¹Diameter of the root neck and number of green stems in alfalfa plants damaged by the larvae of alfalfa longhorn beetle (*Plagionotus floralis* Pall.: Coleoptera, Cerambycidae)

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Abstract

During the period 2010-2015, three field trials with three alfalfa varieties are conducted in the experimental field of the IASS "Obraztsov chiflik" - Rousse: Pleven 6, Prista 3 and Mnogolistna 1 in four replications, perpendicular method with randomization of the plots, in non-irrigating conditions and 12,5 cm distance between rows. The aim of the study is to investigate the correlation between damage caused by alfalfa longhorn beetle larvae, the age of alfalfa plants, the diameter of the root neck and the number of green stems in alfalfa varieties. Two-, three- and four-year-old alfalfa plants are more susceptible to damage by alfalfa longhorn beetle larvae, a prerequisite of which is the larger diameter of the root neck. The largest diameter of the root neck has the damaged four-year-old plants. There is no proven difference between number of green stems of healthy plants and plants damaged by alfalfa longhorn beetle larvae, depending on the age and variety of alfalfa. The reason is their considerable quantitative variability under the influence of genetic features, cultivation technology, climatic conditions, etc.

Keywords: alfalfa; alfalfa longhorn beetle; damage; diameter of the root neck; number of green stems

INTRODUCTION

Alfalfa is the most widely distributed forage crop in the country (MAF, 2015), which has a great potential productivity (Yankov et al., 2002). Its green mass has high content of protein, essential aminoacids, vitamins and trace elements, which makes it the appropriate main component for green meals, hay, senage, vitamin flour and many other applications (Lacefield et al., 1997; Veronesi et al., 2010).

The harmful activity of the larvae of alfalfa longhorn beetle is expressed in grooving of tunnels in the roots of alfalfa plants. Plants with thick roots are being preferred and that is why the damage affects strongly older lucerne crops (Đukić and Erić, 1995). Excessive use of old, thin and heavily weed infested alfalfa crops favors reproduction and possible damages caused by the alfalfa longhorn beetle (Bozsik, 2010, 2013).

Data about root diameter and number of green stems of damaged alfalfa plants, which are key indicators for assessment of crop productivity, can not be found in the specialized literature.

The aim of the study is to investigate the correlation between damage caused by alfalfa longhorn beetle larvae, age of alfalfa plants, diameter of the root neck and number of green stems in alfalfa varieties.

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MATERIAL AND METHODS

The experiments were conducted in the period 2010-2015 in the experimental field and at the laboratory of entomology of IASS "Obraztsov Chiflik" - Rousse. The field parcel trial included three alfalfa varieties: Pleven 6, Prista 3, and Mnogolistna 1 in four replications, set in the perpendicular method with randomization of the plots (Shanin, 1977; Dimova and Marinkov, 1999), under non-irrigation conditions and 12.5 cm distance between rows (Fig. 1).

In order to provide the necessary authenticity of the obtained results for the effects of the age of alfalfa plants over the larval damages, diameter of the root neck and number of green stems, field trial has been repeated in three consecutive years (2010, 2011 and 2012) and each one has been harvested for 4 years (Fig. 2).

In October-November, the plants grown on 0.25 m^2 were rooted out from a depth of 15-20 cm – from

each of the 4 replictes (experimental plots) of each variety during the study period. The roots of all plants were cut under laboratory conditions. The following indicators were traced out:

• diameter of the root neck of the plants (the measurement was made with caliper);

• number of green stems per plant.

Statistical processing of the results was performed using the program SPSS 16.0 for Windows (www. spss.com) at a level of authenticity $P \le 0,05$. In order to prove the comparability of the results and the reliability of the differences between the average values, different tests were applied depending on the type of the analyzed quantities (Dytham, 2003).

