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VARIABILITY IN SPRING PEA (*Pisum sativum* L.) VARIETIES FOR TOLERANCE TO *Bruchus pisorum* L. (*COLEOPTERA*: *BRUCHIDAE*)

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Abstract. During the 2012–2014 period in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria, a study was conducted on tolerance of 5 spring pea varieties to *Bruchus pisorum* L. (*Coleoptera*: *Bruchidae*): Glyans, Modus; Kamerton and Svit (Ukrainian varieties) and Pleven 4 (Bulgarian variety). It was found that the duration of flowering and pods development stages in spring pea varieties affected the seasonal dynamics of *B. pisorum*. Modus variety had the shortest duration of flowering and pods development stages and the lowest density of the pea weevil. The tolerance of Modus was related to earliness of the variety. The resistance to bruchids in spring pea may be related to length of pods, affecting oviposition. Modus had the shortest length of pods, the lowest proportion of infected pods and number of laid eggs per pod. Use of different markers for resistance as discrepancy between the phenological development of the host plant and the life cycle of the phytophagous insects, a length of pods *etc*. in the creation of new pea varieties may be effective methods for defense and control against *B. pisorum.*
Keyword*:* pea weevil, pea varieties, tolerance

Introduction

During the selection process a significant place have the search for sources with important breeding value and tolerance to economical important pests. This has a great meaning for creating new varieties. It is one of the ecological approaches to resolving main problems related to the development of resistant forms among plant–eating insects and establishment of conditions for their rapid multiplication.

Positive results are achieved in *Pisum sativum's* selection in regard to setting up germplasm that is resistant or tolerant of one of the major pests– *Bruchus pisorum* L. (*Coleoptera*: *Bruchidae*) [PARIS and GOVONI, 1998; ZATYAMINA, 1993; DOSS *et al*., 2000; MORTON *et al*., 2000; DARRYE and CLEMENT, 2001; CLEMENT *et al.* 2002].

Specific mechanisms of resistance to insects have been identified in legume although these mechanisms are still not widely integrated into the breeding programs [EDWARDS and SINGH, 2006].

The defense processes in different varieties are related to morphological, physiological and/or biochemical mechanisms. They range from minimum provoked effect on insects' attacks and damages to adverse consequences for their cell processes, growth and development [SINGH, 2002, BUTNARIU *et al*., 2005].

According to some authors [PANDA and KHUSH, 1995; EDWARDS and SINGH, 2006, BUTNARIU *et al*., 2012] the legumes including pea use a set of direct and indirect defenses as morphological barriers, secondary metabolites and antifeeding compounds which are important for the seed and its defence against insects.

Clement and collab. [CLEMENT *et al*., 1996] found that it may be possible to use a flower assay to screen pea germplasm for antixenosis resistance to pea weevil.

Ahmed and collab. [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. He found that resistance to bruchids appeared to be a more heritable trait than the other two damage characters. In addition, he concluded that the number of emergence holes is a better indicator of seed resistance than the number of eggs present on the seeds.

Variety, as a powerful means, influences the abundance and physiological state of phytophagous insects. The tolerant varieties suppress their reproduction and the nonresistant ones favour it.

The use of tolerant varieties decreases the application of insecticides, increases the activity of entomophages, and reduces the negative effect of anthropogenic factor on the agroecosystems.

The aim of the study was to determine the variability in some pea varieties *(Pisum sativum* L.) for tolerance to *Bruchus pisorum* L. (*Coleoptera*: *Bruchidae*).

Material and methods

During the 2012–2014 period in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria, a study was conducted on tolerance of 5 spring pea varieties to *Bruchus pisorum* L. (*Coleoptera*: *Bruchidae*): Glyans, Modus; Kamerton and Svit (Ukrainian varieties) and Pleven 4 (Bulgarian variety).

The field trial was conducted using a long–plot design with a sowing rate of 120 g.s. m^{-2}, size of harvest plot of 4 m^2, three replications and the Ukrainian varieties: Glyans, Modus; Kamerton and Svit. No chemical control of insect pests was conducted during growing season. Bruchids were counted at the budding stage and counting continued until the aboveground biomass has dried.

Over the period, population density was recorded by sweepings with an entomological net through 4–5 days.

