**Assessment of the pure sowing of different legumes and in mixtures
with grass on productivity and damage level by *Sitona* spp.
and *Otiorrhynchus ligustici* (LINNAEUS, 1758)
(Coleoptera: Curculionidae)**

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ABSTRACT. This work examined the impact on productivity of the cultivation system of perennial legumes such as sainfoin (*Onobrychis viciifolia*), bird's-foot trefoil (*Lotus corniculatus*) and alfalfa (*Medicago sativa*), grown pure and in binary mixtures with cocksfoot and on damage caused by root pests of *Sitona* spp. and *Otiorrhynchus ligustici*. It was found that the productivities of aboveground and root biomasses in binary mixtures were significantly higher compared to the corresponding legume monocultures on average by 30.7 and 39.7% respectively in mixtures with sainfoin, by 15.0 and 73.1% in mixtures with bird's-foot trefoil, and by 18.7 and 49.6% in mixtures with alfalfa. The weight of dry aboveground biomass in the pure leguminous crops slightly exceeded that of the corresponding leguminous components in the mixtures, whereas the productivity of cereal plants in mixed crops was significantly lower compared to the pure cocksfoot on average by 38.5, 47.0 and 51.7% in mixtures of sainfoin, bird's-foot trefoil and alfalfa respectively. A similar tendency was observed with regard to dry root biomass. Damage to nodules by *Sitona* larvae in mixtures decreased significantly: on average by 33.5% (sainfoin + cocksfoot), by 50.3% (bird's-foot trefoil + cocksfoot) and by 55.6% (alfalfa + cocksfoot) compared to pure grown legumes. The damage caused by *Otiorrhynchus ligustici*, as expressed by the length of gnawed furrows, in the mixtures with sainfoin, bird's-foot trefoil and alfalfa decreased considerably – by 12.5, 77.8 and 59.5%, respectively – compared to the pure crops. The damage caused by the alfalfa snout beetle had the most significant negative effect on aboveground and root biomass productivities. Mixed cultivation systems of forage crops are an economically and ecologically sustainable method of insect pest control.

KEY WORDS: forage, productivity, *Sitona* spp., *Otiorrhynchus ligustici*.

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INTRODUCTION

Mixed cultivation systems of forage crops are based on ecological principles and can be more stable and productive than monoculture systems. In mixed communities, there is greater species diversity, complementarity between plant species in terms of available resources, symbiosis interactions (PICASSO et al. 2011), for example, which creates necessary prerequisites for high productivity and environmental stability.

There are a number of studies that have investigated the composition of appropriate legume and grass components in forage mixtures for intensive pasture use, as well as for ensuring a more even distribution of dry matter, superior forage quality and greater stability during the pasture season (GERRISH 2001, TRACY & SANDERSON 2004, THARMARAJ et al. 2007, SANDERSON 2010, WOODWARD et al. 2013).

There are relatively fewer studies in mixed associations related to the impact of stress factors such as drought, pests, weeds and diseases, which pose a significant risk and reduce forage productivity. A number of environmental studies have shown that a greater variety of species in grassland ecosystems increases and stabilizes productivity in stressful conditions (TILMAN 1999, MINNS et al. 2001). Intercropping is an important cultural practice for pest control. This is based on reducing insect density by increasing ecosystem diversity (SMITH & MCSORLEY 2000, RISCH 2005, MOONEN & BÀRBERI 2008); in mixed crops, a pest finds fewer acceptable hosts to feed or lay eggs on compared to monoculture crops.

Mixed cultivation of legumes and grasses reduces the number of pests and the damage they cause, improves control through so-called “bottom-up” and “top-down” control mechanisms (TILMAN et al. 2004, SASTAWA et al. 2004, TOOKER & FRANK 2012) and reduces the invasion of weed species (FARGIONE et al. 2003, ZAVALETA & HULVEY 2004) compared to pure crops, thus creating conditions for a stable and functional ecosystem. SKINNER et al. (2006) reported that the growth of plant species diversity in mixed crops may increase forage yield, resistance to weed invasion and carbon sequestration in the soil by including high-yielding forage species. However, the density of a mono- or oligophagous insect pest may not be reduced by a mixed cultivation system (ANDOW 1991). In contrast, polyphagous species that are characterized by a wide range of hosts often adapt better but are present at a lower density in mixed systems (HUREJ & TWARDOWSKI 2003).

