were in close limits and varied from 7.169 to 7.945 cm and from 1.034 to 2.030 cm respectively for primary radicle and shoot. In regard to the weight of primary radicle with significantly the highest weight was distinguished Glyans (0.117g) and at the others it varied from 0.078 to 0.098 g with significant differences between Cvit and Modus (0.098 and 0.078g respectively). At Pleven 4 variety was observed statistically significant the greatest weight of plumule (0.083 g) followed by Cvit (0.055 g). Among the up-growth length of primary radicle and plumule were established positive correlations as strongly expressed ones were between the length and weight of plumule (r = +0.981) and between the length of primary radicle and plumule (r = +0.928).

As a result of the larva feeding was suppressed in some degree the up-growth of primary radicle at seeds type 2 as its length varied from 4.571 to 7.736 cm. Only in Pleven 4 variety was found significantly reduction from 49.3% in the up-growth of radicle compared to this of the healthy seeds till at other varieties it was insignificant (from 2.6 to 11.1% reduction). In regard to the weight a significant decrease from 21.3 to 39.3% was observed at Glyans, Cvit and Pleven 4. The damaged seeds with parasitoid emergence hole were characterized with lower values of the plumule length and weight (from 4.2 to 23.3% and from 3.3 to 25.8% respectively) to the healthy seeds but these values were statistically insignificant. Among the varieties with significantly lower values of the radicle length was distinguished Pleven 4 (4.571 cm) as this variety had and the greatest length of plumule (3.152cm).

The most essential changes as regards the studied parameters were found in the damaged seeds with bruchid emergence hole (type 3). In all varieties was observed significant suppression in the up-growth of primary radicle and its weight (only exception was the weight of Kamerton variety) to the relevant in the healthy seeds from 13.1 to 58.1% and from 27.0 to 56.7%. The suppression was most pronounced for Pleven 4 and the lowest – for Glyans. Significant decrease in plumule length was established for Pleven 4 (39.3%), Glyans (38.5%) and Kamerton (36.7%) and as redards its weight – for Pleven 4 (40.8%) and Kamerton (39.8%). The comparing of varieties showed significant differences in regard to the length and weight of primary radicle and plumule length between Pleven 4 and Glyans. Significant differences between seeds type 2 and type 3 were observed for Glyans in respect to the length of primary radicle and plumule until for other varities such differences were not found.

Table 2. Effect of the degree of damage by *Bruchus pisorum* to pea seeds on the length and weight of primary radicle and plumule

Таблица 2. Влияние на степента на повреда от *Bruchus pisorum* по семената върху дължината и теглото на първичния корен и кълна

Varieties	1		2		3		$LSD_{0.05\%}$
			RL				
Glyans	7.945	b*/ab**	7.736	b / b	6.900	a / b	0.823
Cvit	7.653	b / a	7.032	ab / b	4.923	a / ab	2.153
Kamerton	7.169	b / a	6.656	ab / b	4.473	a / ab	2.435
Modus	7.571	b / a	6.733	ab / b	5.850	a / ab	1.576
Pleven 4	9.015	b / b	4.571	a / a	3.775	a / a	1.969
Average	7.871		6.546		5.184		
$LSD_{5\%}$	1.157		1.198		2.570		
			RW				
Glyans	0.117	b / c	0.092	a / b	0.082	a / b	0.018
Cvit	0.098	b / b	0.067	a / a	0.061	a / ab	0.014
Kamerton	0.083	a / ab	0.069	a / a	0.061	a / ab	0.03
Modus	0.078	b / a	0.069	ab / a	0.055	a / a	0.016
Pleven 4	0.092	b / ab	0.056	a / a	0.040	a / a	0.03
Average	0.094		0.071		0.060		
$LSD_{5\%}$	0.018		0.017		0.028		
			PL				
Glyans	1.660	b / ab	1.514	b / ab	1.021	a / a	0.442
Cvit	2.030	a / b	1.945	a / b	1.755	a / b	0.826
Kamerton	1.231	b / ab	0.944	ab / a	0.780	a / a	0.332
Modus	1.034	a / a	0.974	a / a	0.785	a / a	0.324
Pleven 4	3.773	b / c	3.152	ab / c	2.292	a / c	1.294
Average	1.946		1.706		1.326		
$LSD_{5\%}$	0.879		0.667		0.507		
			PW				
Glyans	0.040	a / ab	0.038	a / a	0.029	a / ab	0.011
Cvit	0.055	a / b	0.051	a / a	0.040	a / ab	0.061
Kamerton	0.025	b / a	0.019	ab / a	0.015	a / a	0.009
Modus	0.027	a / a	0.026	a / a	0.019	a / a	0.009
Pleven 4	0.083	a / c	0.063	a / a	0.049	b / b	0.032
Average	0.046		0.039		0.030		
$LSD_{0.05\%}$	0.024		0.048		0.030		

Legend: 1 – healthy seeds; 2 – damaged seeds with parasitoid emergence hole; 3 – damaged seeds with bruchid emergence hole; RL - radicle length, cm; RW - radicle weight, g; PL - plumule length, cm; PW - plumule weight, g;

* – significance among the type of seeds for a variety

** – significance among the varieties

The obtained results at determination of vigor index of primary radicle (VIr) and plumule (VIp) in Table 3 showed a significant difference among the three type of seeds for stidied varieties.