The following parameters were investigated: phenological stages in pea varieties, proportion of infected pods and number of laid eggs per pod (recorded in pods development stage), peduncle lengths, length and width of pods (the measure was carry out in stage of the ripening of seeds on pods on two bottom nodes to avoid the differences in size between the pods of various nodes [MAKASHEVA, 1973, BUTNARIU *et al.*, 2005], sum of effective temperatures (SET).

A minimum of 20 pods (1 pod per plant) from two bottom nodes were chosen for analysis on each plot.

The statistical processing of experimental data was conducted using the Statgraphics Plus for Windows Ver. 2.1. software program.

Results and discussion

The period of study included years, differing as regards the weather conditions, which affect the population density of the pea weevil (Table 1).

Table 1.

Meteorological characteristics in the Pleven region

Month	Ten–day periods	Temperature,°C			Rainfall, mm			Relative humidity, %		
		2012	2013	2014	2012	2013	2014	2012	2013	2014
April	1–10	12.2	10.5	11.9	14.2	33.1	37.6	57	76	71
	11–20	14.3	12.5	9.9	22.1	17.6	69.9	66	65	82
	21–30	17.8	19.5	14.9	10.0	0	32.3	54	52	76
	Average	14.8	14.2	12.2	46.3	50.7	139.8	59	65	76.3
May	1–10	20.3	20.7	14.7	6.0	3.8	23.7	57	55	72
	11–20	15.7	20.0	15.9	28.9	33.3	16.9	76	57	68
	21–31	16.3	18.2	19.4	50.3	26.6	42.4	79	67	70
	Average	17.4	19.6	16.7	85.2	63.7	83	71	60	70.0
June	1–10	22.5	18.9	19.4	13.1	5.9	19.6	63	63	70
	11–20	24.7	23.3	20.8	1.4	49.9	15.5	56	68	72
	21–30	25.0	21.8	21.7	25.8	55.8	19.2	56	66	60
	Average	24.1	21.3	20.6	40.3	111.6	54.3	58	66	67

Average air temperatures in May 2013 from 19.6°C and 63.7mm rainfall contributed to early appearance and development of weevils and led to the highest density and fecundity.

The lower temperature in May 2012 by an average of 2.2°C, combined with higher rainfall by 33.8 % and a relative humidity by 18.3 % resulted in a lower numbers and activity of pea weevil.

Considerable adverse effects on *B. pisorum* development was observed in 2014 as average air temperatures in May and June were the lowest corresponding to high relative humidity and rainfall.

The reported numbers of weevils in 2014 were the lowest.

Year 2012. The pea weevil appeared in field pea crops at the budding or beginning of flowering stages [DIMITROV, 2008; BRUDEA and MATEIAS, 1998].

In 2012 the appearance of pest was found in 16–20 May at the flowering stage.

The lower air temperature by 3.1°C combined with 32.2 % higher amount of rainfall in the second and third decade of May, compared to 2013 determined the later appearance of pea weevil (and later occurrence of phonological stages).

According to Dochkova and collab. [DOCHKOVA *et al.*, 1990] there was a positive correlation between the average daily air temperature and the numbers of pea weevil. It was observed some differences among the phonological development of the studied varieties that impact on the weevil population dynamic (Figure 1).