Insect pests that have a root-feeding larval stage as one of the stress factors can, under favourable conditions, significantly disturb the stability of an ecosystem. They often cause the most sustained damage to plants because their attrition remains largely unseen, preventing early diagnosis and treatment. The use of a mixed system of cultivation of forage crops is related to its considerable impact on insect pest density and leads to increased activity of predators and pest vulnerability (STRAUB et al. 2013). FENG et al.

(2011) reported that mixed cultivation of alfalfa in tea plantations led to a 77% reduction in harmful species, a 10-17% increase in the number of useful insects and the absence of damage leading to forage yield reduction.

The purpose of this study was to determine the effect of a cultivation system of perennial legumes (sainfoin, bird's-foot trefoil and alfalfa), grown pure and in mixtures with cocksfoot, on productivity and the level of damage caused by the root pests *Sitona* spp. and *Otiorrhynchus ligustici* (LINNAEUS, 1758).

MATERIAL AND METHODS

The trial was performed in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria, from 2014 to 2017. Sainfoin (*Onobrychis viciifolia* SCOP.), bird's-foot trefoil (*Lotus corniculatus* L.) and alfalfa (*Medicago sativa* L.) were sown alone (100%) and in mixtures with cocksfoot (*Dactylis glomerata* GRAEBN.) (50:50%) (Table 1). Perpendicular to the main crops and mixtures in the first year (2014), spring forage pea was sown as a cover crop with a 75% sowing rate. The experimental plot size was 10 m² with four replications according to a methodology of field experience (SHANIN 1977). Four samples of each type of crop and mixture were analysed, i.e. a total of 24 samples in each year.

Table 1. Variants of the experiment.

Variants	Ratio, %	Sowing rate, kg/ha
Sainfoin (<i>Onobrychis viciifolia</i> SCOP.) (local population)	100	120.0
Bird's-foot trefoil (<i>Lotus corniculatus</i> L.) cv. Leo	100	15.0
Alfalfa (<i>Medicago sativa</i> L.) cv. Pleven 6	100	25.0
Sainfoin + cocksfoot (<i>Dactylis glomerata</i> GRAEBN.)	50:50	60.0 + 17.5
Bird's-foot trefoil + cocksfoot	50:50	7.5 + 17.5
Alfalfa + cocksfoot	50:50	17.5 + 17.5

The genus *Sitona* GERMAR, 1817 comprises about 100 species (VELASQUEZ DE CASTRO et al. 2007). All of them feed on Fabaceae in both larval and imaginal stages. The larva of *Sitona* species feed on root nodules, and feeding damage caused by adult beetles is revealed as notches on the leaf margins.

Otiorrhynchus ligustici imagos damage 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 longitudinal furrows on alfalfa roots, thus impairing the plant's growth and development.

To investigate the larval damage of these pests to the root system of the plants in the flowering stage – the beginning of pod formation of legume crops in the second regrowth – soil monoliths 20 (width) x 50 (length) x 40 (height) cm were taken in the period 2015-2017. After rinsing with water, the plants were carefully separated to preserve their integrity and nodule biomass. The following characteristics were recorded: percentage of nodules damaged by *Sitona* weevil larvae calculated as the ratio of the number of damaged nodules x 100 to the total number of nodules; the degree of damage caused by *Otiorrhynchus ligustici* larvae as measured by the length of spiral furrows on the plant roots (cm); the productivity of dry aboveground biomass; dry root biomass (g/plant). The data of the productivity of dry aboveground and root biomass were processed statistically using ANOVA for a one-factor case, the mean being compared by a Tukey test for 5% significance ($P \leq 0.05$). The multifactor regression analysis of STATGRAPHICS PLUS (1995) for Windows Ver. 2.1. was also used.

RESULTS

The results of this study showed that the productivity of dry aboveground biomass in binary mixtures in 2015 slightly exceeded the corresponding pure cultivated species, and that the differences between them were not significant at 95% confidence intervals (Table 2).

Productivity was significantly higher in the alfalfa mixture. During the next two years and on average for the period, differences in productivity increased, with mixed crops having statistically significant higher values than monocultures. The average productivity of aboveground biomasses in binary mixtures was 21.5% higher than in monocultures. It was higher in mixtures of sainfoin and cocksfoot than in pure sainfoin stands from 18.2% (2015) to 39.4% (2017). The increase in productivity in these mixtures was the most pronounced, averaging 30.7%, followed by the mixtures of alfalfa and bird's-foot trefoil – averages of 18.7% and 15.0% respectively.