Table 3. Effect of the degree of damage by *Bruchus pisorum* to pea seeds on vigor index (VI) and germination index (GI)

Таблица 3. Влияние на степента на повреда от *Bruchus pisorum* по семената върху индекса на жизненост (SVI) и индекса на кълняемост (GI)

Varieties	1		2		3		LSD 0.05%
			VIr				
Glyans	673.44	c / ab	547.59	b / c	191.43	a / c	71.36
Cvit	619.14	c / ab	463.45	b / b	130.78	a / b	71.766
Kamerton	569.81	c / a	466.08	b / b	95.80	a / ab	70.984
Modus	612.50	c / ab	431.19	b / b	133.30	a / b	92.101
Pleven 4	694.87	c / b	229.83	b / a	48.78	a / a	172.334
Average	633.95		427.63		120.02		
LSD 0.05%	133.56		83.485		60.594		
			VIp				
Glyans	140.69	c / ab	107.19	b / b	28.33	a / ab	22.793
Cvit	164.25	b / b	128.22	b / bc	46.61	a / b	56.132
Kamerton	97.87	c / ab	66.14	b / a	16.70	a / a	27.932
Modus	83.62	c / a	62.39	b / a	17.89	a / a	17.802
Pleven 4	290.80	c / c	158.46	b / c	29.61	a / a	121.724
Average	155.44		104.48		27.83		
LSD 0.05%	88.63		49.189		12.541		
			GI				
Glyans	15.42	b /a	15.28	b / c	3.13	a / b	3.523
Cvit	14.84	b /a	12.20	b / ab	3.44	a / b	3.719
Kamerton	15.68	b /a	14.01	b / bc	2.55	a / ab	2.201
Modus	15.97	c /a	12.18	b / ab	2.45	a / ab	2.953
Pleven 4	14.48	c /a	9.59	b / a	1.39	a / a	4.68
Average	15.28		12.65		2.59		
LSD 0.05%	4.711		2.955		1.392		

Legend: 1 – healthy seeds, 2 – damaged seeds with parasitoid emergence hole, 3 – damaged seeds with bruchid emergence hole, VIr – vigor index of radicle, VIp- vigor index of plumule, GI - germination index.

With the lowest significant values were damaged seeds with bruchid emergence hole (type 3) followed by damaged seeds with parasitoid emergence hole (type 2). VIr and VIp varied from 48.78 to 191.43 and from 16.70 to 46.61 for type 3 and from 229.83 to 547.59 (VIr) and from 62.39 to 158.46 (SVIp) for type 2 respectively. At comparing among varieties with highest vigor index of healty seeds (type 1) was distinguished Pleven 4 (694.87 and 290.80 respectively for radicle and plumule) followed by Glyans and Cvit. This trend at type 2 and 3 (seeds) was retained only at Glyans in regard to VIr where the values of parameter were significantly higher to other studied varieties (547.59 and 191.43 respectively). In this variety the reduction relative to the healthy seeds was least pronounced – 18.7 and 71.6% respectively for seeds type 2 and 3. The results were not so categorical for Cvit independently of higher vigor index of primary radicle. Pleven 4 variety was distinguished with the lowest vigor index of primary radicle among the varieties (229.83 and 48.78, respectively, for type 2 and 3) and the greatest reduction to the healthy seeds (66.9 and 93.0%). The greater plumule length of healthy seeds for Pleven 4 variety did not secure reduction in VIp values of the damaged seeds. It was important to note that for this variety the reduction in VIp to the healthy seeds was the most essential (45.5 and 89.8% for seeds type 2 and 3 respectively).