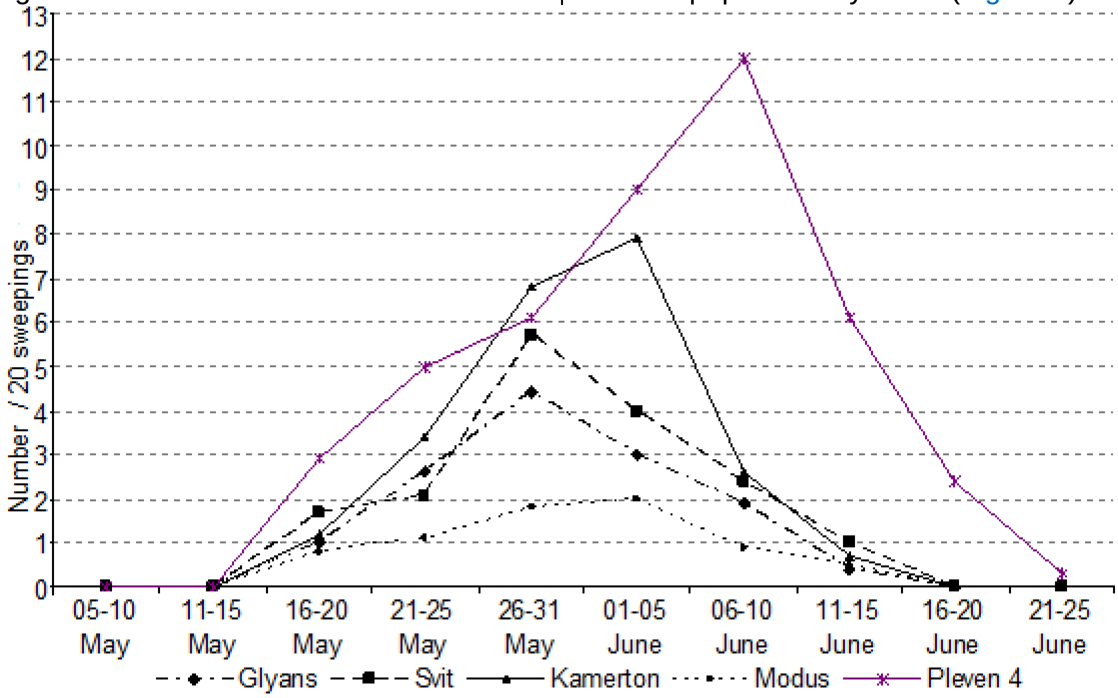

Figure 1. Numerical dynamics of *Bruchus pisorum* in spring pea varieties, 2012

Bulgarian variety Pleven 4 stood out with the highest population density of *Bruchus pisorum* during the vegetation period. The pest appeared on 18 May in the numbers 3 number of individuals per 20 sweepings^{-1}, two days later than other varieties.

The numbers of weevils were high and ranged from 3 to 9 number of individuals per 20 sweepings^{-1} from the stage of flowering to the formation of the first pods (until 5 June).

The highest value of 12 number of individuals per 20 sweepings^{-1} was observed at the stage of beginning of pods development during the period 6–10 June, as the density remained relatively high to beginning of ripening of seeds, registered on 20 June.

Flowering and pods development stages had the longest duration (total of 40 days) and occurred 5 to 10 days later compared to the corresponding stages of Ukrainian varieties. This determined the later mass occurrence of weevils.

The sum of effective temperatures from germination to the formation of the first pods and up to the ripening of seeds were 460.5 and 682.0°C respectively and they were the highest.

Kamerton was the second variety in the numerical participation of weevils, which compared to other Ukrainian varieties, was characterized by the longest duration of flowering and pods development stages (34 days) and, accordingly, the highest SET of 388.4 and 577.1°C respectively.

Especially large differences were found in the duration of flowering stage, which exceeded the corresponding in Glyans, Svit and Modus from 5 to 13 days. The numbers of *B. pisorum* increased dynamically from 1, and reached the maximum value to 8 number of individuals per 20 sweepings^{-1} in the period 1–5 June.

Pods development stage occurred after 6 June and the density decreased in the range 1–3 number of individuals per 20 sweepings^{-1}.

It not observed differences between phonological stages in Glyans and Svit varieties and the population dynamic of pea weevil was similar.

Flowering and pods development had a shorter duration (29 days) corresponding with less presence of weevils. Its density increased consistently throughout flowering and formation of the first pods stages and reached maximum values by 4 and 6 number of individuals per 20 sweepings^{-1} respectively in the period 26–31 May.

The density ranged in the lower limit from 0.4 to 4.0 number of individuals per 20 sweepings^{-1} at the stage of pods development (1–15 June), as the species not found at the stage of seed ripening.

SETs from germination to the formation of the first pods and up to the ripening of seeds were relatively low for both varieties –342.2 and 543.3°C respectively (Table 2).

Table 2.

Sum of effective temperatures,°C

Varieties	2012	2012	2013	2013	2014	2014
	1*	2	1	2	1	2
Glyans	342.2	543.3	355.7	481.0	231.6	277.4
Svit	342.2	543.3	355.7	490.9	231.7	277.5
Kamerton	388.4	577.1	369.6	511.9	258.7	317.8
Modus	284.9	510.2	325.1	451.8	199.1	258.7
Pleven 4	460.5	682.0	439.7	653.1	301.0	435.0

Legend:
*****1–period from germination to the formation of the first pods;
2–period from germination to the ripening of seeds

Modus was distinguished with the lowest numbers of *B. pisorum*, ranging from 1 to 2 number of individuals per 20 sweepings^{-1} and had the shortest duration of flowering and pods development stages (27 days).