A similar tendency was observed with regard to dry root biomass productivity (Table 3). In the three years and on average for the period the mixed crops exhibited a significantly higher productivity than the pure ones. The weight of dry root biomass in mixed crops with sainfoin, bird's-foot trefoil and alfalfa increased on average by 39.7, 73.1 and 49.6%, respectively, compared to the pure crops of legumes.

Table 2. Productivity of aboveground biomass of legume components grown alone and in mixtures (g/m²). avg. – average.

Variants	2015		2016		2017		avg.		SD		min, max	
	min, max	SD	min, max	SD	min, max	SD	min, max	SD	min, max	SD	min, max	SD
Sainfoin	411.98 ^a 548.10	527.22 16.179	537.66 ^a 548.10	14.764	686.16 ^a 690.40	5.996	545.27 ^a 546.35	544.19 546.35	1.527			
Sainfoin + cocksfoot	486.74 ^a 650.00	633.92 18.752	641.96 ^b 650.00	11.370	1008.93 ^b 1016.60	10.847	712.54 ^b 722.20	702.89 722.20	13.65			
LSD_{0.05%}	75.352	–	56.696	–	37.708	–	41.801	–	–			
Bird's-foot trefoil	340.02 ^a 349.12	330.92 12.869	516.60 ^a 527.00	14.707	651.60 ^a 659.20	10.748	502.74 ^a 508.71	499.77 508.71	4.820			
Bird's-foot trefoil + cocksfoot	377.22 ^a 384.62	369.82 10.465	618.44 ^b 633.20	20.874	739.40 ^b 745.10	8.061	578.35 ^b 577.80	570.80 577.80	2.006			
LSD_{0.05%}	50.466	–	77.688	–	40.875	–	13.000	–	–			
Alfalfa	576.72 ^a 584.20	569.24 10.578	679.14 ^a 688.40	13.096	831.24 ^a 838.40	10.126	695.70 ^a 698.89	692.51 698.89	4.511			
Alfalfa + cocksfoot	652.50 ^b 662.90	642.10 14.708	744.88 ^b 754.40	13.142	1050.75 ^b 1056.00	7.425	816.04 ^b 824.43	807.65 824.43	11.865			
LSD_{0.05%}	55.119	–	57.142	–	38.201	–	38.621	–	–			
Cocksfoot	455.60	–	471.78	–	475.74	–	467.71	–	–			

* Means in each column marked by the same letter are not significantly different ($P > 0.05$).

Table 3. Productivity of root biomass of legume components grown alone and in mixtures (g/m²). avg. – average.

Variants	2015		2016		2017		avg.		SD		min, max		SD
	min, max	SD	min, max	SD	min, max	SD	min, max	SD	min, max	SD	min, max	SD	
Sainfoin	202.58a [*] 189.36 215.80	18.695	604.40a 592.70 616.10	16.546	874.40a 865.80 883.00	12.162	560.46a 558.10 562.82	3.337					
Sainfoin + cocksfoot	274.18b 264.88 283.48	13.152	844.80b 836.70 852.90	11.455	1229.50b 1220.80 1238.20	12.304	749.49b 779.53 786.13	4.667					
LSD_{0.05%}	69.546	–	61.278	–	52.635	–	17.456	–					
Bird's-foot trefoil	169.38a 160.26 178.50	12.897	356.04a 349.78 362.30	8.853	456.30a 449.40 463.20	9.758	327.24a 319.81 334.67	10.508					
Bird's-foot trefoil + cocksfoot	270.44b 264.82 276.06	7.948	617.96b 604.72 631.20	18.724	811.40b 800.10 822.70	15.981	566.60b 560.29 572.91	8.923					
LSD_{0.05%}	46.092	–	63.014	–	56.967	–	41.942	–					
Alfalfa	444.74a 435.38 454.10	13.237	672.75a 665.10 680.40	10.819	1030.9a 1025.82 1036.00	7.198	716.13a 713.40 718.40	3.203					
Alfalfa + cocksfoot	649.76b 644.32 655.20	7.693	1012.96b 1007.32 1018.60	7.976	1450.80b 1441.60 1460.00	13.011	1037.84b 1031.10 1044.60	9.560					
LSD_{0.05%}	46.581	–	40.894	–	45.239	–	30.675	–					
Cocksfoot	413.25	–	565.09	–	679.68	–	552.67	–					

*Means in each column marked by the same letter are not significantly different ($P > 0.05$).