The results in regard to the germination index were analogical and indicative. Glyans variety was distinguished with high values in both types (2 and 3) of damaged seeds (15.277 and 3.129%) as well as with lowest degree of reduction to the relevant seeds from type 1. This variety had significant differences to Pleven 4 variety. The last one had the lowest germination index for all types of seeds and the most pronounced reduction in the values of seeds for type 2 and type 3 – 33.7 and 90.4% respectively to the healthy seeds. Independently of the variety factor significantly lowest index (variation in limits 1.388 – 3.438%) was observed in damaged seeds with bruchid emergence hole. The germination index for damaged seeds with parasitoid emergence hole (type 2) was significantly higher to type 3 and significantly lower to type 1 only for Pleven 4 and Modus varieties. It was not observed significant difference in the values of parameter among the varieties for seeds type 1 as they varied from 14.479 to 15.793%.

Table 4. Analysis of variance for studied factors in pea forage varieties

Таблица 4. Влияние на факторите на варианса върху проучваните показатели при сортове пролетен грах

Source of variation	df	GP MS	GP IF, %	RL MS	RL IF, %	RW MS	RW IF, %	PL MS	PL IF, %
Total	59	-	-	-	-	-	-	-	-
Variants	14	5684.8 *	135.0	9.0*	5.0	0.002*	7.8	3.1*	13.8
Factor A - Varieties	4	491.2 *	11.7	5.3*	3.0	0.002*	10.6	9.3*	41.7
Factor B–Types of seeds	2	38281.3 *	909.1	36.1*	20.2	0.006*	29.7	1.9*	8.7
A x B	8	132.5*	3.1	4.1*	2.3	0.000	1.0	0.3	1.2
Pooled error	42	24.4	-	1.3	-	0.000	-	0.2	-

Source of variation	PW MS	PW IF, %	GI MS	GI IF, %	SVIr MS	SVIr IF, %	SVIp MS	SVIp IF, %
Total	-	-	-	-	-	-	-	-
Variants	0.001*	3.0	134.6*	28.0	382277.3*	39.0	36275.9*	12.2
Factor A - Varieties	0.004*	8.6	13.2*	2.7	38019.5*	3.9	29230.5*	9.8
Factor B–Types of seeds	0.001	2.6	897.0*	186.9	2434145.0*	248.3	151248.5*	51.0
A x B	0.000	0.3	4.7	1.0	41439.4*	4.2	11055.4*	3.7
Pooled error	0.001	-	4.8	-	4113.0	-	1536.6	-

Legend: LSD 5%; DF – Degrees of freedom; MS – Mean square; IF – Influence of factor; GP – germination percentage; RL – radicle length; RW – radicle weight; PL – plumule length; PW – plumule weight; GI – germination index; VIr – vigor index of radicle; VIp – vigor index of plumule

The analysis of variance in regard to the germination ability of healthy seeds and damaged seeds by *Bruchus pisorum* in pea varieties showed that dominant factor in all analysed parameters was the type of seeds (factor B) – Table 4. It had the strongest influence on the sowing qualities and significant effect – from 8.7 to 909.1% from the total variation (A). It was observed an exception only in regard to the plumule weight where it was not found significant influence. The varieties were also factor with significant strength of influence but their interaction was significantly lower pronounced – from 3.0 to 41.7%. The interaction between two factors (AxB) as strength of influence was not always significant and varied in much narrow limits – from 0.3 to 4.2%.

Discussion

The emergence of seeds is influenced by many abiotic and biotic factors including damages by different insects. The period for which the seeds emerge and germinate has serious consequences on the whole process of plant growth and development (Kalisz, 1986). According to Southgate (1979) the effects of damaged bruchid seeds on the germination could be unpredictable. The damaged seeds with parasitoid emergence hole (type 2) had significantly lower germination independently that the larva did not reach its full development and was died. This type was distinguished with significantly higher germination and considerably lower inhibitory effect (IE_1) to damaged seeds with bruchid emergence hole (type 3).

The early mortality of bruchid larvae due to parasitism had no negative influence in great degree on seed germination because the larvae were killed before consuming too large a quantity of the seed. Unlike that the larval feeding (in damaged seeds with imago) effectively kills the embryo or removes so much endosperm that a large part of seeds can not germinate (Thomson, 1979; El Atta, 1993; Camargo-Ricalde *et al.*, 2004). Result of damage was the high inhibitory effect (IE_2). Despite these detrimental effects of seed-beetle damage, some proportion of infested seeds germinate successfully. Similar results reported Nakai *et al.* (2011) pursuant to which the seeds from which *Pteromalus* wasps is emerged germinated more successfully than the seeds from which *Bruchus loti* adults emerged. *B. loti* larvae parasitised by the two wasp species consumed with less intensively the seeds of *Lathyrus japonicus* than the unparasitised larvae of weevil. Mateus et al. (2011) are found that the proportion of pea germinated seeds is significantly higher for non-attacked seeds from *B. pisorum* compared to the attacked seeds. Similar results about reduced germination in result of bruchid damages are reported and other authors (Andersen, 1930; Halevy, 1974; Hoffman et al., 1989).