SETs from germination to the formation of the first pods and up to the ripening of seeds were the lowest –284.9 and 510.2°C respectively.

In terms of the density of the pea weevil average in 2012, varieties had following positions in ascending order: Modus, Glyans, Svit, Kamerton and Pleven 4 with relevant the highest numbers by 2, 4, 6, 8 and 12 number of individuals per 20 sweepings^{-1}, recorded during vegetation period. The numbers of pea weevil in Kamerton during the vegetation period exceeded the economic threshold of harmfulness (4 number of individuals per 20 sweepings^{-1}) in the period 26 May–5 June, while in Pleven 4–21 May–15 June.

Year 2013. The high daily average air temperature in May 2013 exceeded the respective temperature by 2.2 and 2.90C in 2012 and 2014.

It was combined with low relative humidity and rainfall and activated early appearance of pea weevil. The weevil appeared in the budding stage compared to 2012 and 2014, when adults migrated later in the flowering stage. The pest had the highest numbers in 2013 (Table 1).

Bruchus pisorum was appeared between 11 and 15 May as its density in the budding stage was similar among the studied pea varieties (Figure 2).

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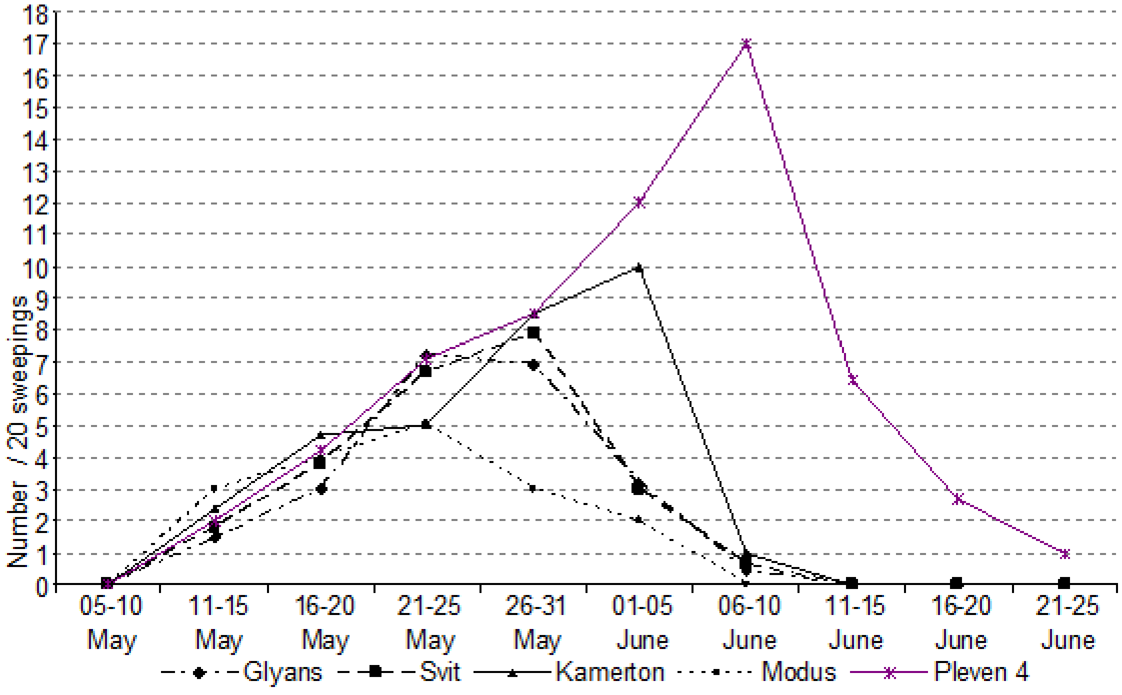

Figure 2. Numerical dynamics of *Bruchus pisorum* in spring pea varieties, 2013

Considering the climatic condition in May 2013 the duration of flowering stage was relatively shorter compared to 2012 and the formation of pods started approximately 1 week earlier.

The numbers of weevils in Pleven 4, due to the prolonged 14–day flowering period intensively increased from 4 to 9 number of individuals per 20 sweepings^{-1}.