The productivity of dry aboveground biomass in the pure leguminous crops slightly exceeded the corresponding leguminous components in the mixtures. Productivity in mixtures with bird's-foot trefoil was on average 20.6% higher than the corresponding figures for the legumes in the binary mixtures with cocksfoot; this was followed by sainfoin – on average by 10.7% and alfalfa – on average by 8.7%. Differences over the years and on average were statistically insignificant (Table 4). In contrast to the leguminous component, the productivity of cereal plants in mixed crops was significantly lower compared to pure cocksfoot on average by 38.5, 47.0 and 51.7%, in mixtures with sainfoin, bird's-foot trefoil and alfalfa respectively.

In relation to the root biomass weight, the productivities of sainfoin, bird's-foot trefoil and alfalfa in the pure and mixed crops in 2015 and 2016 were similar; differences were insignificant (Table 5). In 2017, the weight of pure legumes significantly exceeded the corresponding leguminous plants in mixed crops. The average dry root biomass weight for the study period did not differ statistically between the two stand types, except for the sainfoin variants, where the productivity of the pure crop was 27.1% higher – a significant figure – than sainfoin plants in the mixture. With regard to the cocksfoot average for 2015-2017, the productivity of the cereal component in the mixtures of sainfoin and bird's-foot trefoil was significantly the lowest (53.0 and 43.0% respectively), followed by that of alfalfa (lower by 29.4% compared to the pure cocksfoot crop).

The productivity factor is genetically determined, and the trend among the studied crops over the years remained unchanged. The weights of both dry aboveground and root biomasses increased with plant growth, but the latter increased very much more than the former. Comparative analysis showed that the aboveground and root biomasses both increase the most intensively in sainfoin from 2015 to 2017 (by 66.5 and 331.6%), followed by bird's-foot trefoil (by 65.2 and 169.4%) and alfalfa (by 44.1 and 131.8%) (Tables 4 and 5).

In mixed crops, the dry aboveground biomass of legume components from 2015 to 2017 increased significantly (differences were non-significant between 2015 and 2016 in alfalfa) (Table 6). A similar increasing trend of the grass component biomasses was found, but differences were not always statistically significant. The most pronounced increase in the productivity of the legume component in the mixture from 2015 to 2017 was found in the bird's-foot trefoil stand (by 1.6 times) whereas that of the grass component was in the sainfoin mixture (by 1.5 times).

The dry root biomass weight, like the dry aboveground biomass weight, increased proportionally in the mixed crops over the years (Table 7). The root productivity of each of the two components increased significantly over the years, and in the legumes, the roots of sainfoin grew the most intensively (almost 4.5 times from 2015 to 2017), and in cocksfoot in mixtures with sainfoin (3 times).

Table 4. Dry aboveground biomass weight of legume components grown alone and in mixtures (g/plant). avg. – average, S – Sainfoin; S (S+C) – Sainfoin from the Sainfoin + Cocksfoot mixture; BT – Bird's-foot trefoil; B (BT + C) – Bird's-foot trefoil from the Bird's-foot trefoil+Cocksfoot mixture; A – Alfalfa; A (A + C) – Alfalfa from the Alfalfa + Cocksfoot mixture; C – Cocksfoot; C (S + C) – Cocksfoot from the Sainfoin + Cocksfoot mixture; C (BT + C) – Cocksfoot from the Bird's-foot trefoil+Cocksfoot mixture; C (A + C) – Cocksfoot from the Alfalfa + Cocksfoot mixture.

Variants	2015	min, max	SD	2016	min, max	SD	2017	min, max	SD	avg.	min, max	SD
S	2.289a*	2.264 2.314	0.035	2.987a	2.806 3.168	0.256	3.812a	3.551 4.073	0.369	3.029a	2.874 3.185	0.220
S (S + C)	2.002a	1.864 2.139	0.194	2.684a	2.590 2.777	0.132	3.435a	3.304 3.566	0.185	2.707a	2.586 2.827	0.170
LSD_{0.05%}	0.601	–	–	0.877	–	–	1.257	–	–	0.846	–	–
BT	1.889a	1.672 2.106	0.307	2.870a	2.680 3.059	0.267	3.120a	2.900 3.340	0.311	2.626a	2.464 2.788	0.229
BT (BT + C)	1.123a	1.010 1.281	0.191	2.186a	2.040 2.332	0.206	2.924a	2.808 3.040	0.164	2.078a	1.953 2.218	0.187
LSD_{0.05%}	1.101	–	–	1.029	–	–	1.070	–	–	1.065	–	–
A	3.204a	3.074 3.334	0.184	3.773a	3.658 3.888	0.162	4.618a	4.497 4.738	0.170	3.865a	3.790 3.940	0.106
A (A + C)	2.838a	2.738 2.937	0.140	2.984a	2.800 3.167	0.259	4.763a	4.636 4.890	0.179	3.528a	3.391 3.665	0.193
LSD_{0.05%}	0.704	–	–	0.932	–	–	0.753	–	–	0.789	–	–

Table 4. Continued.

