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Effect of growing legumes as pure and mixed crops on *Otiorrhynchus ligustici* L. (Coleoptera: Curculionidae) damage

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Abstract

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A field trial was carried out at the Institute of Forage Crops, Pleven, Bulgaria to determine the effect of the growing legumes as pure and mixed crops on the damage of *Otiorrhynchus ligustici* L. The study was performed for the period 2015–2017 on sainfoin, birdsfoot trefoil and alfalfa stands (grown alone) and in mixtures with cocksfoot, sowed under cover of spring forage pea. In mixed crops, the *O. ligustici* damage expressed through the number of holes and furrows, as well as their length, was reduced by 22.7, 33.3 and 18.2%, respectively. Alfalfa was the most preference crop by the alfalfa snout beetle and birdsfoot trefoil was less affected. In addition, the distance of the spiral furrows from the root collar and legume root length increased in mixtures by 19.0 and 3.0% to the crops grown alone. The most strongly significant negative effect on the height of legumes had the length of the spiral furrows (-13.1). A less pronounced but significant negative effect had hole number on plant roots (-5.7). The technology of growing of legume crops had an impact on the injury on the plant roots by the soil-inhabiting insects as in mixtures, there are favorable conditions for both, development of plants and reducing the degree of damage.

Keywords: legumes; mixtures; damage; *Otiorrhynchus ligustici*

Introduction

Legumes play an important role in agriculture due to their unique ability to fix atmospheric nitrogen (Graham & Vance, 2003). They are often grown in mixtures where with appropriate components and proportions are stimulated to fix more nitrogen, productivity is higher and resources are used more efficiently (Sessitsch et al., 2002; Luscher et al., 2014).

Management of forage insects is an important component of most forage systems. Although insect species generally differ in legume and grass forage crops, management of beneficial or pest insects in all forage crops generally results in greater protection of potential forage yields. Alfalfa is one of the most common legumes forages grown as pure-stand and /or mixed stands utilizing one to several different grasses. When mixed-stands of alfalfa and grass species are grown together, insect problems are often reduced as compared to pure-stand planting, although economic populations of specific insect pests can occasionally develop (Bailey, 2015). Straub et al. (2013) reported that the damage caused by some main sucking pests such as *Acyrthosiphon pisum* and *Empoasca fabae* in the monoculture cultivation of alfalfa in the United States was significantly higher than that when alfalfa was grown in mixtures. In mixtures, the predator-to-prey ratio was found higher and increased the predator activity and the pest's vulnerability. Similar results reported by Feng et al. (2004). They found the reduction of harmful species (by 77%), an increase in the number of useful insects (by 10–17%) and minimal variation in alfalfa quantity grown in tea plantations. In addition, the authors have not find any damage that lead to a reduction in the forage yield. In mixtures of alfalfa with cocksfoot was established that *Nabis americoferus* was more effective and resulted in a more pronounced decrease in the density of the larvae of the *E. fabae* compared to pure alfalfa cultivation (Straub et al., 2014).

The choice of suitable components in the mixture is important because the degree of their competitiveness determines the productivity of the mixtures as well as its attractiveness to insects (Kusvuran et al., 2014).

Knowledge of composition and changes in plant diversity that affect agro-ecosystems is still a major challenge.

In the present study, the aim was to determine the effect of growing sainfoin, birdsfoot trefoil and alfalfa as pure crops and in mixtures with cocksfoot on the damage of *Otiorrhynchus ligustici* L.

Materials and Methods

The trial was performed in the experimental field of the Institute of Forage crops, Pleven, Bulgaria. Sainfoin, birdsfoot trefoil and alfalfa were sown alone (100%) and in mixtures with cocksfoot (50:50%) (Table 1). Perpendicular to the main crops and mixtures (2014), spring forage pea was sown as a cover crop with 75% of sowing rate in the first year. The experimental randomized plot size was 10 m^2 with four replications according to a methodology of field experience (Shannin, 1977). Four samples of each type of crop and mixture were analyzed or a total of 24 samples in each year.

The chemical composition of a haplic loamy chernozem soil is given in Table 2. The data show that the soil is slightly acidic, poorly supplied with hydrolysable nitrogen, moderately supplied with phosphorus, and rich in total potassium. The plants were cultivated in conditions of organic farming (without the use of any fertilizers and pesticides). Weeds were controlled mechanically during the growth period.

Otiorrhynchus ligustici imago damages leaves, buds and the upper parts of plants. Such damage is of no economic importance. The main damage of these weevils is caused by their larvae, which gnaw deep spiral furrows and holes on alfalfa roots, thus impairing the plant's growth and development.