The formation of pods and ripening of seeds proceeded for a 20–day period, during which the density was high and ranged from 6 to17 number of individuals per 20 sweepings^{-1}. The highest value was recorded on 7 June in mass pods development stage. In the second half of the month, when seeds were beginning to ripen, the numbers of weevils reduced and it presented in the field until 25 June.

In variety Pleven 4 the beginning of stages of budding, flowering and pods development in 2013 compared to other varieties occurred from 3 to 7 days later, which affected the seasonal dynamics of *B. pisorum* and the time of its mass appearance. Pleven 4 was distinguished again with the long duration of flowering and pods development (total 34 days) as SETs from germination to the formation of first pods was 439.7, and up to the ripening of seeds –653.1°C.

This favored the mass emergence and development of weevils and determined the highest numbers of *B. pisorum*. The pea weevil in Ukrainian varieties appeared from two to three days earlier in the budding stage.

As a result of slight differences in the duration of the flowering stage (approximately 9 days), the *B. pisorum* density was similar and ranged from 2 (Modus) to 5 number of individuals per 20 sweepings^{-1} (Kamerton).

Difference in the values was observed in pods development stage (after 20 May) as in Kamerton the numbers increased considerably, ranged in a higher thresholds (5–10 number of individuals per 20 sweepings^{-1}) and reached a maximum of 10 number of individuals per 20 sweepings^{-1} in the period 1–5 June.

Modus was characterized by the lowest numbers of pest (2–5 number of individuals per 20 sweepings^{-1}) and the earliest peak, which found in the period 21–25 May. In Glyans and Svit varieties the density during pods development was similar (ranged from 3 to 8 number of individuals per 20 sweepings^{-1}) as the maximum number of individuals was recorded between 26 and 31st May.

The density of *B. pisorum* was negligible in Ukrainian varieties in the stage of ripeness of seeds after 5th June and it almost not to observe.

The flowering and pods development stages in Kamerton occurred from two to five days later as compared to other Ukrainian varieties, and the stages were distinguished by a longer duration (29 days).

Respectively SETs from germination to the formation of the first pods by 369.6 and up to the ripening of seeds by 511.9°C were higher than relevant in the other three varieties and determined a higher numbers of pea weevil (Table 2).

Modus had the shortest duration of stages (23 days), respectively, the smallest sum of temperatures –325.1 and 451.8°C. The first pods were registered the earliest.

In 2013 the varieties held their positions in regard to the density of the pea weevil in the following ascending order: Modus, Glyans, Svit, Kamerton and Pleven 4.

The highest numbers by 5, 7, 8, 10 and 17 number of individuals per 20 sweepings^{-1} recorded during the vegetation exceeded the economic threshold of harmfulness (ETH) in all studied varieties.

During the vegetation period the numbers of *B. pisorum* exceeded ETH in the period 21–31 May in Glyans and Svit varieties, 16 May–5 June –in Kamerton and 21 May–15 June–in Pleven 4.

Year 2014. In 2014 the numbers of pea weevil during the vegetation period were the lowest and ranged in low levels considering the lower daily air temperature in May and June average by 1.9 and 2.2°C compared to 2013 and 2012 respectively (Table 1).

As a result of the cool and wet weather the weevils appeared between 18 and 20 May in Ukrainian varieties in the flowering stage (Figure 3).