RESULTS AND DISCUSSION

The results show that there is no proven difference between the diameter of the root neck of healthy

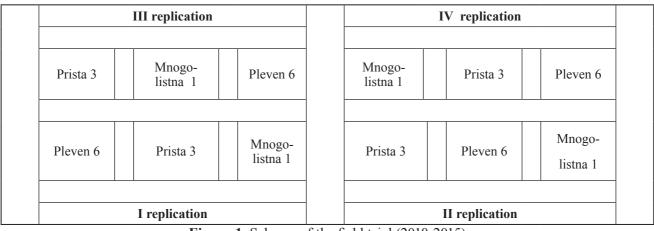


Figure 1. Scheme of the field trial (2010-2015)

Year	Age of plants						
	1 variant	2 variant	3 variant				
2010	sowing						
2011	2-year old	sowing					
2012	3-year old	2-year old	sowing				
2013	4-year old	3-year old	2-year old				
2014		4-year old	3-year old				
2015			4-year old				

Figure 2. Repeatability of the field trials

and damaged annual plants in the three tested varieties (Tabl. 1). The average diameter of root neck ranges from 7.38 mm to 7.78 mm in the healthy and

from 5.80 mm to 9.00 mm in the damaged plants. It is important to note the high coefficient of variation in both types: healthy (34.57% - 38.82%) and

Table 1. Diameter of root neck (mm) of healthy and damaged plants by the larvae of <i>Plagionotus floralis</i> Pall.
in three alfalfa varieties (2010-2015)

Variety	Plants	Ν	Min-max	m±SE	CV%	RSE%
One-year-old plants						
Pleven 6	damaged	6	5-14	9,00±1,31	35,78	14,55
	healthy	60	4-15	7,38±0,34	36,04	4,61
Prista 3	damaged	9	3-13	7,78±0,99	38,43	12,72
Pfista 5	healthy	60	3-15	7,78±0,35	34,57	4,50
Musselistus 1	damaged	10	3-11	$5,80\pm0,90$	49,31	15,52
Mnogolistna 1	healthy	60	2-15	7,65±0,38	38,82	4,97
Two-year-old plants						
Pleven 6	damaged	25	4-22	10,84±1,00	46,12	9,22
Plevell 0	healthy	60	3-21	9,50±0,57	46,53	6,00
	damaged	29	4-29	11,93ª±1,00	45,26	8,38
Prista 3	healthy	60	2-23	9,83 ^b ±0,51	40,08	5,19
				P=0,041*		
	damaged	35	6-25	10,91ª±0,69	37,58	6,32
Mnogolistna 1	healthy	60	1-25	8,67 ^b ±0,58	51,79	6,69
				P=0,017*		
Three-year-old plants						
	damaged	39	5-27	11,90ª±0,78	41,01	6,55
Pleven 6	healthy	60	4-22	9,43 ^b ±0,57	46,98	6,04
				P=0,011*		
	damaged	47	4-30	10,66±0,85	54,41	7,97
Prista 3	healthy	60	4-23	9,62±0,55	44,70	5,72
	damaged	35	6-25	12,06ª±0,72	35,24	5,97
Mnogolistna 1	healthy	60	5-21	10,28 ^b ±0,45	34,14	4,38
				P=0,031*		
Four-year-old plants						
	damaged	58	8-30	14,45ª±0,67	35,57	4,64
Pleven 6	healthy	60	5-26	11,38 ^b ±0,51	34,80	4,48
				P=0,001*		
Prista 3	damaged	44	6-29	13,66ª±0,72	34,92	5,27
	healthy	60	5-22	11,53 ^b ±0,48	32,52	4,16
				P=0,012*		
	damaged	53	7-31	13,66ª±0,67	35,72	4,90
Mnogolistna 1	healthy	60	6-24	10,88 ^b ±0,52	37,04	4,78
				P=0,001*		

N – number of analyzed plants; CV – coefficient of variation; RSE – relative standard error; test of the significance of the differences: * One way ANOVA, a, b – differences proven in P<0.05

damaged plants (35.78% - 49.31%), which reveals a significant variation in the diameter of the root neck towards average assessment.