In the stage of flowering – beginning of the pod formation of legume crops in the second regrowth (in June) soil monoliths 20 (width) x 50 (length) x 40 (depth) cm were taken in the period 2015–2017. After rinsing with water the plants were carefully separated to preserve their integrity. The following characteristics were recorded: number of holes; the degree of damage caused by *O. ligustici* larvae as measured by the length of spiral furrows on the plant roots (cm); a number of furrows; the spacing of the chewed furrows from the root cervix and root length (cm). In addition, the height of plants was measured by a meter after taking soil monoliths.

The mathematical processing of the data was done using an Anova for one factor case, the mean being compared by a Tukey test in 5% provenance ($P \leq 0.05$). A multi-factor regression analysis of Statgraphics Plus (1995) for Windows Ver. 2.1. was also used.

Results and Discussion

The alfalfa snout beetle (*Otiorrhynchus ligustici*) is soil inhabiting insects of great economic importance. It inflicts damage by chewing of spiral furrows and holes, both in pure and mixed crops. The length of the spiral furrows characterizes the degree of attack by the larvae of the alfalfa snout beetle on the root system. It increased with the development of crops over the years and was the highest in the last experimental year (from 0.9 cm (2015) to 3.2 cm/root (2017) where the exceedance of the length was three times as a result of the more active food activity and the larvae number (Table 3). Similar was the trend in the number of holes and furrows/

Table 1. Variants of the experiment

Variant	Ratio, %	Sowing rate, kg da^{-1}
Sainfoin (*Onobrychis viciifolia*) (local population)	100	12.0
Birdsfoot trefoil (*Lotus corniculatus* L.) cv. Leo	100	1.5
Alfalfa (*Medicago sativa* L.) cv. Pleven 6	100	2.5
Sainfoin + cocksfoot	50:50	6.0 + 1.75
Birdsfoot trefoil + cocksfoot	50:50	0.75 + 1.75
Alfalfa + cocksfoot	50:50	1.25 + 1.75

Table 2. Chemical composition of the chernozem soil during the trial in Pleven (2015–2017)

Humus, %	Nitrogen, mg 10^{-3} soil	Phosphorus, mg 10^{-2} soil	Potassium, mg 10^{-2} soil	pH in KCl	pH in H_2O
2.48	0.225	4.29	31.1	5.87	6.54

Table 3. Dgree of damage of *Otiorrhynchus ligustici* L. in perennial legume crops grown alone and in grass mixtures