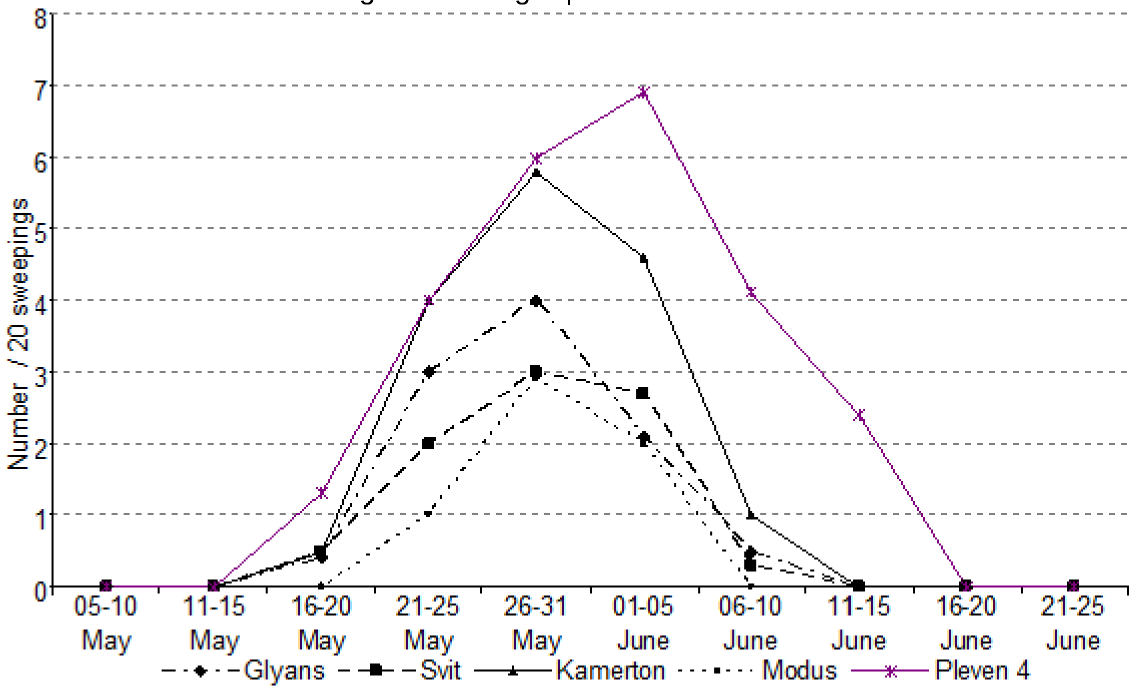

Figure 3. Numerical dynamics of *Bruchus pisorum* in spring pea varieties, 2014

The numbers were under 1 number of individuals per 20 sweepings^{-1}.

The *B. pisorum* density gradually increased and reached maximum values in the formation of the first pods stage during the period 26–31 May in the range of 3 (Modus) to 6 (Kamerton) number of individuals per 20 sweepings^{-1}.

In the beginning of ripeness of seeds in the lower pods between 6–10 June the numbers considerably reduced in the range of 0 to 1, and then it was not observed. The trend for the highest numbers of *B. pisorum* in Kamerton among Ukrainian varieties remained.

Modus had the shortest duration of flowering and pods development stages again and the lowest density of pea weevil. Glyans and Svit hold an intermediate position. Pea weevil in Pleven 4 appeared on 20 May, exceeding 1 number of individuals per 20 sweepings^{-1}. Its numbers in the flowering stage (until 31 May) increased dynamically and in stage of formation of the first pods (during the period 1–5 June) reached a peak by 7 number of individuals per 20 sweepings^{-1}.

The duration of flowering and pods development stages was the largest. The highest numbers during the vegetation were 3, 3, 4, 6 and 7 number of individuals per 20 sweepings^{-1} in Modus, Svit, Glyans, Kamerton and Pleven 4 varieties respectively.

The numbers in the Kamerton and Pleven 4 varieties exceeded the economic threshold of harmfulness in the period from 26 May to 5 June and from 26 May to 10 June respectively. It was observed some differences in terms of the number of laid eggs (Table 3).

Table 3.

Oviposition of *Bruchus pisorum* in pea varieties

Varieties	Proportion of infected pods				Number of laid eggs per pod^{-1}			
	2012	2013	2014	Average	2012	2013	2014	Average
Glyans	52.7ab*	66.2bc	43.6a	54.2ab	1.7a	2.8b	0.6a	1.7b
Svit	56.6bc	63.7b	43.9a	54.7bc	1.8a	2.4b	0.9ab	1.7b
Kamerton	58.8c	69.1c	47.8a	58.6c	2.0a	2.9b	1.0ab	2.0b
Modus	48.2a	56.2a	46.7a	50.4a	1.4a	1.7a	0.7a	1.2a
Pleven 4	67.5d	74.5d	64.7c	68.9d	2.7b	3.6d	1.1b	2.5c
LSD $_{0.05\%}$	4.644	5.017	4.838	4.330	0.646	0.620	0.418	0.358

*Means in each column followed by the same letters are not significantly different ($P > 0.05$)

The high numbers of weevils in 2013 correlated with high fecundity as differences between varieties were considerable. Modus had the lowest proportion of infected pods and number of laid eggs. Differences between Modus and other varieties were statistically significant. Pleven 4 was distinguished with the highest values of the respective parameters.

