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Original article

Biochemical Composition of Root Mass of Healthy and Damaged by the Larva of *Plagionotus floralis* Pall. (Coleoptera: Cerambycidae) Alfalfa (*Medicago sativa* L.) Plants

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Abstract

Alfalfa (*Medicago sativa* L.) is a preferred host for a large number of arthropods. The large diversity of harmful entomofauna is a serious difficulty in its cultivation. The aim of the study is to investigate the impact of damages caused by the larvae of alfalfa longhorn beetle (*Plagionotus floralis* Pall.) on the biochemical composition of the roots of alfalfa plants of different variety and age. Standard methods were used to carry out the field experiments in order to define the damages and the laboratory biochemical analyzes. It was found that significant changes in the composition of important organic compounds and chemical elements occurred in the root mass of the damaged plants during the years. In the roots of the damaged plants, the composition of water-soluble sugars and saponins decreased the amount of raw protein, raw fibers, phenols and calcium increased, whereas difference was not found in dry matter, phosphorus and magnesium content, compared to healthy plants.

Keywords: Alfalfa, *Plagionotus floralis*, Biochemical changes, Root mass.

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INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a major grass-forage crop. It is called the "Queen of forages" because of its high productivity and excellent forage qualities (Lacefield et al., 1997). Besides forage value, the alfalfa also has agro-technical significance as a nitrogen-fixing crop and an anti-salinization agent in soils in regions with irrigation (Veronesi et al., 2010).

The root system and the above ground alfalfa mass are hosts of a large number of insect pests capable of substantially altering the phytosanitary status of alfalfa fields. Among the insects, damaging the roots, the alfalfa longhorn beetle *Plagionotus floralis* (Pallas 1776) (Coleoptera: Cerambycidae) is of great economic importance for the crop. Damages, caused by the larvae that develop in the roots of the attacked plants, lead to thinning the stands, reduce the quantity and quality of the forage and seed yield, worsen the quality of the breeding materials (Kemenesy and Manninger, 1968; Makarov, 1968; Nikolova and Kertikova, 2008).

When comparing the results of the biochemical analysis of healthy roots with those of root rot and damaged by larvae of alfalfa longhorn beetle, it was found that under both stress factors the metabolic response of plants was unidirectional. The content of raw fibers, calcium and total phenols increased and the content of phosphorus, magnesium and sugars decreased. The differences between the impact of root rot and the damages, caused by alfalfa longhorn beetle were that in roots, infected by root rot, the content of crude protein and saponins decreased (Jurzysta, 1979; Ilieva, 1996), and in the damaged by the larva – increased (Petkova et al., 2005). The results showed significant non-specificity in plant responses in the studied traits to the pathogen and the pest. It is possible the size and the nature of the responses to depend mainly on the degree of the damage caused by the stress factors and the degree of resistance of the plants to the same (Edreva, 1989).

The saponins in alfalfa belong to the group of triterpenoid saponins and are mainly concentrated in the roots, with genetic differences found in their concentration in the different varieties and undergrowths (Bickoff et al., 1972; Nowacka and Oleszek, 1994; Nozzolillo et al., 1997; Yazdi-Samadi et al., 2004; Georgieva et al., 2012; Nikolova et al., 2012; Nikolova et al., 2015). The presence of saponins in alfalfa is associated with the resistance to some pests and pathogens (Pedersen et al., 1976; Sutherland et al., 1982; Gorski et al., 1991; Tava and Odoardi, 1996; Sylwia et al., 2006).

The crude protein in the roots plays a major role in the formation of the undergrowths (Avice et al., 1997) and is an indicator of potential protein nutrition of the forages.

Raw fibers include various chemical substances from the group of the carbohydrates (natural cellulose, hemicellulose, lignin, kutin, pectin, etc.), which have an important role to play in increasing the energy nutrition of the forages (Nikolova et al., 2017).

Plant phenols are secondary metabolites and one of the most common and widespread groups of substances in plants. Secondary metabolites are present in all plants, usually as mixtures, which may be too diverse. The necessity of phenolic compounds for plants is related to pigmentation, growth, reproduction, protection from pathogens, insects, competing plants and UV rays, attraction of insects for pollination and grazing livestock for seed spreading, and many other functions. In plants, the secondary metabolites are contained in specialized cells and do not participate in the basic metabolism of the plants (Swain, 1977; Kutchan, 2001; Theis and Lerdau, 2003; Lattanzio et al., 2006). Plant phenols could be divided into two classes: preformed phenols that are synthesized during normal growth of plant tissues and induced phenols that are synthesized by plants in response to physical injury, insect damages and pathogens, or stress from other factors (Castellanos and Espinosa-Garcia, 1997; Nicholson and Hammerschmidt, 1992; Winkel-Shirley, 1998; 2002).