Variants	2015	min, max	SD	2016	min, max	SD	2017	min, max	SD	avg.	min,m ax	SD
C	2.531b	2.362 2.700	0.239	2.261b	2.212 2.310	0.069	2.600b	2.500 2.700	0.141	2.464b	2.358 2.570	0.150
C (S + C)	0.938a	0.808 1.068	0.183	1.210a	1.000 1.420	0.297	2.400b	2.299 2.501	0.143	1.516a	1.369 1.663	0.208
C (C + BT)	1.000a	0.880 1.120	0.169	1.420a	1.330 1.510	0.127	1.500a	1.400 1.600	0.141	1.307a	1.203 1.410	0.146
C (A + C)	0.902a	0.700 1.104	0.286	1.352a	1.204 1.500	0.209	1.313a	1.126 1.500	0.264	1.189a	1.010 1.368	0.253
LSD_{0.05%}	0.623	–	–	0.543	–	–	0.501	–	–	0.540	–	–

* Means in each column marked by the same letter are not significantly different ($P > 0.05$).

Table 5. Dry root biomass weight of legume components grown alone and in mixtures (g/plant). avg. – average, S – Sainfoin; (S + C) – Sainfoin from the Sainfoin + Cocksfoot mixture; BT – Bird's-foot trefoil; B (BT + C) – Bird's-foot trefoil from the Bird's-foot trefoil + Cocksfoot mixture; A – Alfalfa; A (A + C) – Alfalfa from the Alfalfa + Cocksfoot mixture; C – Cocksfoot; C (S + C) – Cocksfoot from the Sainfoin + Cocksfoot mixture; C (BT + C) – Cocksfoot from the Bird's-foot trefoil + Cocksfoot mixture; C (A + C) – Cocksfoot from the Alfalfa + Cocksfoot mixture.

Variants	2015	min, max	SD	2016	min, max	SD	2017	min, max	SD	avg.	min, max	SD
S	1.125a*	1.038 1.212	0.123	3.358a	3.216 3.500	0.201	4.858b	4.596 5.120	0.371	3.114b	3.103 3.125	0.016
S (S + C)	0.640a	0.485 0.794	0.218	2.662a	2.524 2.800	0.195	3.504a	3.418 3.590	0.121	2.269a	2.245 2.292	0.033
LSD_{0.05%}	0.763	–	–	0.852	–	–	1.186	–	–	0.111	–	–
BT	0.941a	0.780 1.102	0.227	1.978a	1.756 2.200	0.314	2.535b	2.370 2.700	0.233	1.818a	1.635 2.001	0.259
BT (BT + C)	0.497a	0.304 0.690	0.273	1.268a	1.190 1.345	0.109	1.710a	1.620 1.800	0.127	1.158a	1.038 1.278	0.170
LSD_{0.05%}	1.081	–	–	1.012	–	–	0.809	–	–	0.942	–	–
A	2.471a	2.321 2.621	0.212	3.737a	3.624 3.850	0.160	5.727b	5.648 5.806	0.112	3.979a	3.864 4.092	0.161
A (A + C)	2.048a	1.965 2.130	0.117	3.623a	3.582 3.664	0.058	5.015a	4.907 5.123	0.153	3.562a	3.485 3.639	0.109
LSD_{0.05%}	0.736	–	–	0.517	–	–	0.576	–	–	–	–	–

Table 5. Continued.

Variants	2015	min, max	SD	2016	min, max	SD	2017	min, max	SD	avg.	min,m ax	SD
C	2.296c	2.192 2.400	0.147	3.139c	2.990 3.287	0.210	4.248c	4.086 4.409	0.228	3.227c	3.197 3.258	0.043
C (S + C)	0.938a	0.805 1.071	0.188	1.210a	1.184 1.236	0.037	2.400a	2.312 2.488	0.124	1.516a	1.434 1.598	0.116
C (C + BT)	0.913a	0.786 1.040	0.180	2.002b	1.854 2.150	0.209	2.600ab	2.500 2.700	0.141	1.838a	1.713 1.963	0.177
C (A + C)	1.464b	1.386 1.542	0.110	2.424b	2.328 2.519	0.135	2.948b	2.788 3.108	0.226	2.279b	2.167 2.390	0.158
LSD_{0.05%}	0.442	–	–	0.455	–	–	0.517	–	–	0.371	–	–

* Means in each column marked by the same letter are not significantly different ($P > 0.05$).