2015						
Variants	Damage traits from *Otiorrhynchus ligustici*				Root length,cm	
	Number of spiral furrows	Number of holes	Length of furrows, cm	Spacing of the chewed furrows from the root collar	Legume	Cocksfoot
Sainfoin**	0.2 a*	0.0 a	1.2 b	1.41 b	12.3 a	–
Birdsfoot trefoil	0.1 a	0.0 a	1.0 b	0.0 a	14.1 b	–
Alfalfa	2.0 b	0.1 a	1.6 c	0.0 a	17.6 d	–
Sainfoin+cocksfoot	0.0 a	0.5 b	0.0 a	0.0 a	13.5 b	7.4 a
Bird. trefoil +cocksfoot	0.0 a	0.0 a	0.0 a	1.25 b	16.0 c	9.1 c
Alfalfa + cocksfoot	0.3a	0.1 a	1.3 bc	1.5 b	18.4 e	8.3 b
$LSD_{0.5\%}$	0.490	0.363	0.330	0.297	0.763	0.774
2016						
Variants	Damage traits from *Otiorrhynchus ligustici*				Root length,cm	
	Number of spiral furrows	Number of holes	Length of furrows, cm	Spacing of the chewed furrows from the root collar	Legume	Cocksfoot
Sainfoin**	1.3 b	2.7 b	1.9 a	2.7 a	18.5bc	–
Birdsfoot trefoil	0.5 a	1.5 b	1.6 a	2.7 a	20.1 d	–
Alfalfa	2.0 c	3.9 c	1.8 a	3.9 b	18.8 c	–
Sainfoin+cocksfoot	0.9 ab	2.4 b	1.8 a	2.9 a	17.7ab	10.2 a
Bird. trefoil +cocksfoot	0.5 a	0.9 a	1.8 a	3.2 ab	16.9a	13.6 c
Alfalfa + cocksfoot	2.0 c	4.6 c	2.3 a	3.5 ab	21.8e	12.0 b
$LSD_{0.5\%}$	0.650	0.762	0.829	0.913	1.100	0.846
2017						
Variants	Damage traits from *Otiorrhynchus ligustici*				Root length,cm	
	Number of spiral furrows	Number of holes	Length of furrows, cm	Spacing of the chewed furrows from the root collar	Legume	Cocksfoot
Sainfoin**	2.3 b	2.4 bc	3.0 b	3.6 bc	24.0 c	–
Birdsfoot trefoil	2.0 b	2.8 c	1.7 a	2.8 b	16.4 a	–
Alfalfa	3.2 c	6.5 e	5.6 d	1.9 a	26.3 d	–
Sainfoin+cocksfoot	1.8 b	1.8 ab	2.9 b	3.1 bc	23.0c	17.6 b
Bird. trefoil +cocksfoot	1.0 a	1.3 a	1.7 a	3.5 bc	19.2b	19.2 c
Alfalfa + cocksfoot	2.4 b	3.8 d	4.2 c	3.9 c	26.5d	14.0 a
$LSD_{0.5\%}$	0.733	0.998	0.898	0.805	1.393	1.540
2015-2017						
Variants	Damage traits from *Otiorrhynchus ligustici*				Root length,cm	
	Number of spiral furrows	Number of holes	Length of furrows, cm	Spacing of the chewed furrows from the root collar	Legume	Cocksfoot
Sainfoin**	1.3 bc	1.7 b	2.0 bc	2.6 bc	18.3 b	–
Birdsfoot trefoil	0.9 ab	1.4 b	1.4 ab	1.8 a	16.9 a	–
Alfalfa	2.4 d	3.5 d	3.0 d	1.9 a	20.9 c	–
Sainfoin+cocksfoot	0.9 ab	1.6 b	1.6 ab	2.0 ab	18.1b	11.7 a
Bird. trefoil +cocksfoot	0.5 a	0.7 a	1.2 a	2.7 c	17.4ab	14.0 b
Alfalfa + cocksfoot	1.6 c	2.8 c	2.6 cd	3.0 c	22. d	11.4 a
$LSD_{0.5\%}$	0.481	0.610	0.618	0.563	1.036	1.752

Legend: *Means in each column followed by the same letters are not significantly different ($P > 0.05$); **the stage was determined on the legume crop root, as values an average of 0.4 and 0.1 in 2015 reached respectively to 2.1 and 3.1 number/root in 2017. The intensity of the hole formation on the roots was many times higher than spiral furrows, as the hole number increased thirty times from the first to the third year, while the furrows – five times.

There was a pronounced preference of alfalfa snout beetle to the pure grown crops, as the values of the aforementioned traits exceeded those in the mixed ones. In the first year of study, the dependence was clearly expressed in terms of the number and length of the furrows, and their values in the mixtures decreased by 87.5% and 69.2% respectively compared to the pure legumes.

Indicative of all three indicators, characterizing less preference and damage caused by *O. ligustici* to the mixtures with cocksfoot, were the second and third years. In 2016, the number and the length of furrows decreased on average by 15.4 and 15.0% respectively, and by 32.0 and 14.7% in 2017. The reduction of the hole number was 3.7 and 41.0%. The average data for the period indicated that in mixed crops the amount of holes and furrows, as well as their length, was reduced by 22.7, 33.3 and 18.2%, respectively.

Depending on the legume crops, the alfalfa snout beetle caused damage to a different extent on the plant roots. Alfalfa was distinguished as the most attractive for *O. ligustici*, as studied indicators occupied the highest values. In the first year, the damage was minimal, but a tendency of preference nevertheless emerged. The alfalfa grown alone had the highest number and length of spiral furrows with a significant diferences compared to other species of legumes. In the mixture was found significant reduction of the number and the length by 85.0 and 18.8%. In 2016, in both in the pure and in the mixed alfalfa crops, the highest damage was observed, as the difference between them was minimal and insignificant. Distinctive was the third year where the three root indicators statistically significant occupied the highest values in pure stand followed by the mixture with cocksfoot. It was found a significant reduction of the damage in the alfalfa mixture by 25.0, 41.5 and 25.0% respectively for the number of holes, furrows and their length in comparison with pure stand.

Sainfoin was a crop, also preferred by *O. ligustici*, as the tendency for less attack in the mixture was remained and the value reduction during the years varied in the range 21.7 – 30.8% and 11.1 – 25.0% relative to the hole and furrow number and 3.3% to 5.3% relative to the length.