In the plant organism, calcium plays an important role in the growth of the root system, and therefore the necessity for it is expressed by the germination of seeds. This facilitates the absorption of nitrogen and is extremely important for the process of photosynthesis, also participates in other physiological processes important for the plant.

The aim of the study is to investigate the impact of damages caused by the larvae of alfalfa longhorn beetle on the biochemical composition of the roots of plants of different varieties and age.

Material and Methods

The experimental work was carried out during the period 2010-2015 at the experimental field of IASS "Obraztsov Chiflik" - Rousse.

The scheme of field experiments is presented in Table 1.

Table 1. Scheme of field experiment

	Age of the alfalfa stand		
	1st variant	2nd variant	3rd variant
2010	sowing		
2011	2-year old alfalfa stand	sowing	
2012	3-year old alfalfa stand	2-year old alfalfa stand	sowing
2013	4-year old alfalfa stand	3-year old alfalfa stand	2-year old alfalfa stand
2014		4-year old alfalfa stand	3-year old alfalfa stand
2015			4-year old alfalfa stand

A field experiment was started with three alfalfa varieties: „Pleven 6”, „Prista 3” and „Mnogolistna 1” at the age of one to four years.

The experiment included three variants. In order to provide the necessary significance of the results of the effects of alfalfa age on the biochemical composition, the various variants were sown in three consecutive years: first variant - 2010, second variant - 2011 and third variant – 2012. Every variant included sowing of the three varieties - „Pleven 6”, „Prista 3” and „Mnogolistna 1”, in four replications each (experimental plots) at a row-spacing of 12.5 cm. The experiment was carried out after the perpendicular method (Shanin 1977; Dimova and Marinkov, 2005).

In October-November, plants of 0.25 m^2 at a depth of 15-20 cm were extracted from the four replications (experimental plots) of each variety during the study period. The roots of all plants were cut under laboratory conditions. The healthy and damaged roots of the alfalfa plants were very well cleansed from soil, plant and biological impurities, after which the milled mass (pulp) was provided for analysis.

The biochemical analyzes included the determination of crude protein (CP) after Kjeldahl (Sandev, 1979), raw fibres (RF) – via Veende analysis (AOAC, 2007), calcium (Ca) - complexometrically (Sandev, 1979), phosphorus (P) –colorimetrically by hydroquinone method (Sandev, 1979), magnesium (Mg) - colorimetrically after the method of Kunkel-Pearson-Schweigert (1947), water soluble sugars – after Ermakov et al. (1987), biologically active saponins - after Jurzysta (1979), total phenols - as relative units after Swain and Hillis (1959).

SPSS 16.0 for Windows was used to process the results at a level of significance - $P \leq 0,05$. To prove the comparability of the results and the significance of the differences between the mean values, different tests were applied depending on the type of the analyzed quantities (Dytham, 2003).

Results and Discussion

Table 2. Content of organic compounds in healthy and damaged by the larvae of *Plagionotus floralis* Pall. root mass at different variety and age alfalfa stands

Variety	Variant	Crude protein. % of abs. dry matter	Water-soluble sugars. % of dry matter	Raw fibers. % of abs. dry matter	Saponins. % of dry matter	Phenols. relative units
		Content (m±SE)				
2-year old plants						
„Pleven 6”	healthy	12.17±0.34	13.53^a±1.05	24.20±2.07	2.93±0.42	0.10^a±0.02
	damaged	13.13±1.31	6.00^b±0.99	30.24±1.20	2.60±0.16	0.31^b±0.03
			P=0.006			P=0.006
„Prista 3”	healthy	12.31±0.65	13.53^a±1.24	25.10^a±1.31	3.03±0.58	0.11^a±0.02
	damaged	11.83±0.73	7.15^b±0.15	31.59^b±0.06	2.46±0.14	0.28^b±0.02
			P=0.029	P=0.032		P=0.009
„Mnogolistna 1”	healthy	11.40±0.52	15.93^a±0.17	22.94±1.08	3.03±0.52	0.11^a±0.03
	damaged	12.79±0.93	8.16^b±0.42	26.74±1.09	2.60±0.14	0.32^b±0.01
			P=0.001			P=0.003
3-year old plants						
„Pleven 6”	healthy	10.89^a±0.49	14.43^a ±0.64	26.06^a±0.84	3.95^a±0.18	0.14^a±0.031
	damaged	14.11^b±0.63	5.13^b±2.30	30.83^b±1.07	2.77^b±0.06	0.36^b±0.05