Table 6. Dry aboveground biomass weight of grass and legume components in binary mixtures (g/plant).

Year	Sainfoin	Cocksfoot	Bird's-foot trefoil	Cocksfoot	Alfalfa	Cocksfoot
2015	2.002a*	0.938a	1.123a	1.000a	2.838a	0.902a
2016	2.684b	1.210a	2.186b	1.420ab	2.984a	1.352a
2017	3.435c	2.400b	2.924c	1.500b	4.763b	1.313a
Average	2.707	1.516	2.078	1.307	3.528	1.189
LSD _{0.05%}	0.429	0.486	0.428	0.436	0.634	0.812

*Means in each column marked by the same letter are not significantly different ($P > 0.05$).

Table 7. Dry root biomass weight of grass and legume components in binary mixtures (g/plant).

Year	Sainfoin	Cocksfoot	Bird's-foot trefoil	Cocksfoot	Alfalfa	Cocksfoot
2015	0.640a*	0.938a	0.497a	0.913a	2.048a	1.464a
2016	2.662b	1.210a	1.268b	2.002b	3.623b	2.424b
2017	3.504c	2.400b	1.710b	2.600c	5.015c	2.948b
Average	2.269	1.516	1.158	1.838	3.562	2.279
LSD _{0.05%}	0.582	0.460	0.589	0.569	0.368	0.524

*Means in each column marked by the same letter are not significantly different ($P > 0.05$).

Fig. 1 shows that the cultivation system influenced the degree of damage caused by *Sitona* species. Among the pure crops, bird's-foot trefoil had the highest proportion of damaged nodules and alfalfa the lowest: the difference between the two variants was significant. Nodules damaged by the larvae of *Sitona* weevils in mixed crops decreased by 33.5% on average (sainfoin + cocksfoot), by 50.3% (bird's-foot trefoil + cocksfoot) and by 55.6% (alfalfa + cocksfoot) compared to pure legumes: the differences were significant at 95% confidence intervals. Damage reduction was the most pronounced in the alfalfa mixture, where the percentage of damaged nodules was the lowest (20.8%). Among the mixed crops, the damage rate varied from 20.8 to 40.5%, but there were no significant differences between them.

Reducing weevil damage in mixed crops increases plant productivity. A strong negative correlation was found between the proportion of damaged nodules and the productivity of root and aboveground biomasses ($r = -0.886$ and $r = -0.846$, respectively).

Fig. 1. Influence of damage of *Sitona* spp. (% damaged nodules) on productivity in pure and mixed crops averaged for 2015-2017. 1 – Sainfoin; 2 – Sainfoin from the Sainfoin + Cocksfoot mixture; 3 – Bird's-foot trefoil; 4 – Bird's-foot trefoil from the Bird's-foot trefoil + Cocksfoot mixture; 5 – Alfalfa; 6 – Alfalfa from the Alfalfa + Cocksfoot mixture.

Fig. 2. Influence of damage of *Otiorrhynchus ligustici* (length of gnawed furrows, cm) on productivity in pure and mixed crops averaged for 2015-2017. Abbreviations like in the Fig. 1.

Damage by *O. ligustici* was reduced to a different extent in the mixed stand as compared to the pure crops (Fig. 2). The damage caused by the alfalfa snout beetle, expressed by the length of the gnawed spiral furrows in mixed crops with sainfoin, bird's-foot trefoil and alfalfa decreased considerably – by 12.5, 77.8 and 59.5%, respectively – compared to the pure crops. The most pronounced reduction in the furrows took place in the mixture of bird's-foot trefoil with cocksfoot, while in the pure stands, the legume crop had the lowest value of this indicator (on average, a furrow length of 1.8 cm per root compared to 2.4 cm in sainfoin and 2.5 cm in alfalfa). The difference in values between those two crops was statistically significant.

In the mixture of alfalfa and cocksfoot, the degree of damage by *O. ligustici* was significantly lower compared to the pure stands, whereas in the sainfoin variants the difference was insignificant. There were no statistical differences in the furrow lengths between the pure perennial crops, whereas between the mixed crops of bird's-foot trefoil and alfalfa the values were significantly lower than the sainfoin mixture. The correlation coefficient between the root and aboveground biomasses with furrow length was negative ($r = -0.151$ and $r = -0.100$, respectively), but unlike the dependence on the damaged nodules by *Sitona* weevils, the correlations were weak.