A change in the indicator is observed in older crops, which is expressed in proved larger diameter dimensions of root necks of damaged plants compared to healthy plants. A proved difference in the diameter of the root neck values of two-, threeand specially four-year-old crops for the three studied alfalfa varieties was determined. The damaged four-year-old plants have the largest diameter of the root neck – 14.45 mm (Pleven 6), 13.66 mm (Prista 3) and 13.66 mm (Mnogolistna 1). The indicated values exceed those of healthy plants by 3.07 mm, 2.13 mm and 2.78 mm - in Pleven 6, Prista 3 and Mnogolistna 1, respectivly.

Statistical analysis of the data about the influence of alfalfa age over the diameter of the root neck of damaged plants convincingly proves that the largest diameter in all three varieties is the root neck of four-year-old plants (Tabl. 2). Variety does not affect the test indicator when plants of the same age are compared. The high values of the coefficient of variation in healthy and damaged plants (Tabl. 1, 2) confirm the diameter variation of root neck as a biological feature of alfalfa, influenced by other factors (genetic features of the variety, breeding technology, climatic conditions, etc.).

It should be noted the accuracy of the experiment, which is lower than 25% in all variants (admissible accuracy for field trials) (Southwood and Henderson, 2000).

Table 3 shows results for the number of green stems of healthy and damaged plants by the larvae of alfalfa longhorn beetle, depending on the age of the three studied alfalfa varieties. There is no proven difference between the two variants of the three varieties and the different aged plants, as well as between the various aged damaged plants of each variety (Tabl. 4). Probably, this is due to

Age of plants	Ν	m±SE	CV%	RSE%
Pleven 6				
One-year-old plants	6	9,00ª±1,31	35,78	14,55
Two-year-old plants	25	10,84ª±1,00	46,12	9,22
Three-year-old plants	39	11,90°±0,78	40,92	6,55
Four-year-old plants	58	14,45 ^b ±0,67	35,57	4,64
		P<0,05*		
Prista 3				
One-year-old plants	9	7,78ª±0,99	38,43	12,72
Two-year-old plants	29	11,93°±1,00	45,26	8,38
Three-year-old plants	47	10,66ª±0,85	54,41	7,97
Four-year-old plants	44	13,66 ^{bc} ±0,72	34,92	5,27
		P<0,05*		
Mnogolistna 1				
One-year-old plants	10	5,80ª±0,90	49,31	15,52
Two-year-old plants	35	10,9 ^b ±0,69	37,58	6,32
Three-year-old plants	35	12,06 ^{bc} ±0,72	35,32	5,97
Four-year-old plants	53	13,66°±0,67	35,72	4,90
		P<0,05*		

Table 2. Influence of the age of three alfalfa varieties on the diameter of root neck (mm) of the damaged plants by the larvae of *Plagionotus floralis* Pall. (2010-2015)

N – number of analyzed plants; CV – coefficient of variation; RSE – relative standard error; test of the significance of the differences: * One way ANOVA: LSD Post Hoc test, ^{a,b,c} – differences proven in P<0.05