		P=0.016	P=0.018	P=0.025	P=0.003	P=0.026
„Prista 3”	healthy	9.88±1.26	14.77^{a}±0.62	26.05±0.83	3.73^{a}±0.23	0.13^{a}±0.04
	damaged	13.77±0.85	5.93^{b}±1.01	29.93±2.69	2.71^{b}±0.21	0.40^{b}±0.07
			P=0.002		P=0.032	P=0.026
„Mnogolistna 1”	healthy	10.04^{a}±0.86	14.77^{a}±1.03	25.51±0.80	3.97^{a}±0.24	0.13^{a}±0.02
	damaged	13.99^{b}±0.04	6.20^{b}±1.45	28.38±1.88	2.93^{b}±0.09	0.39^{b}±0.05
		P=0.010	P=0.009		P=0.016	P=0.010
4-year old plants						
„Pleven 6”	healthy	9.66^{a}±0.60	13.50^{a}±0.42	26.52^{a}±0.93	3.17±0.52	0.14^{a}±0.01
	damaged	11.64^{b}±0.18	5.40^{b}±1.59	32.86^{b}±1.12	2.40±0.40	0.29^{b}±0.05
		P=0.034	P=0.008	P=0.012		P=0.05
„Prista 3”	healthy	8.94^{a}±0.38	12.60^{a}±0.40	28.18±0.92	3.23±0.30	0.14^{a}±0.02
	damaged	11.26^{b}±0.39	4.90^{b}±2.33	32.88±2.18	2.33±0.27	0.31^{b}±0.07
		P=0.013	P=0.005			P=0.036
„Mnogolistna 1”	healthy	9.28^{a}±0.71	14.10^{a}±0.85	26.15^{a}±1.26	3.48^{a}±0.20	0.13^{a}±0.02
	damaged	10.85^{b}±0.30	5.13^{b}±1.36	32.96^{b}±1.20	2.35±^{b}0.27	0.30^{b}±0.03
		P=0.010	P=0.005	P=0.017	P=0.029	P=0.009
One way ANOVA test. $^{a, b}$ - Differences. significant at $P<0.05$						

Table 3. Content of dry matter, calcium, phosphorus and magnesium in healthy and damaged by *Plagionotus floralis* Pall. root mass at different variety and age alfalfa stands

Variety	Variant	Dry matter. g/kg fresh weight	Ca	P	Mg
			% of abs. dry matter		
		Content (m±SE)			
2-year old plants					
„Pleven 6”	healthy	91.28±0.84	0.49^{a}±0.05	0.28±0.08	0.08±0.01
	damaged	90.89±0.89	0.99^{b}±0.13	0.34±0.04	0.14±0.02
			P*=0.022		
„Prista 3”	healthy	91.34±0.80	0.53^{a}±0.02	0.29±0.01	0.09±0.02
	damaged	91.14±1.39	0.97^{b}±0.06	0.30±0.04	0.13±0.01
			P=0.003		
„Mnogolistna 1”	healthy	91.33±0.76	0.51^{a}±0.02	0.30±0.02	0.08±0.01
	damaged	90.92±0.82	0.90^{b}±0.04	0.34±0.01	0.11±0.01
			P=0.001		
3-year old plants					
„Pleven 6”	healthy	89.93±1.89	0.40^{a}±0.91	0.35±0.07	0.12±0.02
	damaged	89.86±1.99	0.89^{b}±0.14	0.30±0.04	0.11±0.02
			P=0.043		
„Prista 3”	healthy	90.06±1.82	0.40^{a}±0.07	0.34±0.05	0.12±0.01
	damaged	89.40±1.67	0.92^{b}±0.06	0.31±0.02	0.12±0.01
			P=0.004		