In order to determine the impact of the damage and plant heights on productivity and whether they had any influence, a regression analysis based on the root and aboveground productivity (g/m^2) was performed (Tables 8 and 9).

The results of the analysis of variance showed that the linear component in the regression of the dry aboveground biomass and dry root biomass with respect to the damaged traits was significant.

Models were obtained (1 and 2) from the comprehensive study of the traits; they demonstrated the complex character of the change in productivity of the dry aboveground and root biomasses, depending on the variability of the damage traits.

The usual types of regression equations were:

$$(1) Y = 791.723 - 5.492 * X_1 - 67.082 * X_2 + 0.255 * X_3$$

where Y – productivity of the dry aboveground biomass; X_1 – % damaged nodules, cm; X_2 – length of the gnawed furrows, cm; X_3 – height, cm;

$$(2) Y = 957.208 - 11.105 * X_1 - 123.425 * X_2 + 0.0562 * X_3$$

where Y – productivity of the dry root biomass; X_1 – % damaged nodules, cm; X_2 – length of the gnawed furrows, cm; X_3 – height, cm.

Regression analysis (Table 8) showed that the damage from *O. ligustici* as expressed by the length of the gnawed furrows had the most negative effect on the productivity of aboveground biomass and that the regression coefficient of - 67.082 was statistically significant ($P = 0.0135$ and P is no greater than 0.05; this term is statistically significant at the 95% confidence level). The regression between the damaged nodules percent and

productivity of aboveground biomass was weaker ($r = -5.492$), but with significant difference ($P = 0.0001$).

Table 8. Analysis of variance and regression coefficients of the productivity of aboveground biomass of pure and mixed leguminous crops with regard to damage indicators and plant height. Cf – coefficients, SE – standard error, L 95% – lower 95%, U 95% – upper 95%.

Analysis of Variance					
Source	Sum of Squares	Cf	Mean Square	F-Ratio	P-Value
Model	166201.0	3	55400.4	16.50	0.0001*
Residual	47018.9	14	3358.49	–	–
Total (Corr.)	213220.0	17	–	–	–

*If the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between the variables at the 95% confidence level.

Regression coefficients						
Trait	Cf	SE	t Stat	P-value	L 95%	U 95%
Constant	791.723	90.7671	8.72257	0.0000	597.046	986.399
% damaged nodules	-5.4921*	0.9775	-5.61874	0.0001	-7.5885	-3.3956
Length of the gnawed furrows, cm	-67.0817*	23.7529	-2.82415	0.0135	-16.1367	-118.027
Height, cm	0.2548	1.1438	0.2228	0.8269	2.7081	2.1985

*There is a significant difference ($P < 0.05$) between the trait and the dry aboveground biomass.

The impact of the damage traits on the productivity of the root biomass was more pronounced (Table 9). The length of spiral furrows has the highest significant negative effect on the root weight (regression coefficient = - 123.425 at $P = 0.0120$), followed by the degree of damaged nodules with a significant negative influence (regression coefficient = - 11.105). The height of the plants had no effect on root biomass productivity.

DISCUSSION

The productivity and quality of high-protein forage crops increasingly needed for livestock development in Bulgaria is an important and permanent task in agriculture. It is usually accomplished through the use of high-yielding forage species, which at the same time can provide balanced agricultural production and the production of competitive agricultural and animal products.

Table 9. Analysis of variance and regression coefficients of the **productivity of root biomass** of pure and mixed leguminous crops with regard to damage indicators and plant height. Cf – coefficients, SE – standard error, L 95% – lower 95%, U 95% – upper 95%.

Analysis of Variance						
Source	Sum of Squares	Cf	Mean Square	F-Ratio	P-Value	
Model	718303.0	3	239434.0	21.96	0.0001*	
Residual	152622.0	14	10901.6	–	–	
Total (Corr.)	870925.0	17	–	–	–	

*If the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between the variables at the 95% confidence level.

Regression coefficients						
Trait	Cf	SE	t Stat	P-value	L 95%	U 95%
Constant	957.208	163.531	5.8534	0.0001	606.468	1307.95
% damaged nodules	-11.105*	1.7611	-6.3059	0.0020	-14.8821	-7.3279
Length of the gnawed furrows, cm	-123.425*	42.7947	-2.8841	0.0120	-31.6393	-215.211
Height, cm	0.0562	2.0608	0.0273	0.9786	4.4762	4.3637

*There is a significant difference ($P < 0.05$) between the trait and the dry aboveground biomass.