Birdsfoot trefoil was less affected and damaged. However, in the mixture with cocksfoot was established a significant reduction in the number of furrows in 2015 and 2017, holes – in 2016 and 2017 and the furrow length – in 2015. One of the reasons for the less pronounced preference of the alfalfa snout beetle is probably related to the biochemical composition of the root system. Ilieva et al. (2015) found that the birdsfoot trefoil roots had considerably higher condensed tannins than the sainfoin, making them more difficult to digest and not sufficiently appetizing for *O. ligustici* larvae.

On average for the period, in mixtures of sainfoin, birdsfoot trefoil, and alfalfa, the number of furrows decreased by 30.7; 44.4 and 33.3%, holes – by 5.9; 50.0 and 20.0%, and the furrow length by 20.0; 14.3 and 13.3% relative to their pure crops.

According to Hurej et al. (2013) in fields with a mixture of crops a given pest will find fewer acceptable hosts to feed or lay eggs in comparison to fields with a single crop. That was one of the possible reasons reducing alfalfa snout beetle damage.

The statistical significance of reduced plant root injury in the mixed crops (average for the period) was found between the following variants:

– In terms of the furrow number – between alfalfa and alfalfa with cocksfoot;

– In terms of the hole number – between birdsfoot trefoil and birdsfoot trefoil with cocksfoot; between alfalfa and alfalfa with cocksfoot.

Similar results for control of alfalfa beetle reported Bailey (2015), where mixed planting of alfalfa and orchardgrass (*Dactylis glomerata* L.) in ratio 12:2, reduced weevil density by 48.0%, while in the mixture with tall fescue (10:4) the decrease was 50.2%. According to Bowman (1992), a mixed planting of alfalfa and grasses reduced weevil levels depending on the crop ratio. In another intercropping system, Svensson (2006) found that *Otiorrhynchus sulcatus* avoided strawberry crops with parsley and/or spruce as intercrop plants and it preferred to feed and reproduce in plantations with only strawberries. Interactions between component crops make intercropping systems more complex and at the same time frequently reduce pest attack.

The development of the root system of plants through the years of cultivation was accompanied by increasing the distance of the spiral furrows from the root collar. In pure crops, the trait values were lower, and in the mixtures increasing by 19.1%.

Significant differences were observed between alfalfa stands and birdsfoot trefoil stands. A similar trend was also observed with respect to plant root length, which increased by 3.0% in mixtures. That was probably due to the more favourable conditions of development of the root system of legume crops in mixtures with grasses and a more active root system growth compared to their pure growing. Vasileva et al. (2017) reported analogous results wherein mixtures of

Table 4. Regression analysis (ANOVA) of the height of alone and mixture legume crops in regard to the damage traits of *Otiorrhynchus ligustici* L.

A

Dispersion	df	SS	MS	F-Ratio	P-value
Model	3	2816.88	938.961	6.07	0.04
Residual	5	773.845	154.769		
Total (Corr.)	8	3590.73			

B

Trait	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Constant	72.961	7.589	9.613	0	53.451	92.471
Number of furrows	-0.174	7.698	-0.022	0.982	-19.964	19.616
Length of furrows	-13.113	4.865	-2.695	0.043	-25.62	-0.605
Number of holes	-5.745	4.257	-1.349	0.035	-5.199	1.691

birdsfoot trefoil with perennial ryegrass had a higher dry root mass productivity (by 20%) as compared to the pure grown legume.

The regression analysis (Table 4A) indicated that the linear dependence in regression of plant height in relation to the traits tested for damage by the larvae of *O. ligustici* in pure and mixture crops was significant. The applied analysis in Table 4B showed that on the height of legumes the most strongly significant negative effect had the length of the spiral furrows (- 13.1). A less pronounced but significant negative effect had hole number on plant roots (-5.7). The influence of the number of furrows was also of negative value, but there was insignificant.

Conclusions

In mixed crops, the *Otiorrhynchus ligustici* damage expressed through the number of holes and furrows, as well as their length, was reduced by 22.7, 33.3 and 18.2%, respectively. Alfalfa was the most preference crop by the alfalfa snout beetle and birdsfoot trefoil was less affected.

The distance of the spiral furrows from the root collar and legume root length increased in mixtures by 19.0 and 3.0% to the crops grown alone.

The most strongly significant negative effect on the height of legumes had the length of the spiral furrows (-13.1). A less pronounced but significant negative effect had hole number on plant roots (-5.7).

The technology of growing of legume crops had an impact on the injury on the plant roots by the soil-inhabiting insects as in mixtures, there are favorable conditions for both, development of plants and reducing the degree of damage.

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