„Mnogolistna 1”	healthy	89.91±2.11	0.43[a]±0.06	0.32±0.06	0.11±0.01
	damaged	89.71±1.80	0.84[b]±0.09	0.35±0.04	0.12±0.01
			P=0.024		
4-year old plants					
„Pleven 6”	healthy	89.32±0.21	0.66[a]±0.08	0.25±0.02	0.63±0.27
	damaged	89.71±0.52	1.03[b]±0.06	0.26±0.01	0.11±0.02
			P=0.024		
„Prista 3”	healthy	89.35±0.19	0.63[a]±0.02	0.31±0.04	0.12±0.01
	damaged	89.36±0.18	1.03[b]±0.11	0.24±0.02	0.10±0.02
			P=0.020		
„Mnogolistna 1”	healthy	89.33±0.20	0.59[a]±0.05	0.25±0.02	0.12±0.01
	damaged	89.25±0.21	0.96[b]±0.04	0.24±0.02	0.12±0.03
			P=0.005		
One way ANOVA test. [a, b] - Differences significant at $P<0.05$					

Table 4. Biochemical composition of damaged root mass by the larvae of *Plagionotus floralis* Pall. at different variety (A) and age (B) alfalfa stands

	Crude protein. % of abs. dry matter	Water-soluble sugars. % of dry matter	Raw fibers. % of abs. dry matter	Saponins. % of dry matter	Phenols. relative units	Dry matter. g/kg fresh weight	Ca. % of abs. dry matter	P. % of abs. dry matter	Mg. % of abs. dry matter
	Content (m±SE)								
(A)	2-year old plants								
„Pleven 6”	13.13	6.00	30.24	2.60	0.31	90.89	0.99	0.34	0.14
„Prista 3”	11.83	7.15	31.59	2.46	0.28	91.14	0.97	0.30	0.13
„Mnogolistna 1”	12.79	8.16	26.74	2.60	0.32	90.92	0.90	0.34	0.10
3-year old plants									
„Pleven 6”	14.11	5.13	30.83	2.77	0.36	89.86	0.89	0.30	0.11
„Prista 3”	13.77	5.93	29.93	2.71	0.41	89.40	0.92	0.31	0.12
„Mnogolistna 1”	13.99	6.20	28.38	2.93	0.39	89.71	0.84	0.35	0.12
4-year old plants									
„Pleven 6”	11.64	5.40	32.86	2.40	0.29	89.71	1.03	0.26	0.11
„Prista 3”	11.26	4.90	32.88	2.33	0.31	89.36	1.03	0.24	0.10
„Mnogolistna 1”	10.85	5.13	32.96	2.35	0.30	89.35	0.96	0.24	0.12
(B)	„Pleven 6”								
2-year old plants	13.13	6.00	30.24	2.60	0.31	90.89	0.99	0.34	0.14
3-year old plants	14.11	5.13	30.83	2.77	0.36	89.86	0.89	0.30	0.11

4-year old plants	11.64	5.40	32.86	2.40	0.29	89.71	1.03	0.26	0.11
„Prista 3”									
2-year old plants	11.83	7.15	31.59	2.46	0.28	91.14	0.97	0.30	0.13
3-year old plants	13.77	5.93	29.93	2.71	0.40	89.40	0.92	0.31	0.12
4-year old plants	11.26	4.90	32.88	2.33	0.31	89.36	1.03	0.24	0.10
„Mnogolistna 1”									
2-year old plants	12.79[ab]	8.17	26.74[a]	2.60	0.32	90.92	0.90	0.34[a]	0.11
3-year old plants	13.99[a]	6.20	28.38[ab]	2.93	0.39	89.71	0.84	0.35[a]	0.12
4-year old plants	10.86[b]	5.13	32.96[b]	2.35	0.30	89.17	0.96	0.24[b]	0.12
	P=0.022		P=0.050					P=0.04	
One way ANOVA test. [a, b] - Differences significant at P≤0.05									

The biochemical composition of the roots - healthy and damaged by the larvae of alfalfa longhorn beetle, at different age of three alfalfa varieties was presented in Tables 2 and 3. It was found that in the root mass of the damaged plants significant changes occurred in the content of important organic compounds and chemical elements through the years. At the roots of attacked plants, the content of water-soluble sugars and saponins decreased, the amount of crude protein, raw fibres, phenols and calcium increased, whereas compared to the healthy plants, difference in the content of dry matter, phosphorus and magnesium was not observed.