In 2012 and 2014 among Ukrainian varieties commonly not observed significant differences due to the narrow limits of variation of the proportion of infected pods and number of laid eggs.

Average for the period Modus had the lowest proportion of infected pods – 50.4% and the lowest number of laid eggs –1.2 number per pod^{-1}.

Table 4.

Size of pods in pea varieties, cm

Parameters	Years	Glyans	Svit	Kamerton	Modus	Pleven 4	LSD $_{0.05\%}$
Length of pod	2012	5.41b*	5.66bc	5.58bc	5.14a	5.79c	0.247
	2013	5.41ab	5.72ab	5.87b	5.26a	5.91b	0.555
	2014	5.24a	5.29a	5.42a	5.18a	5.20a	0.687
	Average	5.37b	5.56c	5.62c	5.19a	5.63c	0.128
Width of pod	2012	0.87b	0.90b	0.93b	0.92b	0.74a	0.072
	2013	1.03a	1.18b	1.07a	1.00a	1.07a	0.108
	2014	0.79a	0.95b	0.98b	0.97b	0.78a	0.060
	Average	0.90a	1.01b	1.00b	0.96b	0.87a	0.065
Peduncle length	2012	0.79a	0.85a	0.88a	0.77a	0.80a	0.206
	2013	0.83bc	0.89c	0.79b	0.62a	0.65a	0.072
	2014	0.68bc	0.58a	0.73c	0.67bc	0.66b	0.066
	Average	0.77b	0.77b	0.80b	0.68a	0.70a	0.069

* Means in each row followed by the same letters are not significantly different ($P > 0.05$)

Differences between Modus and other varieties were statistically significant (except Glyan about proportion of infected pods). The highest values and marked preference for oviposition had Pleven 4, where the proportion of infested pods reached 68.9% and the number of laid eggs per pod^{-1} –2.5. Glyans, Svit and

Kamerton hold an intermediate position with similar values as the differences among them were mostly not significant.

According to Makasheva [MAKASHEVA, 1973, BUTU *et al.*, 2014, BUTNARIU and BOSTAN, 2011] the size of the pods according to the agro–climatic conditions was variable value, but the ratio between varieties under the same conditions was reserved. It was found that the length of pods had an effect on the choice of oviposition despite size differences of pods over the years.

The pea weevil had a less pronounced preference for varieties which had a shorter length of pods (Table 4).

In the comparative analysis between varieties Modus had the shortest length of pods –5.19cm, followed by Glyans (5.37cm) average for 2012–2014.

Differences between Modus and Glyans and other varieties were statistically significant. It was observed a higher values in Svit, Kamerton and Pleven 4. It was found the strong positive correlation between the length of pods and the proportion of infected pods (r = +0.765), as well as between the length of pods and the number of laid eggs per pod^{-1} (r= +0.853). According to Pesho and collab. [PESHO *et al.,* 1977, BUTNARIU *et al*., 2014, BUTNARIU *et al.,* 2006, CAUNII *et al.,* 2015, BOSTAN *et al.,* 2013] peduncle lengths of resistant introductions were significantly shorter than those of susceptible cultivars and were highly correlated with lower percentages of infested pods and seeds.

In this study a similar trend was not observed. The width of the pods was an indicator varying slightly and was no effect on the oviposition. Use of different markers for resistance as discrepancy between the phenological development of the host plant and the life cycle of the phytophagous insects, a length of pods *etc*. in the creation of new pea varieties may be effective methods for defense and control against *Bruchus pisorum.*

Conclusions

The duration of flowering and pods development stages in spring pea varieties affected the seasonal dynamics of *Bruchus pisorum*. Modus variety had the shortest duration of flowering and pods development stages and the lowest density of *B. pisorum*. The tolerance of Modus was related to earliness of the variety. The resistance to bruchids in spring pea may be related to length of pods, affecting oviposition. Modus had the shortest length of pods, the lowest proportion of infected pods and number of laid eggs per pod.

Use of different markers for resistance as discrepancy between the phenological development of the host plant and the life cycle of the phytophagous insects, a length of pods *etc*. in the creation of new pea varieties may be effective methods for defense and control against *Bruchus pisorum.*

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