The variety factor influnced the germination only for damaged seeds with bruchid emergence hole (type 3). The established highest germination and low inhibitory effect for Glyans variety probably was due to the higher content of nutrients. It was found that depending on the sensitivity of different pea varieties in regard to attack of *B. pisorum* occurred biochemical changes related to increased content of crude protein, proteins, total phenols, water-soluble sugars, phosphorus and decreased content of calcium and trypsin inhibitory activity. The sensitive varieties had the most pronounced increase in regard to crude protein, proteins, total phenols which due to the protective reaction of plant to compensate the losses from damage (Nikolova et al., 2009).

Result of larva feeding of pea weevil was suppression in the growth of primary radicle and plumule as well as decrease of their weight. The damaged seeds with parasitoid emergence hole were less affected than the damaged seeds with bruchid emergence hole where was observed significant suppression in the growth and weight of primary radicle for all varieties. The depletion of cotyledon reserves in seeds type 3 probably slowed the growth and development of plant and hence reduced the probability of establishment. Glyans variety was distinguished as the most tolerant to damage by *B. pisorum* which likely was related to the different quantity of reserve nutrients accumulated in seeds and their potential. As the most sensitive was manifested Pleven 4 which independently of the greatest length and weight of plumule to other varieties was the most affected by the damages and with reduced sowing characteristics of seeds.

Mateus et al. (2011) found that the mean weight and mean length of primary radicle and plumule were significantly greater than these of damaged seeds by *B. pisorum.* At the same time comparing the two types (2 and 3) of seeds did not show significant differences between them in regard to seed germination and seedling vigor. A similar tendency was also observed in the present study (Glyans variety was an exception) as there was significant decrease in the length of primary radicle and plumule between seeds from type 2 and type 3. According to Bonal et al. (2007) and Mack (1998) in some plants, part of the cotyledon may serve to buffer the negative impact of bruchid damage whereas any loss of cotyledon tissue in other plants greatly reduces seedling mass. Probably this was the reason for different reaction of studied varieties to pea weevil attack.

In support of the above were the results regarding vigor index and germination index. Pleven 4 variety had a high vigor index of healthy seeds but at damage by pea weevil it could not overcome or compensate the negative consequences. It was distinguished and with the lowest germination index in regard to three types of seeds. As opposed to it glyans variety (independently of lower vigor index of healthy seeds) overcomed the detrimental effects from bruchid damage and had higher vigor index and germination index in damaged seeds to other varieties.

Irrespective of varieties the damage by pea weevil was related to reduction of germination and vigor of seeds and the possibility for fast and simultaneously emergence and development. The damaged seeds with parasitoid emergence hole (type 2) had better potential for growth and development whereas the damaged seeds with bruchid emergence hole had significantly low germination, vigor and sowing characteristics. These seeds could not provide the establishment of well-garnished stand and stable yields. As the most tolerant to damage by *B. pisorum* was distinguished Glyans variety which can be used as a germplasm source for selection.

Conclusions

Result of damage by *Bruchus pisorum* in seeds with parasitized larva was significant decrease of the germination by 16.4% percentage points (from 9.45 to 26.8 percentage points), the length and weight of primary radicle by 16.8 and 24.5% (from 2.6 to 49.3% and from 11.8 to 39.3% respectively), the length and weight of plumule by 12.3 and 14.1% (from 4.2 to 23.3% and from 3.3 to 25.8% respectively), the vigor index of primary radicle and plumule by 32.5 and 32.8% as well as the germination index by 17.4%. The inhibitory effect was on average 17.8%.

Essential significant changes in regard to the studied parameters were found for damaged seeds with bruchid emergence hole. In these seeds the germination decrease by 58.3% percentage points, the length and weight of primary radicle by 34.1 and 36.2%, the length and weight of plumule by 31.8 and 34.3%, the vigor index of primary radicle and plumule by 81.1 and 82.1% as well as the germination index by 83.1%. The inhibitory effect was on average 58.3%.

The damaged seeds with parasitoid emergence hole (type 2) provided better possibility for growth and development of plants whereas the damaged seeds with bruchid emergence hole had significantly low germination, vigor and sowing characteristics. These seeds could not provide the establishment of well-garnished stand and stable yields.

As the most tolerant to damage by *Bruchus pisorum* was distinguished Glyans variety for which the values of parameters related to germination and vigor of seeds were influenced in the lowest degree from damage unlike the sensitive Pleven 4 variety. It was found that the dominant factor influencing germination ability of seeds for all analyzed parameters was the type of seeds compared to varietal appurtenance.

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