To the fullest extent the damaging activity of larvae affected the energy reserve of alfalfa plants during the three years of the study. The content of water-soluble sugars in the roots of the damaged 1-year old plants was 1.9 („Prista 3") to 2.3 („Pleven 6") times lower than in healthy plants, and in 3 and 4-years old alfalfa stands – from 2.4 („Mnogolistna 1") to 2.8 („Pleven 6"), respectively from 2.5 („Pleven 6") to 2.7 („Mnogolistna 1”) times lower, compared with the healthy plants (Table 2). It should be noted that the content of water-soluble sugars in the damaged root mass did not change depending on the age and variety of alfalfa stand (Table 4).

The results of the analyzes showed that unlike the water-soluble sugars, the proven lower content of saponins in the damaged roots was found in the three varieties in the third year, and in the fourth year - only in „Mnogolistna 1” variety (Table 2). Their quantity varied slightly from one variety to another and ranged from 2.35% („Mnogolistna 1", 4th year) to 2.93% („Mnogolistna 1", 3rd year), which was about 1.4-1.5 times lower than the healthy plants. The differences in the content of saponins between the damaged and healthy 2-year old („Pleven 6”, „Prista 3”, „Mnogolistna 1") and 4-year old („Pleven 6" and „Prista 3") alfalfa stands were not proven. Significant differences were not also found in the content of saponins in the damaged roots depending on the variety and age of the plants (Table 4).

The data in Table 2 showed that with the highest content of crude protein, 3-year old alfalfa stand was distinguished. Its quantity in the attacked roots as a percentage of the absolute dry matter in the individual varieties was as follows: 13.77% („Prista 3"), 13.99% („Mnogolistna 1") and 14.11% („Pleven 6”). The average values were 3.89%, 3.95% and 3.22% respectively higher, compared to the studied trait in the roots of the healthy plants. Differences were significant in „Pleven 6” and „Mnogolistna 1" (Table 2).

In the 4-year old alfalfa stand, the crude protein content of the damaged roots was 1.98% („Pleven 6”), 2.32% („Prista 3”) and 1.57% („Mnogolistna 1”) higher than the healthy, with significant differences (Table 2).

In 2-year old stands, the differences in the values of the trait between healthy and damaged plants were not statistically significant. Significant differences were not also found in the crude protein content in the damaged roots between the different varieties. The influence of the factor „alfalfa age” on the studied trait was found only in „Mnogolistna 1" variety, where the lowest crude protein content of the 4-year old plants was significant (Table 4).

In the damaged roots the content of raw fibers was higher than in healthy ones during the three years of study (Table 2). The differences between the two variants in the 2-year old alfalfa stands were significant in „Prista 3” variety, in the 3-year old - in „Pleven 6”, and in the 4-year old - in „Prista 3” and „Mnogolistna 1". The absence of significant differences in the other variants suggested that the content of raw fibers between healthy and damaged roots did not show well pronounced dependence, and the observed differences could be considered as random.

Statistically significant differences in the content of raw fibers in damaged roots, depending on the variety, were not defined. The influence of the age factor on the plants was observed only in „Mnogolistna 1", where the content of raw fibres was significant higher at 4-year alfalfa stands compared with the 2-year old alfalfa stands. (Table 4).

The results of the analyzes (Table 2) showed that the content of phenols was significant higher in the damaged roots by the larva. Their amount ranged from 0.28 to 0.32 relative units in the 2- and 4-year old alfalfa stands and from 0.36 to 0.40 relative units in the 3-year-old alfalfa stands which was from 2.1 to 3.1 times higher than in healthy plants. The results proved that plants responded to the damage caused by the larvae of alfalfa longhorn beetle with increasing the synthesis of induced phenols in the roots, which are believed to serve as a barrier to nutrition in the literature (Castellanos and Espinosa-Garcia, 1997). Significant differences in the content of phenols depending on the variety and age factors, were not defined (Table 4).

The content of calcium in the damaged roots was 1.56 to 2.3 times higher than in the healthy ones, the differences being statistically significant (Table 3). It should be noted that significant differences were not defined with respect to the amount of calcium in the damaged roots depending on the variety and age of the plants (Table 4).

Conclusions

The harmful activity of the larvae of alfalfa longhorn beetle is accompanied by significant changes in the biochemical composition of the root mass.

In the roots of the damaged plants, the content of water-soluble sugars and saponins decreases, the amount of crude protein, raw fibers, phenols and calcium increases, and the content of dry matter, phosphorus and magnesium is without any difference to healthy plants.

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