The productivity of legume components (alfalfa, sainfoin and bird's-foot trefoil) and cocksfoot in the mixtures were lower than that of pure legumes and grass during the years of this study. An analogous tendency was reported by BERDAHL et al. (2001) with regard to a higher dry matter yield of a grass species in a pure stand as compared to a mixture of alfalfa and grass.

Productivity is an indicator influenced not only by the system of cultivation but also by many other factors, the key to which is the presence of economically important pests. Among them, high numbers of polyphagous root pests *Otiorrhynchus ligustici* and *Sitona* weevils can reduce or even completely destroy the harvest (SHIELDS et al. 2008). As the use of plant protection products for managing pests is difficult because many of them lead a hidden lifestyle, the application of alternatives to conventional methods is of great importance: one of these approaches is to diversify the plants in cultivation systems

In our study, the damage caused by *Sitona* spp. and *Otiorrhynchus ligustici* in mixed crops was reduced to varying extents in relation to the monocultures. The reduction in damage by nodule weevils was the most pronounced in the alfalfa mixture. Similar results for significantly lower numbers of *Sitona* weevils and less damage caused by them in a mixture of peas with white mustard versus pure crops were reported by WNUK & WIECH (1996).

It was found that the nodules preferred and damaged by the *Sitona* weevil larvae in pure crops were those of sainfoin, followed by alfalfa and bird's-foot trefoil. Similar results were

reported by BARRATT & BYERS (1991), according to which alfalfa was significantly more preferred and susceptible to damage by *Sitona hispidulus* (FABRICIUS, 1776) than bird's-foot trefoil. Possible reasons for the preference of *Sitona* weevils for some species may be the existence of a signalling mechanism enabling the larvae to find the roots and the nodules formed on them, as well as a higher nodule density (ZHANG et al. 2006). The feeding of *Sitona* larvae had a negative effect on alfalfa growth, reducing this to 40% per year according to ARBAB et al. (2008). The inclusion of *Medicago sativa* in a mixture with other crops (for example, with sainfoin) decreased weevil densities and led to higher forage yields (DIZAJA et al. 2015).

ROSHANDEL & NOORBAKHSIAN (2009) found that average numbers of *Hypera postica* (GYLLENGAL, 1813) larvae, pest population size and percentage of infestation were all significantly lower in mixed treatments in comparison with pure alfalfa and red clover. In addition, according to those authors, the forage yield was higher in mixed treatments than in pure legume crops.

There is significant evidence showing that the increase in species diversity in the crop systems considerably improves the management and control of harmful insects, which in itself is an economically and ecologically sustainable method of control (LUNDGREN et al. 2008, GLINWOOD et al. 2009). One of the reasons for this is the reduced ability of pests to find their preferred host plants, i.e. associative resistance (AGRAWAL et al. 2006, RANDLKOEFER et al. 2010).

CONCLUSIONS

- The productivities of aboveground and root biomasses in binary mixtures were significantly higher compared to corresponding legume monocultures on average by 30.7 and 39.7% respectively in the mixture with sainfoin, by 15.0 and 73.1% in the mixture with bird's-foot trefoil and by 18.7 and 49.6% in the mixture with alfalfa.
- The weight of dry aboveground biomass in the pure leguminous crops slightly exceeded the corresponding leguminous components in the mixtures, whereas the productivity of cereal plants in mixed crops was significantly lower compared to pure cocksfoot on average by 38.5, 47.0 and 51.7% in mixtures with sainfoin, bird's-foot trefoil and alfalfa respectively. A similar tendency was observed with regard to dry root biomass.
- Damage to nodules by *Sitona* larvae in mixtures decreased significantly, on average by 33.5% (sainfoin + cocksfoot), by 50.3% (bird's-foot trefoil + cocksfoot) and by 55.6% (alfalfa + cocksfoot) compared to pure grown legumes. A strong negative correlation was found between the proportion of damaged nodules and the productivity of root and aboveground biomass ($r = -0.886$ and $r = -0.846$, respectively).

- The damage caused by *Otiorrhynchus ligustici* expressed by the length of gnawed furrows in the mixtures with sainfoin, bird's-foot trefoil and alfalfa decreased considerably by 12.5, 77.8 and 59.5%, respectively, compared to the pure crops. Significant furrow reduction was found in the mixture of bird's-foot trefoil and alfalfa with cocksfoot compared to pure crops. The damage caused by the alfalfa snout beetle had the most significant negative effect on aboveground and root biomass productivity.

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