Variety	Plants	Ν	Min-max	m±SE*	CV%	Mediana**	Q ₂₅ -Q ₇₅
One-year-old plants							
Pleven 6	damaged	6	0-13	5,17±2,40	113,70		
i leven o	healthy	60	1-24	8,28±0,78	73,43		
	damaged	9	0-24	6,00±2,56	128,33		
Prista 3	healthy	60	0-18	7,38±0,59	61,79		
	damaged	10	0.05	5 00 10 40	151 04		
Mnogolistna 1		10	0-25	5,20±2,49	151,34		
	healthy	60	1-48	9,98±1,19	92,48		
Two-year-old plants							
Discour (damaged	25	0-29	8,20±1,73	105,24		
Pleven 6	healthy	60	1-38	7,05±0,88	96,45		
Duinte 2	damaged	29	0-34	7,65±1,94	136,60		
Prista 3	healthy	60	0-28	8,72±0,94	89,27		
Musselistus 1	damaged	35	0-27	7,48±1,27	100,67		
Mnogolistna 1	healthy	60	0-52	8,90±1,11	96,40		
Three-year-old plants							
Pleven 6	damaged	39	0-29	7,28±1,27	102,75		
Pleven o	healthy	60	0-34	6,75±1,11	104,00		
Drigto 2	damaged	47	0-42	6,04±1,18	134,44		
Prista 3	healthy	60	0-28	6,60±0,82	96,21		
Mnogolistna 1	damaged	35	0-59			8,0	2,0-17,0
Milogonstila 1	healthy	60	0-23			6,0	2,25-11,5
Four-year-old plants							
Pleven 6	damaged	58	0-43			5,0	2,0-16,25
r leveli 0	healthy	60	0-36			5,0	2,25-9,0
Dristo 3	damaged 44 0-34	0-34			5,50	2,0-12,0	
Prista 3	healthy	60	0-27			7,0	4,0-10,0
Mnogolistna 1		2,0-18,50					
Mnogolistna 1	healthy	60	0-48			9,0	4,0-14,0

Table 3. Number of green stems in healthy and damaged plants by the larvae of *Plagionotus floralis* Pall. of three alfalfa varieties (2010-2015)

N – number of analyzed plants; CV – coefficient of variation; RSE – relative standard error; test of the significance of the differences: * One way ANOVA test; ** – Mann-Whitney U test; Q_{25} - Q_{75} – first (25%) and third (75%) levels of the quantils (the usual fluctuations of the index)

the considerable variability of the number of green stems as a biological feature of the alfalfa, which is influenced by other factors too (genetic features of the variety, breeding technology, climatic conditions, etc.). High values of coefficients of variation and high levels of the usual (Q75) and terminal (Q90) fluctuations of the feature prove this (Tabl. 3, 4).

Age of plants	Ν	Mediana	$Q_{25}-Q_{75}$	$Q_{10} - Q_{90}$
Pleven 6				
One-year old plants	6	3,0	0,0-12,25	0,0-13,0
Two-year-old plants	25	5,0	2,0-15,50	0,0-23,8
Three-year-old plants	39	5,0	2,0-10,0	0,0-21,0
Four-year-old plants	58	5,0	2,0-16,0	0,0-22,3
Prista 3				
One-year old plants	9	3,0	0,0-8,50	0,0-24,0
Two-year-old plants	29	2,0	0,0-12,0	0,0-33,0
Three-year-old plants	47	4,0	0,0-9,0	0,0-18,0
Four-year-old plants	44	5,5	2,0-12,0	0,0-20,5
Mnogolistna 1				
One-year old plants	10	2,5	0,0-7,5	0,0-23,7
Two-year-old plants	35	6,0	2,0-12,0	0,0-19,0
Three-year-old plants	35	8,0	2,0-17,0	0,0-31,2
Four-year-old plants	53	6,0	2,0-18,5	0,0-25,0

Table 4. Influence of the age of three alfalfa varieties on the number of green stems of the damaged plants by the larvae of *Plagionotus floralis* Pall. (2010-2015)

N – number of analyzed plants; test of the significance of the differences: Mann-Whitney U test; $Q_{25}-Q_{75}$ – first (25%) and third (75%) levels of the quantils (the usual fluctuations of the index); $Q_{10}-Q_{90}$ – the final fluctuations of the attribute

CONCLUSIONS

1. Two-, three- and four-year-old alfalfa plants are more susceptible to damage by larvae of alfalfa longhorn beetle, a prerequisite of which is the larger diameter of the root neck. Four-year-old damaged plants have the largest diameter of the root neck.

2. The number of green stems of healthy plants and plants damaged by larvae of alfalfa longhorn beetle has no proven difference depending on the age and variety of alfalfa. The reason is their considerable quantitative variability, influenced by other factors (genetic features of the variety, breeding technology, climatic conditions, etc.).

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